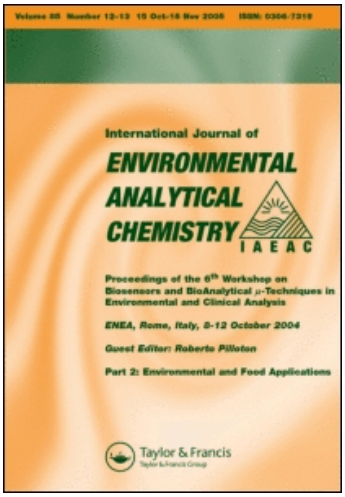

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Determination of chlorinated by-products of parabens in swimming pool water

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We describe the determination of trace amounts of chlorinated parabens, i.e. disinfection by-products, in swimming pool water, using gas chromatography-mass spectrometry with selected ion monitoring (GC-MS-SIM). A dichlorinated by-product of isopropylparaben was detected at levels of up to 25 ng L^{-1}. Further, a dichlorinated by-product of methylparaben and a monochlorinated by-product of benzylparaben were present in concentrations lower than the limit of quantification. Benzylparaben, the parent compound, was also detected at concentrations of up to 28 ng L^{-1}. Thus, in this study, chlorinated parabens were detected and quantified for the first time as disinfection by-products in swimming pool water. The results of this study have raised concerns regarding the chlorinated by-products of active ingredients used in personal care products.

Keywords: hydroxybenzoate esters; chlorinated by-products; gas chromatography-mass spectrometry; pool water

1. Introduction

Hydroxybenzoate esters (parabens) are a group of alkyl esters of para-hydroxybenzoic acid and typically include methylparaben (MeP), ethylparaben (EtP), *n*-propylparaben (*n*PrP), isopropylparaben (*i*PrP), *n*-butylparaben (*n*BuP), isobutylparaben (*i*BuP) and benzylparaben (BnP) (Figure 1). Parabens or their salts are widely used as preservatives in pharmaceuticals and personal care products (PPCPs) since they exhibit a broad spectrum of antimicrobial activity, stability over a wide pH range and sufficient solubility in water [1,2]. Many PPCPs are used daily in various human activities and are continuously released in the aquatic environment. Therefore, the existence and transportation pathways of active ingredients in PPCPs into the aquatic environment have been receiving particular attention [3–5]. Some studies have confirmed the presence of parabens in sewage treatment plant influents [6,7] and effluents [7], river water [8] and raw sewage water [9]. Moreover, several *in vitro* [10,11] and *in vivo* [12,13] studies on the estrogenic activity of parabens have been published recently. These studies have revealed that paraben compounds exert weak activities with a potency ranging from 10^{-4}- to 10^{-7}-fold compared to the activity of 17β-estradiol.

The presence of pollutants originating from the use of PPCPs has also been reported in swimming pool water. For example, 2-hydroxy-4-methoxybenzophenone and octyldimethyl-*p*-aminobenzoic acid have been detected at concentrations of several

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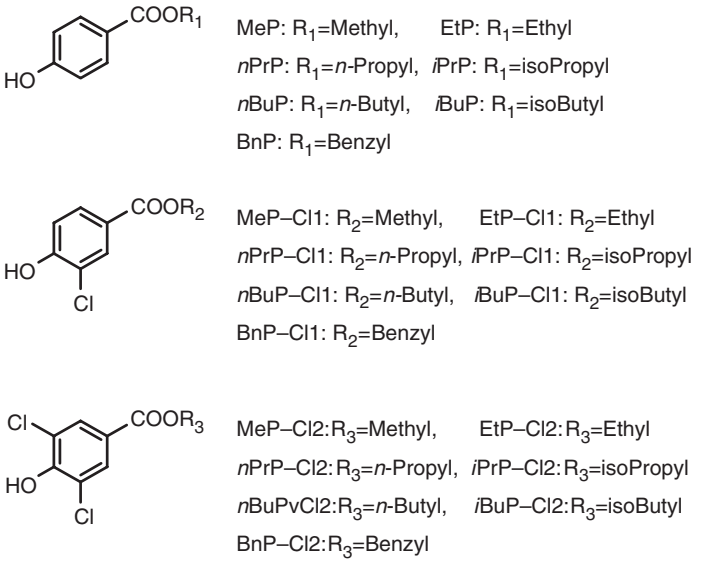

Figure 1. Chemical structures of parabens and mono- and dichlorinated parabens.

micrograms per litre by gas chromatography-mass spectrometry (GC-MS) [14]. These compounds are commonly used as ultraviolet (UV)-absorbing chemicals in commercial sunscreen formulations. Moreover, Zwiener *et al*. [15] have studied several sunscreen agents by a reported method based on liquid chromatography coupled with tandem mass spectrometry (LC-MS) for the identification of five compounds in outdoor pools [15].

