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Scientific paper

Stability and Toxicity of Selected Chlorinated Benzophenone-type UV Filters in Waters

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Abstract

In our study, the transformation of two most widely used UV filters, benzophenone-3 (BP3) and benzophenone-4 (BP4), in chlorinated water with disinfection reagents sodium hypochlorite (NaClO) and trichloroisocyanuric acid (TCCA) was studied. Based on the HPLC/MS and UV-Vis analysis the formation of two different chlorinated products (5-chloro-2-hydroxy-4-methoxybenzophenone and 3,5-dichloro-2-hydroxy-4-methoxybenzophenone) was established. Identity of chlorinated products was confirmed by means of comparison of retention times with independently synthesized standards. Photostability study showed that dichloro-derivative in water is less stable then parent compounds, which is not the case for monochloro-derivatives. Toxicity of chlorinated compounds tested by *Vibrio fischeri* was found to be in the same range as that of the starting compounds. Preliminary testing of real water samples from swimming pools and sea swimming areas confirmed the presence of BP3 and its 3,5-dichloro derivative.

Keywords: UV filter, photostability, chlorination, toxicity, *Vibrio fischeri*

1. Introduction

Organic (also known as chemical) ultraviolet (UV) filters, responsible for the absorption of solar UV radiation, are increasingly used as ingredients in personal-care products (e.g., sunscreens, lipsticks, shampoos and hair sprays) as a result of growing concern about exposure to sunlight causing skin cancer. These products are used primarily under special conditions, such as swimming in the sea and swimming pools, and skiing on the snow and in the mountains, where a really thorough protection is needed. Although UV filters must be stable on exposure to UV radiation, recent studies have revealed that several organic UV filters undergo degradation.[1–3] Usually, two types of reactions occur: a) direct photolytic reactions, and b) chlorination of aromatic rings or side chain in the presence of chlorine and chlorates in water (such as pools, salty seawater).[4] The photo-instability of organic UV filters is recognized as a major problem, since they lose their photo-protective properties and generate photoproducts that may cause allergies or other harmful effects.[5–8] It is essential to study the products formed from these substances, since UV-filters are added to formulations in substantial amounts and applied (e.g., to skin and hair) in large quantities (e.g., US Food and Drug Administration recommends a minimum of 2 mg sunscreen/cm^2 of skin).[5]

The group of organic UV filters can be divided into two sectors, depending on the spectral range covered: the first consists of the UVA (320–400 nm) filters including benzophenones, anthranilates and dibenzoylmethanes, and the second group are UVB (290–320 nm) filters including PABA derivatives, salicylates, cinnamates and camphor derivatives.[6,9] Two of these UV filters, 2-hydroxy-4-methoxybenzophenone (BP3, CAS No. 131-57-7) and 2-hydroxy-4-methoxybenzophenone-5-sulfonic acid (BP4, CAS No. 4065-45-6) are the most widely used UVA filters.[5,10,11] However, little information is available regarding the stability and the transformation of these compounds under natural or photo-induced conditions, as well as about their degradation products. It is notable that both compounds are benzophenones, substituted with strong electron-donor groups (-OH, -OMe), which gives rise to high reactivity of these compounds towards electrophilic halogenating agents. The latter compounds are used for disinfection of drinking and swimming pool water (chlorine, hypochlorites and trichloroisocyanuric acid). It can be expected that these benzophenones would react with the above-mentioned chlorinating agents present in water, producing chloro-substituted benzophenones. 3-Chloro- and 5-chloro derivatives of BP3 are known[4,12] but not well characterized and their degradation pathways were not investigated. Negreira et al.(2008)[4] found that the reaction of BP3 and NaOCl in concentrations, typical for chlorinated water, takes place within minutes. The products of chlorination were identified on the basis of their mass spectra, and it was found that monochloro and dichloro derivatives of BP3 are formed. In the presence of bromide, substantial amounts of bromo derivatives are also produced.

In the last decade there has been extensive discussion regarding the quality of swimming pool waters. Opinions from different experts representing different sectors are quite opposite. On one side some of them claim, that the quality of swimming waters is constantly improving, but on the other side some others warn about the presence of various compounds pool waters contain, from cosmetics to pharmaceuticals (especially residues and metabolites) and other contaminants, and about the possible effects of water consumption (also as inhalation of aerosols), which means increased health risk including an increased incidence of cancer.

