Aquatic Fate of Sunscreen Agents Octyl-4-methoxycinnamate and Octyl-4-dimethylaminobenzoate in Model Swimming Pools and the Mutagenic Assays of Their Chlorination Byproducts

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Reactions of sunscreen agents, octyl dimethyl-*p*-aminobenzoate (ODPABA) and octyl-*p*-methoxycinnamate (OMC), with hypochlorite in aqueous solution were investigated under the conditions that simulate swimming pool disinfection sites. Chlorination byproducts were determined by GC-MS. At a concentration of 9 μM, ODPABA reacted rapidly with free chlorine in the buffered solution at pH 7.0, OMC reacted with hypochlorite reasonably slowly under the same condition. ODPABA and OMC produced chlorine-substituted compounds as intermediates, which were decomposed to cleavaged products of ester-bond during the aqueous chlorination process. The chlorination intermediates of OMC exhibited weak mutagenic on *Salmonella typhimurium* TA100 strain without the S9 mix. The extent of the reactions depended on the chlorine dose, solution pH, and compound structures.

Key words —— octyl-*p*-methoxycinnamate, octyl dimethyl-*p*-aminobenzoate, water chlorination, mutagenicity

INTRODUCTION

The focus of environmental research has been extended from traditional pollutants, such as polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and pesticides, to emerging pollutants such as pharmaceuticals and personal care products (PPCPs), some of which may be carcinogenic, mutagenic and reproductively toxic.[1–3] Pharmaceutical substances are used in human and veterinary medicines and can enter to the aquatic environment following their manufacture, use or ingestion/excretion. The rapid increase in the use of pharmaceutical products is a new environmental concern and has attracted considerable attention worldwide.[4–6]

Recent studies illustrate the omnipresence of numerous PPCPs in municipal wastewater effluents and affected surface waters.[7] A class of compounds that are known as sunscreen agents as well as their structurally related transformation products are expected to present in aquatic environments[8–10] on the basis of their production and use. However, none of the major field studies completed thus far have included parent compounds in their analysis suite. It has been estimated that than less 15% of the PPCPs predicted to occur in the environment are actually sought analytically.[11]

Structures of the sunscreen agents that were investigated in the present study are shown in Table 1. The increased use of sunscreens has raised questions regarding the environmental impact of sunscreen ingredients. Sunscreens applied to the skin may be introduced into the chlorine-treated water and then the surface water when they are released from the skin during swimming and bathing. Although several papers[8–10] have reported the detection of sunscreens in chlorinated water and natural waters, only few papers have reported the fate of sunscreen agents in chlorinated waters such as that swimming pools.[12, 13] The possible endocrine disrupting effect of certain sunscreens is a matter of concern[14] and some of these compounds have been

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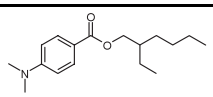
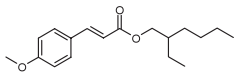
Table 1. Physicochemical Properties of the Sunscreen Agents, ODPABA and OMC: Chemical Structure, Molar Formula, CAS Number, Molecular Mass and Octanol-water Partition Coefficients (Kow)

Compounds	Chemical structure	Chemical formula	CAS-No.	Molecular mass	Log Kow
Octyl dimethyl-*p*-aminobenzoate (ODPABA)		$C_{17}H_{27}NO_2$	21245-02-3	277.41	5.76
Octyl-*p*-methoxy-cinnamate (OMC)		$C_{18}H_{26}O_3$	466-77-3	290.41	5.80

detected in fish from lakes used for bathing[15] and also from sediments and soils.[16]

In order to provide further insight into the possible role of organic compounds in the formation of chlorine-substituted compounds and that of chlorine-induced mutagens, we continued our study on the chemistry of aqueous chlorination of organic compounds.[17,18] The objectives of the present study are to quantify the reaction kinetics of octyl dimethyl-*p*-aminobenzoate (ODPABA) and octyl *p*-methoxycinnamate (OMC) with free available chlorine (HOCl) at various pH values, to identify the probable transformation products under conditions relevant to chlorine-based processes for swimming pool disinfection, and to evaluate mutagenic responses of both the reaction products and the parent compounds.

MATERIALS AND METHODS

Chemicals —— ODPABA and OMC of analytical-reagent grade were purchased from Kanto Chemical Co. Ltd. (Tokyo, Japan) and their purities were over 98% in gas chromatographic analysis. Several OMC degradation products, such as *p*-methoxycinnamic acid, *p*-methoxybenzoic acid, *p*-methoxybenzaldehyde, methoxybenzene and octanone, which are probably formed when OMC reacts with hypochlorite in dilute aqueous solution, are commercially available reagents. The standards of these compounds, both individually and as a mixture, were prepared by dissolving the compounds in methanol and performing subsequent dilutions. The organic solvents (methanol 300, *n*-hexane 300, and ethyl acetate 300) used in this experiment were of analytical-reagent grade for pesticide residue analysis (Wako Pure Chemical Industry Co. Ltd., Osaka, Japan). Hypochlorite solution were prepared by diluting the sodium hypochlorite solution (*ca.* 5% available Cl; Kanto Chemical Co. Ltd.) with distilled water. The pH values of the solutions were adjusted to the required level by adding 0.1 M phosphate buffer solution. The hypochlorite concentrations were determined by means of iodometric titrations.