Hygiene and safety are of particular concern with regard to the quality of swimming pool water. Serious diseases may be caused by micro-organisms present in pool water. Therefore, disinfection of pool water is indispensable. This is mainly achieved by chlorination because chlorine reacts fast and has a lasting disinfection potential. Chlorine is usually applied to swimming pool water as gaseous chlorine or sodium hypochlorite. However, chlorine is known to produce disinfection by-products (DBPs) by reacting with organic constituents. Trihalomethanes were the first to be identified and are the most abundant organic DBPs in chlorinated water [16,17]. Certain DBPs are toxic species that might pose a potential risk to the human health [18]. One of these, namely, nitrosamine, is a potent carcinogen and has recently been detected in swimming pools [19]. The presence of the mutagen halofuranone explains the importance of identifying even traces of DBPs [20]. The formation of DBPs in chlorinated water, particularly compounds containing phenolic hydroxyl groups, exhibit favourable chlorination kinetics [21–23]; therefore, DBPs may be produced when PPCPs containing parabens come in contact with chlorinated water, for example, in a tap water and a swimming pool. In fact, DBPs of methyl and propyl paraben have been found in raw sewage water samples, but they were not quantified by chemical analysis [9].

Although a variety of chlorinated DBPs have been found in pool waters, the presence of parabens and their DBPs, however, remained unnoticed for a long time; these parabens are routinely used as preservatives in cosmetics and sun-care products. As DBPs can be generically found in the pool in trace amounts, preconcentration techniques are essential

Table 1. Characteristics of swimming pools and water samples.

	Pool 1	Pool 2	Pool 3	Pool 4	Pool 5	Pool 6
Date and time	8/25 11:00	8/23 15:00	8/23 16:30	8/23 16:00	8/25 14:00	8/25 12:00
Type	Indoor	Outdoor	Indoor	Outdoor	Outdoor	Outdoor
Size, tons	650	380	360	150	900	500
Visitors, per day	800	200	300	150	1200	270
Temperature	31	31	30	28	31	31
(Water temperature), (°C)	(29)	(30)	(29)	(27)	(28)	(22)
pH	7.9	7.9	7.6	7.5	8.0	7.0
Free Cl ($mg\,L^{-1}$)	0.6	1.0	0.6	0.9	0.6	0.4
$KMnO_4$, consumption ($Mg\,L^{-1}$)	1.7	0.6	2.4	3.1	1.5	0.7

to enrich these analytes and allow their subsequent analysis. Moreover, only limited spectroscopic analysis data are available for chlorinated parabens. In this context, we have modified previously published methods for analysis of chlorinated phenolics such as chlorinated bisphenol A and estrone [24,25]. This procedure involved concentration of analytes in water samples by liquid–liquid extraction, followed by derivatisation with a silylation reagent to improve the performance of the gas chromatographic separations. Furthermore, fragmentation mechanisms for chlorinated parabens have been studied by applying electron impact ionisation to mass spectrometry by the selected ion monitoring (SIM) method.

2. Experimental

2.1 *Test chemicals*

MeP, EtP and *n*BuP were purchased from Wako Pure Chemical Industries, Osaka, Japan. *n*PrP, *i*PrP, *i*BuP and BnP were purchased from Tokyo Chemical Industry, Tokyo, Japan. These test chemicals were used without purification. 3-Chloro-4-hydroxy-benzoic acid methyl ester (MeP–Cl1), ethyl ester (EtP–Cl1), *n*-propyl ester (*n*PrP–Cl1), isopropyl ester (*i*PrP–Cl1), *n*-butyl ester (*n*BuP–Cl1), isobutyl ester (*i*BuP–Cl1) and benzyl ester (BnP–Cl1) were synthesised according to the procedures reported in the literature [24]. 3,5-Dichloro-4-hydroxybenzoic acid methyl ester (MeP–Cl2), ethyl ester (EtP–Cl2), *n*-propyl ester (*n*PrP–Cl2), isopropyl ester (*i*PrP–Cl2), *n*-butyl ester (*n*BuP–Cl2), isobutyl ester (*i*BuP–Cl2) and benzyl ester (BnP–Cl2) were also synthesised as reported previously [26].