As it was already mentioned organic UV filters from the group of benzophenones might form chlorinated products when they are exposed to the disinfection agents.[4] In order to elucidate the stability of chlorinated products in the presence of sunlight, the experiments with chlorinated compounds under UV-A irradiation were performed. In addition, chlorinated products were previously identified only on the basis of mass spectra. From that reason we have decided to synthesize them individually for the purpose of products characterisation and toxicity assessment with *Vibrio fischeri*.

Several papers deal with determination of benzophenone type UV filters in swimming pool and bathing water,[13,14] with no previous research regarding the presence of chlorinated products in real samples of swimming pool water. In this work, concentration of BP3 and BP4 UV filters and their chlorinated by-products were determined in water samples from several bathing areas.

2. Experimental

2. 1. Reagents

The chemicals in this study were used as purchased: BP3 (2-hydroxy-4-methoxybenzophenone) (96% purity), BP4 (2-hydroxy-4-methoxybenzophenone-5-sulfonic acid) (97% purity), sodium hypochlorite (NaClO) (6–14% active chlorine), and trichloroisocyanuric acid (TCCA) (97% purity) from Aldrich; $NaH_2PO_4 \cdot 2H_2O$ and Na_2HPO_4 more than 99% purity from Acros Organics; acetonitrile (HPLC grade) from Sigma; and NH_4OOCCH_3 at least 96% purity from Merck. *N*-Chlorosaccharin was synthesized by literature procedure.[15] Dichloromethane, sodium hydrogen carbonate, sodium bisulfite, anhydrous sodium sulfate, petroleum ether, and silica gel were purchased from Aldrich or Fluka.

2. 2. Chlorination Experiments

2. 2. 1. Chlorination Experiments in Water

Chlorination experiments were performed with solutions of BP3 (5.0 mg L^{-1}) or BP4 (10 mg L^{-1} in 0.01 M $KH_2PO_4 + Na_2HPO_4$ buffer (pH = 6.9). Firstly, 0.29 mL of 30% of NaOCl or 0.4 mg of TCCA were added to 100 mL solution of BP3 or BP4 and stirred in darkness at room temperature. The final active chlorine content of NaClO was 1.4 mg L^{-1}, and the concentration of TCCA was 4.0 mg L^{-1}. The active chlorine content of NaClO was checked via Standard Method 4500-Cl F DPD-FAS titrimetric method[16] because of concern over the decomposition of chlorine over time. Reaction mixtures were left to stand at room temperature for 24 h and were afterwards analyzed by HPLC-DAD and UV-Vis spectrophotometer.

The same set of chlorination experiments for BP3 and BP4 was performed with NaOCl in the simulated natural water, composed by Na^+ (18.0 mg L^{-1}), Mg^{2+} (4.8 mg L^{-1}), Ca^{2+} (47.0 mg L^{-1}), SO_4^{2-} (9.0 mg L^{-1}), Cl^- (17.0 mg L^{-1}), and HCO_3^- (174.8 mg L^{-1}).

2. 2. 2. Synthesis of Chloro Derivatives of BP3 and BP4

Synthesis of 3-chloro- and 5-chloro-2-hydroxy-4-methoxybenzophenone. 2.28 g (10.0 mmol) of 2-hydroxy-4-methoxybenzophenone was dissolved in 20 mL of dichloromethane, cooled in an ice bath and 2.30 g (10.6 mmol) of *N*-chlorosaccharin was added portion wise un-

der stirring. After 10 minutes, the reaction mixture was washed with aqueous sodium hydrogen carbonate and sodium bisulfite, again with water and dried with anhydrous sodium sulfate. After evaporation of solvent under reduced pressure, 2.54 g of a yellow crystalline solid remained; this consisted of 5-chloro-2-hydroxy-4-methoxybenzophenone, as a principal product, and approximately 15% of a 3-chloro-isomer. Recrystallization of the crude product from dichloromethane-petroleum ether yielded 1.20 g (46%) of pure 5-chloro-2-hydroxy-4-methoxybenzophenone, mp. 113–116 °C. Mother liquor after the crystallization of 5-chloro-isomer was evaporated and purified using column chromatography (silica, diethyl ether-petroleum ether 1:6) and 232 mg (9%) of pure 3-chloro-2-hydroxy-4-methoxybenzophenone was isolated, mp. 135–137 °C.