Aqueous Chlorination of Sunscreen Agents —— A mixture of 100 ml buffered solution of sunscreen agents and 1 ml hypochlorite solution was shaken in a brown separatory funnel to avoid its photodegradation at room temperature (*ca.*, 20°C). After the required reaction time, unreacted chlorine was removed by bubbling with dry nitrogen gas for 15 min at room temperature. The reaction mixture was then acidified to pH 2 by using 6 M hydrochloric acid before extracting 20-ml portions of *n*-hexane/ethyl acetate (1 : 1). The extracts were dried over anhydrous sodium sulfate and concentrated under reduced pressure at 40°C to volumes that are suitable for GC analyses, GC-MS analyses and mutagenicity tests.

Product Resolution and Determination —— The extracts were analyzed using a Varian CP-3800 gas chromatograph coupled to a Saturn 2200 ion trap GC-MS (Varian, Walnut Creek, CA, U.S.A.) equipped with a TC-1 capillary column (30 m × 0.25 mm i.d., and 0.12 μm film thickness, GL Science Inc., Tokyo, Japan) under temperature ranging from 80 to 270°C at the rate of 10°C/min. The helium gas flow-rate was 1 ml/min. The electron ionization conditions were as follows: ion energy, 70 eV; ion source temperature, 230°C; m/z = 50–600 full scan for qualitative analysis. A sample volume of 1 μl was splitlessly injected into the GC-MS at 270°C. The products were identified by comparing their retention times and mass spectra with those of authentic compounds.

Quantitative analyses were performed using a Shimadzu GC-14B gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame ionization de-

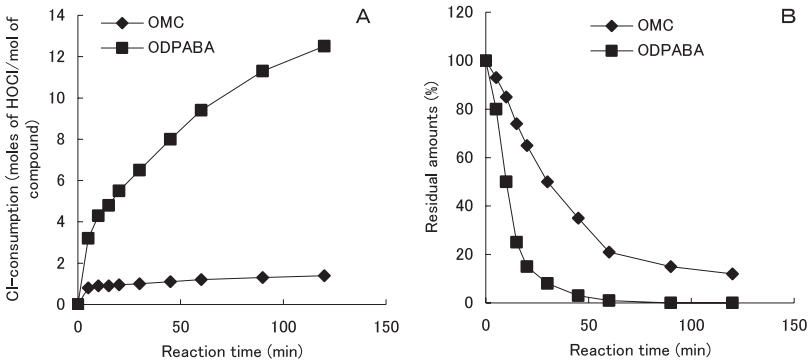


Fig. 1. Time-course Changes in HOCl Consumption by the Sunscreen Agents (A) and Residual Amounts of OMC and ODPABA (9 μM) (B) in Buffered Water at pH 7.0 and Room Temperature

tector (FID). The analytical column and chromatographic conditions were the same as those described above. Both injector and detector temperatures were set at 270°C. A Shimadzu C-R6A data processor was used to determine the retention times and peak areas of the chromatograms. Octadecane was used as an internal standard throughout the GC analyses of the reaction mixtures.

Mutagenicity Assay —— Mutagenicity of the samples was tested according to the methods of Malon and Ames[19] with some minor modifications. Because most of the mutagens formed during the reactions of the organic compounds with the chlorine in water have been shown to be positive in *Salmonella typhimurium* strain TA100 strain without the S9 mix,[20] this strain was used throughout the experiments. The samples were dissolved in dimethyl sulfoxide (DMSO) and preincubated with a test strain in a phosphate buffer at 37°C for 20 min. After the addition of the test samples, the plates were incubated at 37°C for 2 d. The assay was performed in triplicate for each sample, and the mutagenic responses were expressed as the mean of the total number of revertants observed. The mutagenic responses of the sunscreen agents and chlorination products were estimated by the least-squares regression analysis of the initial slopes of the dose-response curves.

RESULTS AND DISCUSSION

Kinetics of the Hypochlorite Reactions

In a preliminary examination of the reactions of the sunscreen agents (ODPABA and OMC) with hypochlorite in a dilute aqueous solution at pH 7.0, the decrease in the concentration of HOCl during contact with each of these compounds was measured by using a Model RC-1 Resiclocorder (Bionics Instrument, Tokyo, Japan) at 20°C. The decrease in the concentrations of these sunscreen agents was also determined under the same conditions by GC-FID analyses of the reaction mixtures. Furthermore, the individual reactions of the sunscreen agents with hypochlorite in water at pH 5.0, 7.0, and 9.0 were measured by iodometric titrations.

Figure 1 shows the time-course changes in HOCl consumption induced by the sunscreen agents (initial concentration = 9 μM) and the residual concentrations of the parent compounds in the buffered solution at pH 7.0. As shown in the figure, HOCl was rapidly consumed by ODPABA during the first 10 min and considerably slower thereafter: approximately 50% of the active chlorine was consumed within the first 1 hr of the 24-hr reaction (*ca.*, 18.0 moles of HOCl/mol of ODPABA) with ODPABA (Fig. 1A). The original ODPABA in the buffered solution at pH 7.0 disappeared completely within 1 hr after exposure to aqueous chlorine under the same condition (Fig. 1B).

In contrast to the ODPABA-hypochlorite reaction, a rapid but small consumption of HOCl by OMC occurred during the first few minutes, followed by a considerably slower secondary reaction (Fig. 1A). In addition, the GC analyses showed that original OMC was decomposed rapidly within the first reaction step, but some OMC remained in the buffered solution at pH 7.0, despite of the presence of excess of chlorine (Fig. 1B). The difference in the reactivity of ODPABA and OMC toward chlorine in water can be explained by the chemical structures of their substituents (methoxy and dimethylamino groups) in the benzene ring (Table 1).