2.2 *Pool water*

The samples were collected on 2 days – 23 and 25 August 2007 – from six public swimming pools. Samples were characterised by the department staff, and the results of this characterisation are listed in Table 1. The free chlorine level was within the acceptable range of 0.4–1.0 ppm, and the pH value was within the acceptable range of 5.8–8.6 for public swimming pools in Japan. Potassium permanganate consumption was also within the acceptable limit of 12 ppm for public swimming pools. Water samples (3 L) were collected in glass bottles with stoppers, quenched with 1 mL of sodium thiosulphate

solution (0.05 M), transported to the laboratory in ice boxes, and stored at 4°C until analysis (within 24 h).

2.3 *Extraction and analytical methods*

The pH of water samples (0.5 L) was adjusted to 3 with 2 M hydrochloric acid, sodium chloride (15 g) was added, and extracted in ethyl acetate (2×100 mL) using a shaker (Recipro Shaker SR-2s; Taitec, Saitama, Japan) at 265 rpm for 10 min. The ethyl acetate extracts were combined and dried over anhydrous sodium sulphate. After removing the solvent under reduced pressure, the residue was pipetted with ethyl acetate in a test tube and concentrated to approximately 1 mL under a nitrogen stream. The extracted samples were trimethylsilylated before the GC-MS analysis. A total of 200 μL of *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) was added to the test tubes containing the extracts, and the solution was allowed to stand for 1 h at room temperature to complete the trimethylsilylation. The extracted samples were concentrated to approximately 0.1 mL under a nitrogen stream and were reconstituted to 0.5 mL with ethyl acetate; then, 5 μL of ethyl acetate containing 100 μg mL^{-1} of an internal standard (pyrene-d_{10}) was then added to the samples and examined by GC-MS. The standard and sample extracts were analysed by GC (HP 6890 Series; Hewlett-Packard, Palo Alto, CA, USA)-MS (HP 5972A Series mass-selective detector; Hewlett-Packard) on an HP-5 fused-silica capillary column (length, 30 m; inner diameter, 0.32 mm; film thickness, 0.25 μm; J&W Scientific, Folsom, CA, USA). The column temperature was initially maintained at 60°C for 1 min, and then programmed to reach 280°C at a rate of 10°C min^{-1}, with a final hold time of 7 min. Helium was used as the carrier gas at a flow rate of 1.3 mL min^{-1}, and the column head pressure was maintained at 6894 Pa. The injector temperature was maintained at 250°C, and the injection volume was 1.0 μL in the splitless mode. The electron multiplier voltage for the MS was 1988 V, and the interface temperature was maintained at 280°C. Mass spectra were obtained by electron impact ionisation at a voltage of 70 eV and scanned over an m/z range of 50–550 atomic mass units at a rate of 1.5 scans per second; the ion source temperature was maintained at 250°C. Each compound in the samples was identified by comparing the GC retention times and mass fragmentograms for the selected ions of the samples with those of the authentic standards. These compounds were quantified by correlating the ratio of the peak area of the compound of interest to that of the internal standard with the calibration curve of the standard solution. The standard calibration solution, containing each compound, was prepared in ethyl acetate.

2.4 *Quality assurance and quality control*

Data are expressed as means of three separate experiments. Calibration curves consisted of five concentrations ranging from 20 to 320 ng mL^{-1} in ethyl acetate. The recoveries were determined by performing four separate experiments using distillated water spiked to 200 ng L^{-1}. The characteristics of this method are shown in Table 2. The relative standard deviation of the results obtained was within 11% for recoveries and within 38% for the samples. The recoveries varied between 91% and 108%, and all standards were linear for target compounds. The coefficient of determination (r^2) ranged from 0.937 to 0.995 over five concentration levels. The limit of quantification (LOQ) was defined as a

Table 2. Monitored ions and quality-control data for trimethylsilyl derivatives of target compounds.