3-chloro-2-hydroxy-4-methoxybenzophenone. ^{1}H NMR, δ/ppm (acetone d_6): 4.02 (s, 3H), 6.80 (d, J = 9.0 Hz, 1H), 7.55 (m, 6H), 13.0 (s, 1H). ^{13}C NMR, δ/ppm ($CDCl_3$): 56.5 (CH_3), 102.4 (CH), 109.4 (C), 114.1 (C), 128.4 (CH), 128.9 (CH), 131.9 (CH), 133.3 (CH), 137.7 (C), 160.7 (C), 161.1 (C), 200.1 (C). MS (ESI+), m/z (%): 265 (MH^++2, 27), 263 (MH^+, 74), 187 (33), 185 (100), 105 (18), 77(8). Elemental analysis, calcd for $C_{14}H_{11}ClO_3$: C 64.01%, H 4.23%, measured: C 64.06%, H 4.22%.

5-chloro-2-hydroxy-4-methoxybenzophenone. ^{1}H NMR, δ/ppm (acetone d_6): 4.03 (s, 3H), 6.74 (s, 1H), 7.50 (m, 3H), 7.60 (m, 3H), 12.57 (s, 1H). ^{13}C NMR, δ/ppm ($CDCl_3$): 56.7 (CH_3), 101.2 (CH), 113.16 (C), 113.18 (C), 128.7 (CH), 129.0 (CH), 132.0 (CH), 134.2 (CH), 137.8 (C), 161.4 (C), 165.1 (C), 199.5 (C). MS (ESI+), m/z (%): 265 (MH^++2, 12), 263 (MH^+, 35), 187 (40), 185 (100), 149 (13), 105 (28), 97 (15), 77 (16). Elemental analysis, calcd for $C_{14}H_{11}ClO_3$: C 64.01%, H 4.23%, measured: C 64.01%, H 4.18%.

Synthesis of 3,5-dichloro-2-hydroxy-4-methoxybenzophenone. 1.13 g (5.0 mmol) of 5-chloro-2-hydroxy-4-methoxybenzophenone was treated with 1.31 g (5.0 mmol) of *N*-chlorosaccharin in 10 mL of dichloromethane at room temperature. After 10 minutes the reaction mixture was washed with aqueous sodium hydrogen carbonate and sodium bisulfite, dried with anhydrous sodium sulfate and the solvent was evaporated on a rotary evaporator. The resulting yellow solid was recrystallized from dichloromethane-hexane and 0.965 g (65%) of light yellow 3,5-dichloro-2-hydroxy-4-methoxybenzophenone (mp. 98–101 °C) was isolated. ^{1}H NMR, δ/ppm ($CDCl_3$): 4.01 (s, 3H), 7.56 (m, 3H), 7.65 (m, 3H), 12.74 (s, 1H). ^{13}C NMR, δ/ppm ($CDCl_3$): 61.5 (CH_3), 118.0 (C), 118.6 (C), 119.2 (C), 129.6 (CH), 130.1 (CH), 133.1 (CH), 133.6 (CH), 138.0 (C), 159.1 (C), 200.6 (C), 206.2 (C). MS (ESI+), m/z (%): 301 (MH^++4, 14), 299 (MH^++2, 36), 297 (MH^+, 56), 263 (20), 223 (12), 221 (71), 219 (100), 185 (32), 105 (42). Elemental analysis, calcd for $C_{14}H_{10}Cl_2O_3$: C 56.59%, H 3.39%, measured: C 56.75%, H 3.35%.

Reaction of BP4 with TCCA in basic medium. 0.92 g (3.0 mmol) of BP4 and 0.25 g (3.0 mmol) of sodium hydrogen carbonate was dissolved in 4 mL of dist. water. After that, 0.28 g (1.2 mmol) of TCCA was added and the reaction mixture left overnight. The solid product was filtered, dissolved in diethyl ether and extracted with aqueous sodium hydrogen carbonate and water. After evaporation of solvent, 0.295 g (33%) of yellow crystalline substance remained, which was identified by mp. and ^{1}H NMR as 3,5-dichloro-2-hydroxy-4-methoxybenzophenone.