Table 2 summarizes the chlorine consumptions by the 2 individual sunscreen agents in the presence of buffer reagent in chlorinated water at pH

Table 2. Chlorine (Cl) Demands of ODBABA and OMC in a 9 μmol/l Solution for 1 and 24 hr, Respectively, with Excess of Hypochlorite at 20°C

Chemicals tested	Solution pH	Cl demand (mol/mol)	
		after 1 hr	after 24 hr
ODPABA	5.0	9.50	18.35
	7.0	9.30	18.10
	9.0	8.50	18.00
OMC	5.0	1.75	2.50
	7.0	1.50	2.00
	9.0	1.45	1.85

5.0, 7.0, and 9.0. As a general trend, the rate of chlorine consumption by both the sunscreen agents decreased with an increase in the solution pH during the first 1 hr of the hypochlorite reactions. The chlorine demands of both the sunscreens in acidic solutions after 24 hr were also found to be somewhat larger than those observed under alkaline and neutral conditions. This can be explained in terms of the concentrations of undissociated hypochlorous acid (pKa = 7.5) in the chlorinated water.

Chlorination Byproducts of Sunscreen Agents

Figure 2 shows the typical chromatograms (with FID) of *n*-hexane/ethyl acetate extracts of the chlorinated ODPABA and OMC solutions at pH 7.0 after treatment with hypochlorite at 20 equivalents of HOCl per mol of the compound for 24 hr in the dark. The mass spectra of the typical peaks in Fig. 2B are also presented in Fig. 3.

The chromatogram (Fig. 2A) of the *n*-hexane/ethyl acetate extracts obtained from the chlorinated ODPABA solution shows 7 reaction products with comparatively large GC peaks and several compounds with small GC peaks. A previous study[12] has reported that chlorine-substituted compounds and dealkylation intermediates are major products of ODPABA after its exposure to an aqueous solution of chlorine. In the present study, similar chlorination byproducts, such as $C_{17}H_{25}Cl_2NO_2$ [Cl_2-ODPABA] (peak 1 in Fig. 2A), $C_{17}H_{26}ClNO_2$ [Cl-ODPABA] (peak 2), $C_{16}H_{24}ClNO_2$ [Cl-octyl-*p*-monomethylamino benzoate (OMPABA)] (peak 4), $C_{16}H_{23}Cl_2NO_2$ [Cl_2-OMPABA] (peak 5), $C_{15}H_{22}ClNO_2$ [Cl-octyl-*p*-aminobenzoate (O-PABA)] (peak 6), and $C_{15}H_{21}Cl_2NO_2$ [Cl_2-O-PABA] (peak 7) were detected in the *n*-hexane/ethyl acetate extracts.

Considerable reaction products including compounds that had a retention time less than and greater than that of octadecane (C_{18}: used as an internal standard) were observed on the chromatogram (Fig. 2B) of the *n*-hexane/ethyl acetate extracts obtained from the chlorinated OMC solution. However, the nature of the compounds having small retention time, except for the octanone (C_8), could not be determined because these compounds exhibited smaller GC peak areas and different retention times as compared to the standard compounds tested in the present experiment.

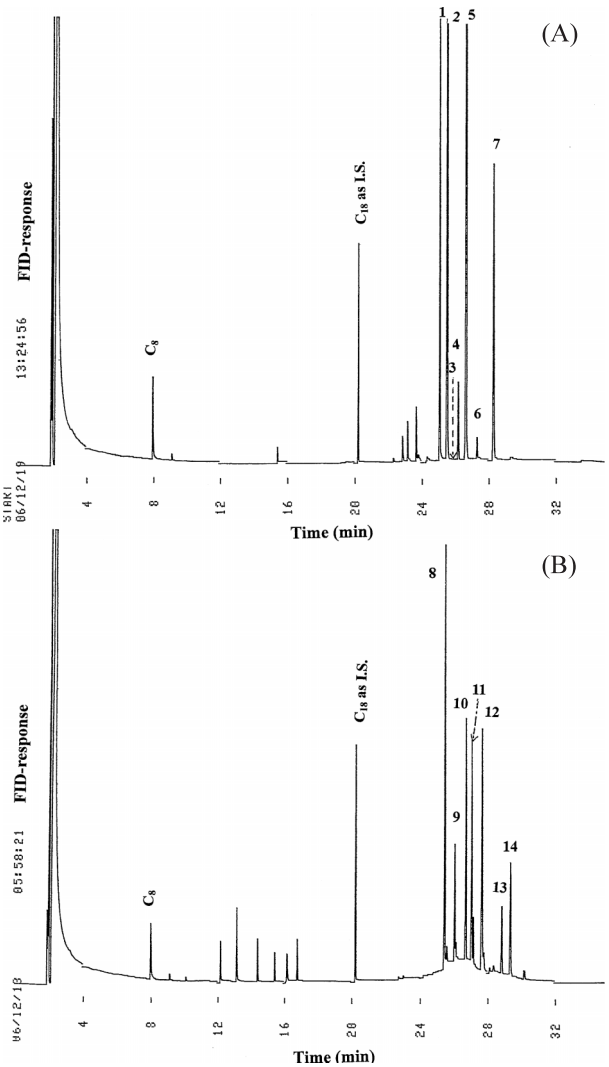


Fig. 2. Gas Chromatograms (FID) of *n*-Hexane/ethyl Acetate (1 : 1) Extracts of Aqueous ODPABA (A) and OMC (B) Solutions (70 μM) after Treatment with Hypochlorite (20 Equivalents of HOCl per mol of the Compound) at pH 7.0 and Room Temperature for 24 hr