Compound	CAS No.	Mass ions (m/z) Quant[a]	Conf[b]	Recovery % ± SD ($n=4$)	LOQ[c] (ng L^{-1})
MeP	99-76-3	209	193, 224	108 ± 4.7	15
MeP–Cl1	3964-57-6	243	245, 258	92 ± 7.3	10
MeP–Cl2	3337-59-5	277	279, 281, 292	98 ± 6.3	10
EtP	120-47-8	193	223, 238	106 ± 4.5	15
EtP–Cl1	16357-41-8	257	259, 272	91 ± 4.9	10
EtP–Cl2	17302-82-8	291	293, 295, 306	95 ± 7.9	10
*n*PrP	94-13-3	195	237, 252	102 ± 6.1	15
*n*PrP–Cl1	37470-49-8	271	229, 273, 286	95 ± 6.7	10
*n*PrP–Cl2	101003-80-9	305	307, 309, 320	91 ± 8.8	10
*i*PrP	419173-5	195	237, 252	104 ± 4.7	15
*i*PrP–Cl1	37470-50-1	271	229, 273, 286	94 ± 3.9	10
*i*PrP–Cl2	15533-29-6	305	307, 309, 320	91 ± 9.0	10
*n*BuP	94-26-8	195	251, 266	98 ± 7.2	10
*n*BuP–Cl1	37470-51-2	229	285, 287, 300	92 ± 9.2	10
*n*BuP–Cl2	909404-90-6	319	321, 323, 334	92 ± 8.5	5
*i*BuP	4247-02-3	195	251, 266	100 ± 6.7	10
*i*BuP–Cl1	37470-52-3	229	285, 287, 300	93 ± 7.5	10
*i*BuP–Cl2	–	319	321, 323, 334	92 ± 10	10
BnP	9418-8	193	285, 300	90 ± 1.3	10
BnP–Cl1	–	227	319, 321, 334	92 ± 2.8	10
BnP–Cl2	22071-55-2	353	355, 357, 368	92 ± 11	5

Notes: The underlined sections denotes molecular ion peaks.
[a]For quantification.
[b]For confirmatory purposes.
[c]Limits of quantification.

signal-to-noise ratio (S/N) of 10. Based on a concentration factor of 1000, the LOQs for target compounds ranged from 5 to 15 ng L^{-1}. In order to avoid laboratory contamination, all glassware was heated at 500°C for 3 h before use.

2.5 *Calculation method for atomic charges*

To estimate the reactivity of the analytes for chlorination, we calculated the molecular orbital coefficients of the highest occupied molecular orbital (HOMO) and the total electric population for each atom in the parabens. Gauss View ver.2.1 was used for three-dimensional molecular modelling of the parabens, and the optimised molecular structure was also obtained by using this software [27]. Gaussian 98W ver.5.4 was used for the quantum chemical calculation [28]. The density functional theory in terms of B3LYP as the exchange and correlation function and the 6-31G** basis set were applied to the calculation, respectively. In this study, we requested that the calculation be performed in the presence of an aqueous solvent, using the polarised continuum overlapping spheres model of Tomasi and coworkers [29]. The permittivity (ε) value was set at 78.39.

3. Results and discussion

3.1 *Mass spectrometric fragmentation patterns of trimethylsilyl ethers of chlorinated parabens*

The MS data of the trimethylsilyl (TMS) derivatives of mono- and dichlorinated parabens are shown in Figures 2 and 3. The MS spectra of the authentic compounds were dominated by the mass fragment ions at m/z [$M^+ - 15$], resulting from the loss of a methyl moiety in the derivatives rather than by the molecular ion peaks (M^+). Each of the ion peaks at [$M^+ - 15$] and [M^+] also presented the typical isotopic pattern corresponding to mono- and dichlorinated compounds. As a typical example, the relative isotopic ion intensities of MeP–Cl1–TMS are 3 : 1 at m/z 243: 245 and m/z 258 : 260 and those for MeP–Cl2–TMS, 9 : 6 : 1 at m/z 257 : 259 : 261, and m/z 277 : 279 : 281. The MS spectra of the chlorinated parabens with a linear side chain and a branched side chain were identical (data not shown). The structures of fragment ions for chlorinated parabens are described in Figure 4. In the MS spectra of chlorinated MeP, fragment ions at m/z 227 for MeP–Cl1 and at m/z 261 for MeP–Cl2 indicate the loss of a methoxy group in the ester side chain