Reaction of BP4 with NaOCl in acidic medium. 0.21 g (0.7 mmol) of BP4 was dissolved in 5 mL of dist. water and cooled in an ice bath. The resulting solution was acidified with 0.15 mL of conc. HCl and a solution of NaOCl, containing 0.91 mmol of NaOCl was slowly added. After 2 h, the reaction mixture was filtered, the filtrate evaporated under reduced pressure and the solid residue extracted with ethanol. The extract was purified by column chromatography (SiO_2, EtOH:CH_2Cl_2 = 1:3) and 39 mg (18%) of 5-benzoyl-3-chloro-4-hydroxy-2-methoxybenzenesulfonic acid were isolated as yellow crystals. mp. 160 °C (dec.) ^{1}H NMR, δ/ppm (acetone d_6): 4.08 (s, 3H), 5.62 (s, 1H), 7.67 (m, 5H), 8.21 (s, 1H), 12.92 (s, 1H). MS (ESI–), m/z (%): 343 ($M–H^-$+2 42), 241 ($M–H^-$ 100), 212 (65), 165 (38),144 (42), 141 (94), 135 (82), 114 (57), 103 (65), 89 (64), 73 (48). HRMS calcd for $C_{14}H_{10}ClO_6S$ 340.9887, found 340.9890.

2. 3. Photostability of Chlorinated Products

The photo-degradation experiments were performed in a custom-made photoreactor[17] with six UVA lamps (CLEO 20 W, 438 mm × 26 mm, Philips; broad maximum at 355 nm) equipped with borosilicate glass cell (240 mm long, inner diameter 40 mm) with the effective volume 250 mL. The solutions of 250 mL were irradiated for fixed periods of time (15, 30, 45, 60, 90 and 120 min). Samples were kept in continuous contact with room atmosphere. During the irradiation, 10 mL of irradiated samples were taken from the cell and analyzed by HPLC-DAD (UV-Vis).

2. 4. Analytical Procedures

Absorption spectra (200–500 nm spectral range) of aqueous solutions were recorded on HP 8453 UV-Vis spectrophotometer.

The HPLC analyses were made on an Agilent 1100 Series chromatograph, coupled with DAD detector. The chromatographic separations were run on a Zorbax C8 column (4.6 mm ID × 250 mm, 5 μm) using a 60: 40 mixtu-

re of acetonitrile and acetic acid (pH 3) as the mobile phase. The column temperature was kept at 25 °C with the flow rate of 1.0 mL/min, injection volume 75 μL and the duration was 25 min with 5 min of post run. BP3, BP4 and their chlorinated products were monitored at 240 nm. All the analyses were done in triplicates and are presented as mean values.

^{1}H NMR spectra were recorded on a Bruker Avance DPX 300 spectrometer(300 MHz). Chemical shifts are reported against the tetramethylsilane reference.

Mass spectra were recorded on a 6224 Agilent Accurate-Mass TOF mass spectrometer.

Elemental analyses were performed on a Perkin Elmer 2400 Series II CHNO/S elemental analyser.

2. 5. Determination of BP3, BP4 and Chlorinated Products in Swimming Pool Waters

Water samples of 1 L volume were taken from selected swimming pools in August 2011 at the summer season. Samples were divided in two replicates and extracted using strata-X 33 μm Polymeric Sorbent (200 mg/6 mL) and eluted according to the protocol prescribed by the Phenomenex. After evaporation of solvents, the samples were diluted with 0.5 mL of mobile phase and analyzed with HPLC-DAD as described in the previous section. For quantification purposes calibration curves in the range from 0.125 to 3 μg L^{-1} was prepared for BP3 and 0.125 to 7.5 μg L^{-1} for 3,5-diCl-BP3. The r^2value of the regression line for BP3 was 0.9955 and for 3,5-diCl-BP3 0.9962.

2. 6. Toxicity Measurements

A stock solution of BP4 was prepared in water at a concentration of 1 g L^{-1}. Stock solutions of BP3 and chlorinated BP3 were prepared in acetone (100 g L^{-1}) and dimethyl sulfoxide (DMSO) (75 g L^{-1}), respectively. Final tested concentrations in toxicity tests of BP3 and chlorinated BP3 were prepared according to ISO standard.[20] Regarding the highest recommended solvent concentration (0.1 mL L^{-1}).

Marine liquid-dried *Vibrio fischeri* NRRL-B-11177 were obtained from the manufacturer (Dr. Lange GmbH, Düsseldorf, Germany). The luminescence of bacteria was measured on a LUMIStox 300 luminometer (Dr. Lange GmbH, Düsseldorf, Germany) at 15 ± 0.2 °C after 30 min of exposure according to ISO standard.[21] The results for each concentration were calculated as the percentage inhibition relative to the control. The 30 min inhibitory concentrations, IC_{20}, IC_{50} and IC_{80} values were calculated using a log-linear regression analysis and represents the concentration of a tested compound that is required for 20, 50 and 80% luminescence inhibition after 30 minutes of exposure.