Pattanaargson *et al.*[21] and Huong *et al.*[22] have demonstrated that when exposed to sunlight, octyl-*p*-methoxy-*trans*-cinnamate (E-OMC) rapidly transformed into octyl-*p*-methoxy-*cis*-OMC (Z-OMC) in several polar solvents, as shown in Fig. 4. In the previous paper,[23] the presence of a new peak at 24.34 min, which seems to be Z-OMC,

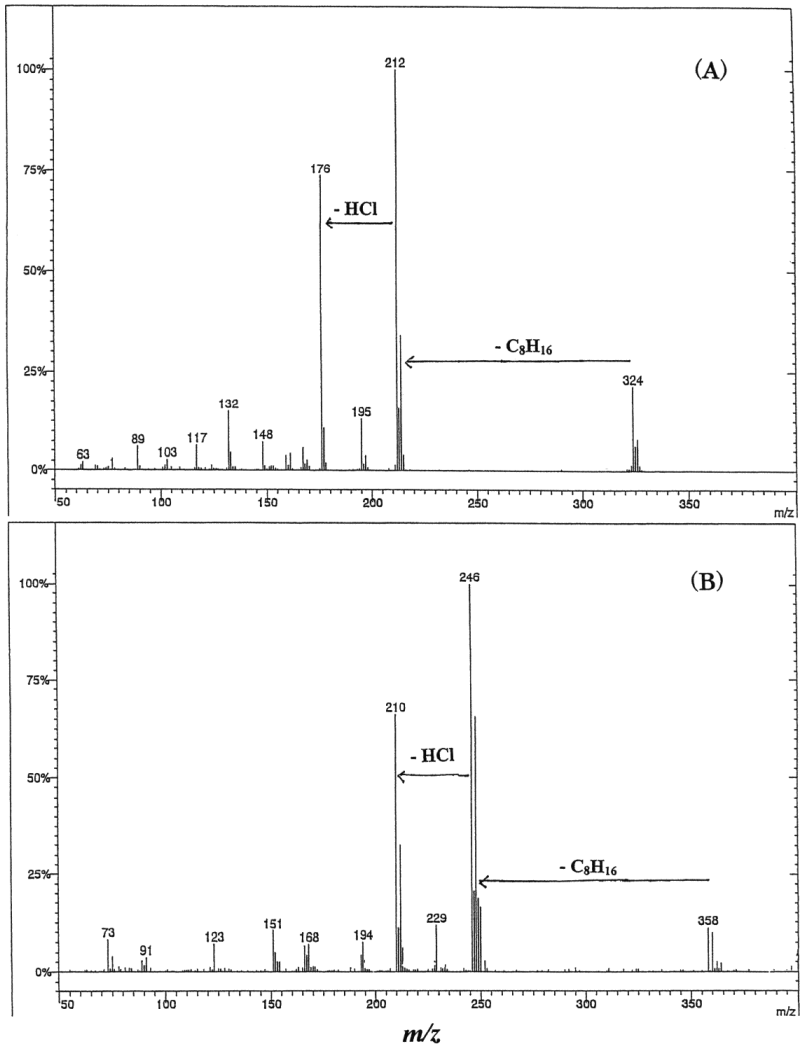


Fig. 3. Mass Spectra of Peaks 8 (A) and 13 (B) in Fig. 2B

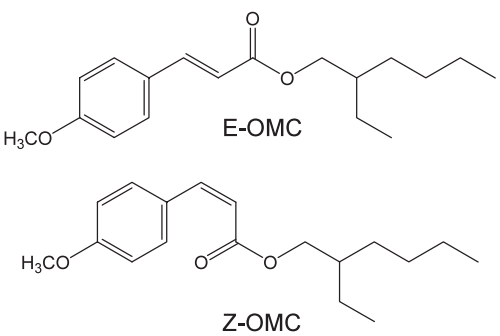


Fig. 4. Structures of E-OMC and Z-OMC

and the original peak at 26.15 min due to E-OMC could be observed on the gas chromatogram of the *n*-hexane/ethyl acetate extract from the aqueous OMC solution after exposure to sunlight. Further experiments[23] also showed the presence of similar chromatographic peaks to that given in Fig. 2B when the aqueous OMC solutions exposed to sunlight and then followed by treatment with hypochlorite.

Compounds corresponding to peaks 8, 10, 11, and 12 in Fig. 2B yielded the same molecular ion at m/z 324 ($C_{18}H_{25}ClO_3^+$) [Cl-OMC] having 1 chlorine atom and the most abundant fragment ions at m/z 212 ($C_{10}H_9ClO_3^+$), 195 ($C_{10}H_8ClO_2^+$), 176 ($C_{10}H_8O_3^+$), 148 ($C_9H_8O_2^+$), and 132 ($C_9H_8O^+$) (Fig. 3). On the basis of their mass spectra and GC retention times, these compounds were considered to be present in the chlorinated water, as their isomers of the E-OMC and Z-OMC. Furthermore, the compounds corresponding to peaks 13 and 14 in Fig. 2B yielded the same molecular ion at m/z 358 ($C_{18}H_{24}Cl_2O_3^+$) [Cl_2-OMC] that had 2 chlorine atoms and the most abundant fragment ions at m/z 246 ($C_{10}H_8Cl_2O_3^+$), 229 ($C_{10}H_7Cl_2O_2^+$), and 210 ($C_{10}H_7ClO_3^+$) (Fig. 3). The molecular ions and their fragmentation patterns also indicate the occurrence