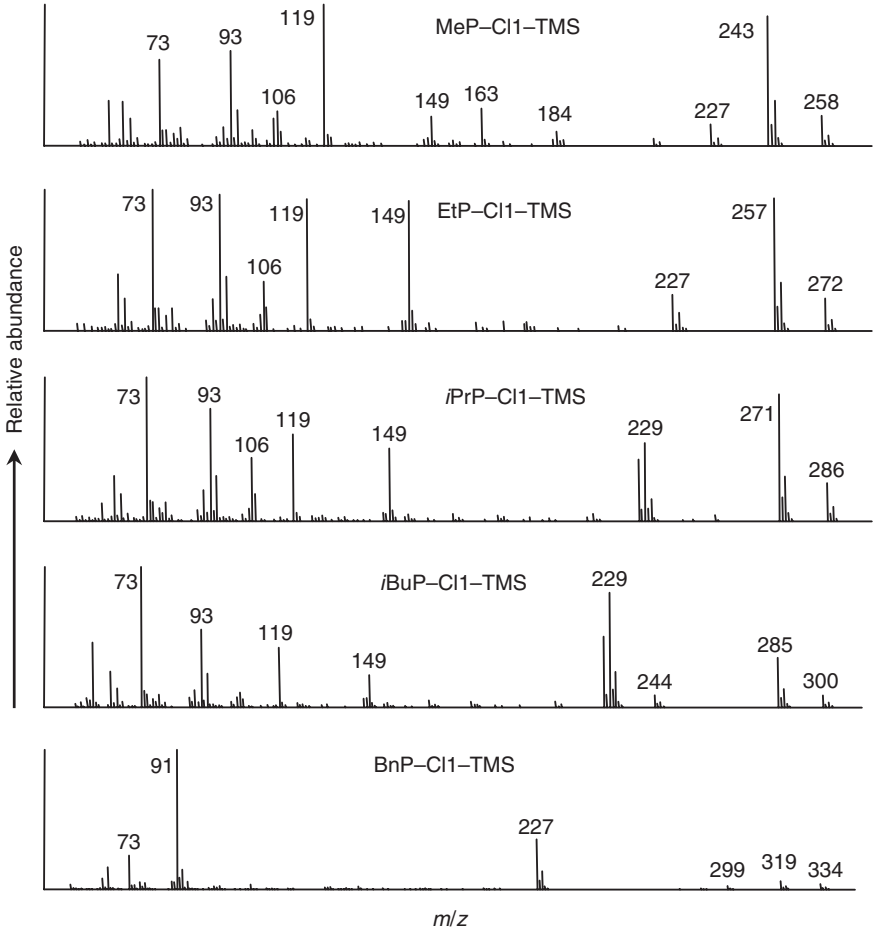

Figure 2. Mass spectra of the TMS derivatives of the authentic monochlorinated parabens.

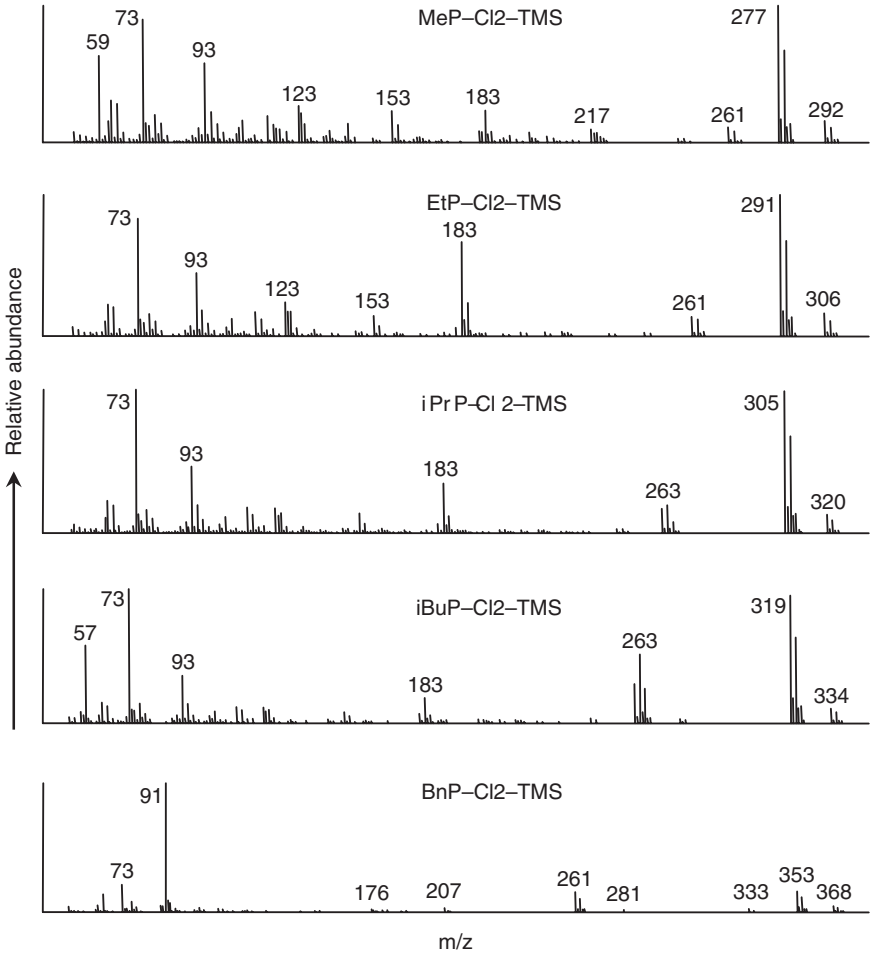

Figure 3. Mass spectra of the TMS derivatives of the authentic dichlorinated parabens.

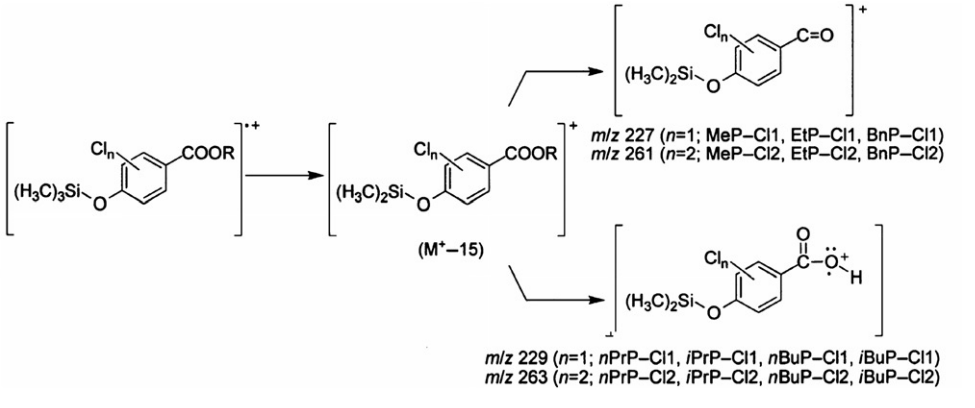

Figure 4. Fragmentation of the TMS derivatives of chlorinated parabens.

of a derivative. The same fragment ions resulting from the loss of a benzyloxy group in derivatives were observed in the case if the chlorinated BnP (BnP–Cl1 and BnP–Cl2). On the other hand, fragment ions of *n*PrP, *i*PrP, *n*BuP and *i*BuP, the ions at m/z 229 for monochlorinated parabens and at m/z 263 for dichlorinated parabens are believed to be formed via McLafferty rearrangement of the chain and one hydrogen atom, followed by the expulsion of an alkyl radical via a 6-membered transition state. These fragment ions might be useful for the identification of chlorinated parabens by GC-MS analysis.

3.2 *Detection and quantitative analysis of parabens and their chlorinated by-products in the swimming pool water samples*