Table 3. GC Retention Times and Mass Spectral Data of Major Chlorination Byproducts of Ethyl Acetate/*n*-hexane Extracts Obtained from Chlorinated Aqueous ODPABA and OMC Solutions

Peak No. in Fig. 2	Retention time (min)	Molecular ion (*m*/*z*)	Fragment ion *m*/*z* (relative intensity, %)	Proposed formulae	(abbreviation)
ODPABA-hypochlorite reaction					
1	25.160	345	345 (76), 232 (100), 216 (26), 118 (17)	$C_{17}H_{25}Cl_2NO_2$	(Cl_2-ODPABA)
2	25.587	311	311 (74), 198 (100), 182 (32), 118 (14)	$C_{17}H_{26}ClNO_2$	(Cl-ODPABA)
3	25.663	277	277 (47), 165 (100), 148 (51), 120 (7)	$C_{17}H_{27}NO_2$	(ODPABA)
4	26.282	297	297 (15), 185 (100), 168 (31), 140 (7)	$C_{16}H_{24}ClNO_2$	(Cl-OMPBABA)
5	26.713	331	331 (26), 219 (100), 202 (30), 147 (57)	$C_{16}H_{23}Cl_2NO_2$	(Cl_2-OMPABA)
6	27.368	283	283 (4), 171 (100), 154 (52), 126 (12)	$C_{15}H_{22}ClNO_2$	(Cl_2-O-PABA)
7	28.362	317	317 (4), 205 (100), 186 (40), 124 (24)	$C_{15}H_{21}Cl_2NO_2$	(Cl_2-O-PABA)
OMC-hypochlorite reaction					
8	25.477	324	324 (25), 212 (100), 195 (11), 176 (75), 132 (19)	$C_{18}H_{25}ClO_3$	(Cl-OMC-1)
9	26.147	290	290 (4), 178 (100), 161 (47), 133 (15)	$C_{18}H_{26}O_3$	(OMC)
10	26.875	324	324 (19), 212 (100), 195 (11), 176 (64), 132 (19)	$C_{18}H_{25}ClO_3$	(Cl-OMC-2)
11	27.208	324	324 (21), 212 (100), 195 (12), 176 (60), 132 (17)	$C_{18}H_{25}ClO_3$	(Cl-OMC-3)
12	27.708	324	324 (21), 212 (100), 195 (13), 176 (74), 132 (15)	$C_{18}H_{25}ClO_3$	(Cl-OMC-4)
13	28.405	358	358 (12), 246 (100), 210 (66), 151 (11)	$C_{16}H_{24}Cl_2O_3$	(Cl_2-OMC-1)
14	29.407	358	358 (17), 246 (100), 210 (35), 151 (11)	$C_{16}H_{24}Cl_2O_3$	(Cl_2-OMC-2)

of 2 isomers of dichloro-substituted OMC in the *n*-hexane/ethyl acetate extract.

A summary of the chlorination products identified or determined from the GC retention times and mass spectra is presented in Table 3. According to Knowland *et al.*[24] ODPABA is similar to an industrial chemical that generates free radicals when illuminated and is harmless in the dark but mutagenic in sunlight, where it attacks DNA directly. It is, therefore, necessary to reveal the fate of these compounds reported here (Table 3) in chlorinated water or in an outdoor aquatic system.

Factors Affecting the Sunscreen Agent-hypochlorite Reactions

Although several reports have been published on the photodecomposition of the sunscreen agents in water,[12,21,22] no study has been reported on the fate of the sunscreen agents-OMC and ODPABA-on exposure to hypochlorite in water. Figure 5 shows the time-course changes in the reactions of the sunscreen agents with an excess of hypochlorite at room temperature in the buffered solutions at pH 7.0.

On exposure of ODPABA to aqueous hypochlorite solution, the amount of the original compound rapidly decreased with the formation of chloro-substituted compounds (Fig. 5A). Dichlorinated ODPABA (Cl_2-ODPABA) and OMPABA (Cl_2-OMPABA) were present at higher concentrations in water when ODPABA was treated with hypochlorite for a short time (Fig. 5A) and at low molar ratios of hypochlorite to the compound (data not shown). These findings suggest that chloro-substitution to the parent compound and demethylation of Cl_2-ODPABA to form Cl_2-OMPABA and then dichlorinated O-PABA (Cl_2-O-PABA) in the chlorinated water occurs rapidly during the first reaction step.

Monochloro-OMC isomers were present at higher concentrations in water when the parent compound was treated with hypochlorite for a short reaction time (Fig. 5B) and at low molar ratios of hypochlorite to the compound (data not shown). This was followed by the formation of a small amount of dichloro-OMC isomers and subsequent destruction of these chloro-substituted compounds. The amounts of *n*-hexane/ethyl acetate extracts and those of compounds that could be studied chromatographically decreased with increasing contact time. These findings indicate the formation of highly water-soluble and non-volatile compounds during the secondary reactions in chlorine-treated OMC solution.