The SIM chromatograms of the authentic compounds and the water samples (pool 1) are shown in Figure 5. The retention time of each peak* in the sample is in fair agreement with that of the authentic standards. The relative intensities of each molecular ion and each fragment ion in the water sample are also consistent with those of the authentic standards. These results strongly supported the presence of chlorinated parabens, i.e. DBPs, in the pool water. Table 3 summarises the concentrations of the parabens and the chlorinated by-products present in pool water samples. BnP was detected in water samples from two pools and was the most abundant paraben among the samples investigated; its maximum measured concentration was comparable to that of *N*-nitrosodimethylamine found in indoor pool water, as reported previously ($32\,ng\,L^{-1}$) [19]. With regard to chlorinated by-products, *i*PrP-Cl2 was detected in two pools; the concentration levels ranged from LOQ to $25\,ng\,L^{-1}$. MeP–Cl2 and BnP–Cl1 were also detected in two pools, and their measured concentrations were LOQ or less. The parent compounds of these chlorinated by-products were not detected except for BnP. A probable reason is that parent compounds readily react with free chlorine. Canosa *et al*. [9] have evaluated the stability of methyl, ethyl, propyl and butyl paraben in presence of free chlorine. This study demonstrates that the half-lives of alkylated parabens were probably insignificant – it was approximately 30 min in the presence of tap water containing free chlorine at the same level as that in pool water. In addition, the dichlorinated parabens are highly resistant to further oxidation reactions. The accumulation of chlorinated parabens and the absence of their parent compounds indicate the remarkable reactivity of parabens with free chlorine and the stability of the chlorinated transformation products. Meanwhile, the abundance pattern of benzyl paraben species was obviously different from that of other parabens; the concentration of parent compound (BnP) was higher than those of the chlorinated by-products. One of the reasons for this occurrence is the incorporation time of PPCPs containing BnP into pool water may differ from those of other parabens. Chlorine substitution of the phenol in the paraben compound is considered to be a typical electrophilic substitution [30]. In the experiments, it was observed that HOCl and ClO^- could both attack the phenolic ring as an electrophile. According to this mechanism, the presence of a higher negative charge on nucleophilic substrate will facilitate the reaction. We have performed a quantum chemical calculation for negative charges in aromatic ring carbon (C3 and C5) of parabens. The average of results obtained were −0.140 for MeP, −0.140 for EtP, −0.138 for *n*PP, −0.136 for *n*BuP, and −0.139 for BnP. These data suggest that BnP is as reactive as other alkylated paraben terms of susceptibility to chlorine substitution. Therefore, it seems that the observed higher concentration of

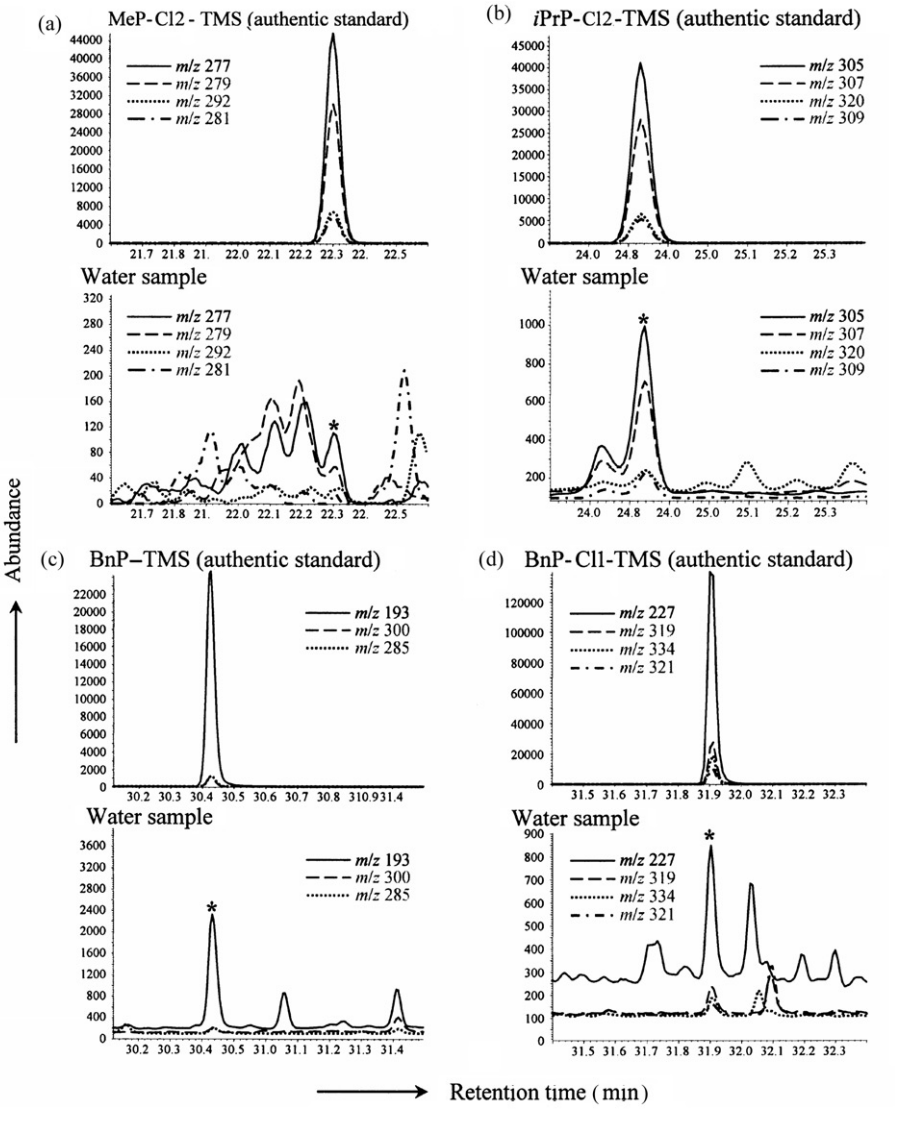

Figure 5. Selected ion monitoring (SIM) chromatograms for the TMS derivatives of an authentic standard and water sample (pool 1). Each asterisk (*) represents the target peak in the sample.

BnP is caused by not its reactivity but the difference in incorporation time of the parent compound. The observed higher concentration of BnP, trace amounts of BnP–Cl1, and an undetected BnP–Cl2 may indicate the first stage of the chlorination process for this paraben that inputs by bather in just minutes. Thus, in this study, the chlorinated parabens (MeP–Cl2, *i*PrP–Cl2 and BnP–Cl1) were detected and quantified for the first time as DBPs in pool water. Parabens are used as preservatives in numerous cosmetic products at concentrations of up to 1% weight per volume. Parabens in PPCPs vary from product to product, e.g. aqueous cosmetics such as hair shampoos mainly contain methyl paraben, while benzyl paraben is observed in relatively large amounts in cream

Table 3. Concentrations ($ng\,L^{-1}$) of parabens and their chlorinated by-products in pool water samples.

Compound	Pool 1	Pool 2	Pool 3	Pool 4	Pool 5	Pool 6
MeP	ND[a]	ND	ND	ND	ND	ND
MeP–Cl1	ND	ND	ND	ND	ND	ND
MeP–Cl2	<LOQ[b]	ND	ND	ND	ND	ND
EtP	ND	ND	ND	ND	ND	ND
EtP–Cl1	ND	ND	ND	ND	ND	ND
EtP–Cl2	ND	ND	ND	ND	ND	ND
*n*PrP	ND	ND	ND	ND	ND	ND
*n*PrP–Cl1	ND	ND	ND	ND	ND	ND
*n*PrP–Cl2	ND	ND	ND	ND	ND	ND
*i*prP	ND	ND	ND	ND	ND	ND
*i*PrP–Cl1	ND	ND	ND	ND	ND	ND
*i*PrP–Cl2	25 ± 5.5	ND	ND	ND	<LOQ	ND
*n*BuP	ND	ND	ND	ND	ND	ND
*n*BuP–Cl1	ND	ND	ND	ND	ND	ND
*n*BuP–Cl2	ND	ND	ND	ND	ND	ND
*I*BuP	ND	ND	ND	ND	ND	ND
*i*BuP–Cl1	ND	ND	ND	ND	ND	ND
*i*BuP–Cl2	ND	ND	ND	ND	ND	ND
BnP	28 ± 11	ND	ND	ND	<LOQ	ND
BnP–Cl1	<LOQ	ND	ND	ND	<LOQ	ND
BnPCl2	ND	ND	ND	ND	ND	ND

Notes: Values are presented as the mean $\pm$ standard deviation ($n = 3$).
[a]Not detected.
[b]Limit of quantification.

products used for the body or the face [31,32]. In this study, the correlation between usage of PPCPs and the compound patterns of these parabens found in pool waters was poor. The compound patterns and concentrations of parabens and the DBPs in pool waters also differed. These results could be attributed to the use of different sunscreen products. A high concentration of parabens was detected in pools 1 and 5, which have a large number of visitors. Therefore, it is considered that paraben species are introduced via the PPCPs used by the bathers.

This study provides an insight into the likelihood of the presence of chlorinated paraben by-products in swimming pool water. The use of free chlorine is a common practice in all countries. The complete assessment of the toxicological risk to human health requires further investigation in terms of the quantitative analysis of chlorinated parabens in different types of pools. Another important point is that the effects of chlorinated parabens on human health are not known. In particular, several parent species have recently been reported to possess estrogenic activity in *in vitro* and *in vivo* assay systems. In addition, a yeast estrogen screen test has shown that chlorinated phenolics, such as bisphenol A, have a 10–100-fold higher activity than parent species [33]. More systematic investigation on the fate of parabens in swimming pool and their estrogenicities is necessary to understand their potent chronic effects and to conduct a comprehensive risk assessment. A more systematic investigation of the fate of parabens in swimming pools and their estrogenicities is necessary to understand their potent chronic effects and to conduct a comprehensive risk assessment.

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