Figure 6 shows the GC determinations of *n*-hexane/ethyl acetate extracts from aqueous solutions of the sunscreen agents after treatment with hypochlorite at 20 equivalents of HOCl per mol of the compound and at various pH values for 1 hr in the dark. High concentrations of Cl_2-ODPABA (column 2 in Fig. 6A) and Cl_2-OMPABA (column 6) were detected in the chlorinated wa-

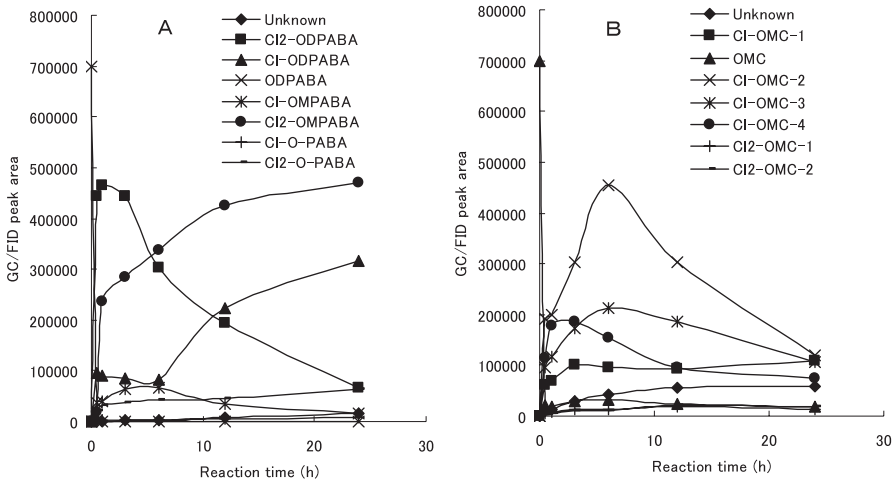


Fig. 5. Residual Amounts of the Reaction Products in Aqueous ODPABA (A) and OMC (B) Solutions (70 μM) after Treatment with Hypochlorite (20 Equivalents of HOCl per mol of the Compound) at pH 7.0 and Room Temperature

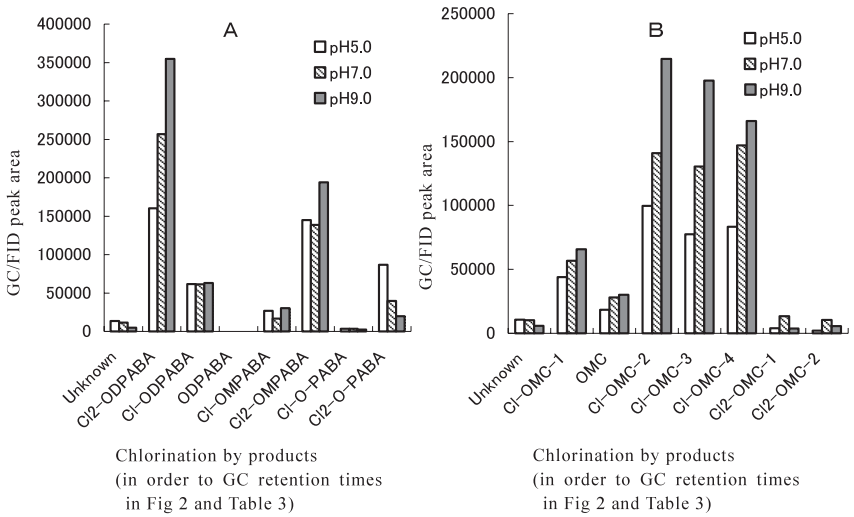


Fig. 6. Residual Amounts of the Reaction Products of Aqueous ODPABA (A) and OMC (B) Solutions (70 μM) after Treatment with Hypochlorite (20 Equivalents HOCl per mol of the Compound) at Room Temperature for 1 hr and at Various pH Values

ter, whereas lower concentrations of total degradation products (column 1) and Cl-O-PABA (column 7) were detected in the chlorinated ODPABA solutions. These findings indicate that the former 2 products are stable, but the latter 3 chlorination byproducts are unstable in the chlorinated water. In addition, the total residual amounts of chlorination byproducts were found to be fairly higher in acidic chlorinated water than in the neutral and basic solutions.

In contrast to the results of ODPABA-hypochlorite reactions, aqueous solutions contained monochloro-substituted OMC isomers (columns 2, 4, 5, and 6 in Fig. 6B) at higher concentrations. This tendency was remarkable in neutral and basic conditions. Low concentrations of unreacted OMC (column 3 in Fig. 6B) indicate that OMC reacts with hypochlorite more rapidly in acidic water than in neutral and basic solutions.

At the typical pH ranges observed during the course of most water treatment processes (pH 5–9), the major form of activated chlorine species can range from entirely hypochlorite ($^{-}$OCl) to entirely hypochlorous acid (HOCl, pKa = 7.5 at 20°C). It has been observed that chlorine is more readily incorporated into aromatic systems at low pH values: this result is parallels with the observation that disinfection ability increases with decreasing pH. Thus, it seems that reactions of the sunscreen agents, ODPABA and OMC react with chlorine in an aqueous solution rapidly when acidic swimming pool water or wastewater is treated with an excess of hypochlorite.

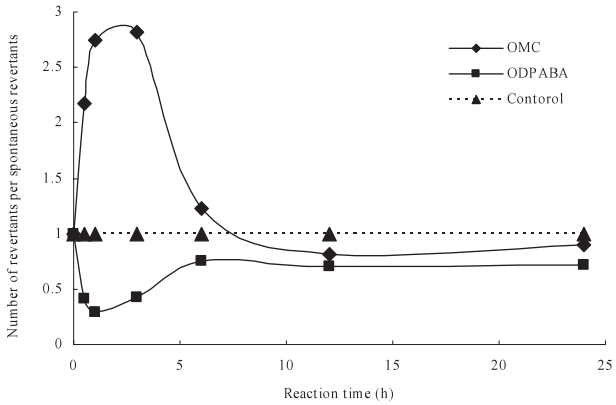


Fig. 7. Time-course Changes in the Mutagenicity of Aqueous ODPABA and OMC Solutions (0.5 mM) after Treatment with Hypochlorite (20 Equivalents of HOCl per mol of the Compound) at Room Temperature and pH 7.0

Mutagenic activity was expressed as the mutagenic ratios at 1000 μg of the starting material per plates.

Table 4. Mutagenicity Tests of Sunscreen agents ODPABA and OMC on Buffered Solutions (Initial Conc., 200 mg/l) Performed Using *Salmonella typhimurium* TA100 without the S9 Mix after Treatment with Hypochlorite at 20 Equivalents of HOCl per mol of the Compound and Room Temperature for 1 hr

Exp. no.	Compound or product tested	Mutagenicity ratios (number of revertants/spontaneous revertants)[a] dose (μg) as starting material					
		50	100	250	500	1000	2000
1	ODPABA standard	1.00	1.00	0.93	1.16	0.97	1.08
2	Chlorination product of ODPABA at pH 5.0	1.20	1.22	1.19	1.05	0.54	0.28
3	Chlorination product of ODPABA at pH 7.0	0.91	1.38	1.13	0.81	0.63	0.56
4	Chlorination product of ODPABA at pH 9.0	1.27	1.31	1.41	1.31	0.92	0.63
5	OMC standard	0.85	1.14	1.36	0.87	0.91	1.13
6	Chlorination product of OMC at pH 5.0	0.89	1.02	1.28	1.59	2.02	4.72
7	Chlorination product of OMC at pH 7.0	1.24	1.35	1.61	1.81	2.75	5.09
8	Chlorination product of OMC at pH 9.0	0.97	1.35	1.61	1.38	2.55	4.52
Spontaneous control (DMSO)		60– 80					
Positive control (NaN_3) 1 μg/plate		500–700					

a) Average of 3 plates per dose in each test.

Mutagenicity Tests of the Chlorination Byproducts

Treatment of ODPABA and OMC compounds with chlorine in the aqueous solution and subsequent mutagenicity assays of their *n*-hexane/ethyl acetate extracts were performed to evaluate the mutagenic potentials of the parent materials and their reaction products. Table 3 shows a summary of the experimental conditions for aqueous chlorination of sunscreen agents and the results of Ames tests for the *n*-hexane/ethyl acetate extracts.

ODPABA and OMC compounds were not mutagenic before aqueous chlorination in the *Salmonella typhimurium* TA100 strain without the S9 mix (Table 4). Aqueous chlorination not only generated mutagens from the non-mutagenic OMC compound but also toxicants from ODPABA against the tester strain TA100 without the S9 mix. The addition of the S9 mix reduced both mutagenic and toxic effects of these chlorination byproducts on the tester strain. In general, most of the mutagens formed during the reactions of organic compounds with chlorine in water were detected in the *Salmonella typhimurium* strain TA100 without the S9 mix[20] except for the polycyclic aromatic hydrocarbons.[25,26]

Figure 7 shows the time-course changes in the mutagenicity of aqueous sunscreen agent solutions after treatment with hypochlorite (20 equivalents of

HOCl per mol of compound) at room temperature and pH 7.0. The increase in the mutagenicity during the first reaction step indicated the formation of mutagenic substances due to the hypochlorite-OMC reaction in water. However, a decrease in the mutagenicity of the OMC solution was observed for 6 hr after the completion of the reaction. This indicates that the mutagenic substances formed in the first step are unstable in chlorinated water and are then converted into non-mutagenic compounds in the subsequent reactions. Therefore, no further experiments to investigate the chemical structure of the mutagen formed in the first step were performed in this study.

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REFERENCES

1) Richardson, S. D. and Ternes, T. A. (2005) Water analysis: emerging contaminants and current issues. *Anal. Chem.*, **77**, 3807–3838.
2) Ashton, D., Hilton, M. and Thomas, K. V. (2004) Investigating the environmental transport of human pharmaceuticals to streams in the United Kingdom. *Sci. Total Environ.*, **333**, 167–184.
3) Tixier, C., Singer, H. P., Oellers, S. and Muller, S. R. (2003) Occurrence and fate of carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen and naproxen in surface water. *Environ. Sci. Technol.*, **37**, 1061–1068.
4) Van DeSteene, J. C., Mortier, K. A. and Lambert, W. E. (2006) Tracking matrix effects during development of a liquid chromatographic-electrospray ionization tandem mass spectrometric analysis of nine basic pharmaceuticals in aqueous environmental samples. *J. Chromatogr. A*, **1123**, 71–81.
5) Lin, W. C., Chen, H. C. and Din, W. H. (2005) Determination of pharmaceutical residues in waters by solid-phase extraction and large-volume on-line-derivatization with gas chromatography-mass spectrometry. *J. Chromatogr. A*, **1065**, 279–285.
6) Petrovic, M., Hernando, M. D., Diaz-Cruz, M. S. and Barcero, D. (2005) Liquid chromatography-tandem mass spectrometer for analysis of pharmaceutical residues in environmental samples: a review. *J. Chromatogr. A*, **1067**, 1–14.
7) Kolpin, D. W., Furlong, E. T., Meyer, M. T., Thurman, E. M., Zaugg, S. D., Barber, L. B. and Buxton, H. X. (2002) Pharmaceuticals hormones, and other organic wastewater contaminants in US streams, 1999-2000 a national reconnaissance. *Environ. Sci. Technol.*, **36**, 1202–1211.
8) Lambropoulou, D. A., Giokas, D. L., Sakkas, V. A., Albanis, T. A. and Karayannis, M. I. (2002) Gas chromatographic determination of 2-hyroxy-4-methoxybenzo-phenone and octyl-dimethyl-*p*-aminobenzoic acid sunscreen agents in swimming pool and bathing waters by solid-phase microextraction. *J. Chromatogr. A*, **967**, 243–253.
9) Giokas, D. L., Sakkas, V. A. and Albanis, T. A. (2004) Determination of residues of UV filters in natural waters by solid-phase extraction coupled to liquid chromatography-photodiode array detection and gas chromatography-mass spectrometry. *J. Chromatogr. A*, **1026**, 289–293.
10) Giokas, D. L., Sakkas, V. A., Albanis, T. A. and Lambropoulou, D. A. (2005) Determination of UV-filter residues in bathing waters by liquid chromatography UV-diode array and gas chromatography-mass spectrometry after micelle mediated extraction-solvent back extraction. *J. Chromatogr. A*, **1077**, 19–27.
11) Ternes, T. A., Joss, A. and Siegris, H. (2004) Scrutinizing pharmaceutical and personal care products in wastewater treatment. *Environ. Sci. Technol.*, **38**, 392A–398A.
12) Sakkas, V. A., Giokas, D. L., Lambropoulou, D. A. and Albanis, T. A. (2003) Aqueous photolysis of the sunscreen agent octyl-dimethyl-*p*-amino benzoic acid. Formation of disinfection products in chlorinated swimming pool water. *J. Chromatogr. A*, **1016**, 211–222.
13) Yamamoto, T., Nakajima, D., Goto, S., Onodera, S., Yasuhara, A., Sakai, S. and Souma, M. (2004) Mutagenicity of chlorination products of benzophenone and its derivatives. *J. Environ. Chem.* (*Kankyo Kagaku, in Japanese with English Abstract*), **14**, 335–342.
14) Kuntz, P. Y., Galicia, H. F. and Fent, K. (2006) Comparison of in vitro and in vivo estrogenic activity of UV filters in fish. *Toxicol. Sci.*, **90**, 349–361.
15) Balmer, M. E., Buser, H. R., Muller, M. D. and Poiger, T. (2005) Occurrence of some organic UV filters in wastewater, in surface water, and in fish from Swiss Lakes. *Environ. Sci. Technol.*, **39**, 953–962.

16) Buser, H. R., Balmer, M. E., Schmid, P. and Kohler, M. (2006) Occurrence of UV filters 4-methylbenzylidene camphor and octocrylene in fish from various Swiss rivers with inputs from wastewater treatment plants. *Environ. Sci. Technol.*, **40**, 1427–1431.
17) Tomiyama, S., Fujie, S., Takahashi, Y., Yaguchi, K. and Onodera, S. (2008) Chlorination by-products of Epoxy resin hardener in aqueous solution. *J. Health Sci.*, **54**, 17–22.
18) Onodera, S., Takahashi, T., Takemoto, S. and Oh-i, T. (2008) Characterization of polyhalogenated 4-methylphenol dimers (Br/Cl-predioxins) formed during aqueous chlorination of 4-methylphenol solution in the presence of bromide ion. *J. Health Sci.*, **54**, 423–431.
19) Maron, D. M. and Ames, B. N. (1983) Revised methods for Salmonella mutagenicity assay test. *Mutat. Res.*, **113**, 173–215.
20) Onodera, S., Yoshimatsu, K., Saitoh, H. and Uchida, A. (1998) Behavior of mutagenic formation from phenolic compounds in water disinfection with chlorine and their mutagenic potential formation. *Jpn. J. Toxicol. Environ. Health* (*Eisei Kagaku*, *in Japanese with English Abstract*), **44**, 289–299.
21) Pattanaargson, S., Munhapol, T., Hirumsupachot, P. and Luangthongaram, P. (2004) Photoisomerization of octyl methoxycinnamate. *J. Photochem. Photobiol. A*, **161**, 269–274.
22) Huong, S. P., Andrieu, V., Reynier J.-P., Rocher, E. and Fourneron, J.-D. (2007) The photoisomerization of the sunscreen ethylhexyl p-methoxy cinnamate and its influence on the sun protection factor. *J. Photochem. Photobiol. A*, **186**, 65–70.
23) Nakajima, M. and Onodera, S. (2006) Chemical fate of the sunscreen agents in model swimming pool water (II). *Proc. 15th Symp. Environ. Chem. Jpn.*, **16**, 480–481.
24) Knowland, J., McKenzie, E. A., McHugh, P. J. and Cridland, N. A. (1993) Sunlight-induced mutagenicity of a common sunscreen ingredient. *FEBS Lett.*, **324**, 309–313.
25) Onodera, S., Muratani, T., Igarashi, K., Fukuda, A. and Suzuki, S. (1990) Chemical changes of organic compounds in chlorinated water. XVI. Production of mutagens in reactions of naphthalene compounds with hypochlorite in aqueous solution. *Eisei Kagaku* (*Jpn. J. Hygenic Chem.*), **36**, 201–210.
26) Onodera, S., Igarashi, K., Fukuda, A., Ouchi, J. and Suzuki, S. (1994) Mutagenic potentials of anthracene and phenanthrene compounds during water disinfection with chlorine. *Jpn. J. Toxicol. Environ. Health* (*Eisei Kagaku*), **40**, 233–243.