3. Results and Discussion

3. 1. Chlorination of BP3 and BP4 UV Filters

Diluted aqueous solutions of BP3 and BP4 were treated with NaOCl or TCCA at room temperature and after certain period of time reactions were stopped by addition of Na_2SO_3. We have observed very fast reaction of both compounds with disinfection reagents. The starting material was in both cases completely consumed in less than 15 min and there is almost no difference in the composition of reaction mixture in15 min, 1 h or 24 h after the setup of the reaction. In simulated natural water conditions with added NaOCl, the same products were formed. As shown on Figure 1, the chlorination of BP3 with TCCA leads to the formation of 5-chloro and 3,5-dichloro derivatives with the small amount of 3-chloro derivative. After 24 hours no presence of BP3 was observed.

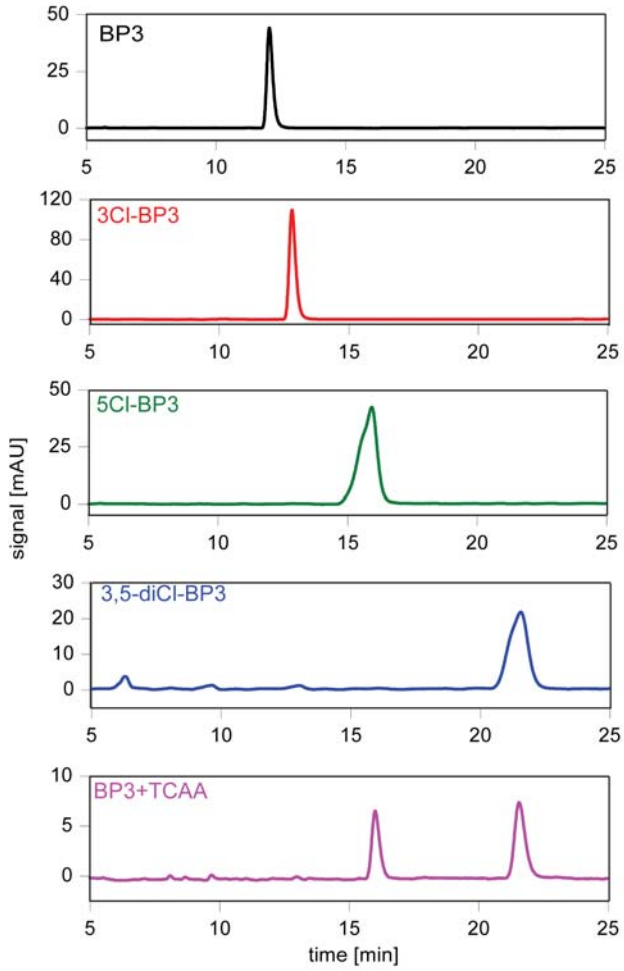

Figure 1. HPLC chromatograms of **BP3**, three possible chlorinated products and a reaction mixture formed from **BP3** with excess TCCA

3. 2. Synthesis of Chloro Derivatives of BP3 and BP4

Preparative chlorination of BP3 was carried out in the organic solvent (CH_2Cl_2) due to its low solubility in water. In an organic medium, besides the main product, 5Cl-BP3, also an appreciable amount of 3Cl-BP3 was formed, which enabled isolation and characterization of this compound (Scheme 1). With the excess of chlorinating agent, also 3,5-dichloro-2-hydroxy-4-methoxybenzophenone (3,5-diCl-BP3) is formed.

Chlorination of BP4 in neutral aqueous environment leads to the formation of 5Cl-BP3 and 3,5-diCl-BP3. Interestingly, no 5-benzoyl-3-chloro-4-hydroxy-2-methoxybenzenesulfonic acid (3Cl-BP4) was formed, indicating that in neutral aqueous medium, where sulfonic group is fully ionized, an *ipso* substitution (replacement of sulfonate group by chlorine) is preferred. Only in strongly acidic medium, where the sulfonic group is protonated, 3Cl-BP4 appears as a product.

All chloro-derivatives were also fully characterized by spectroscopic methods (NMR, IR, MS), and were employed as chromatographic standards.

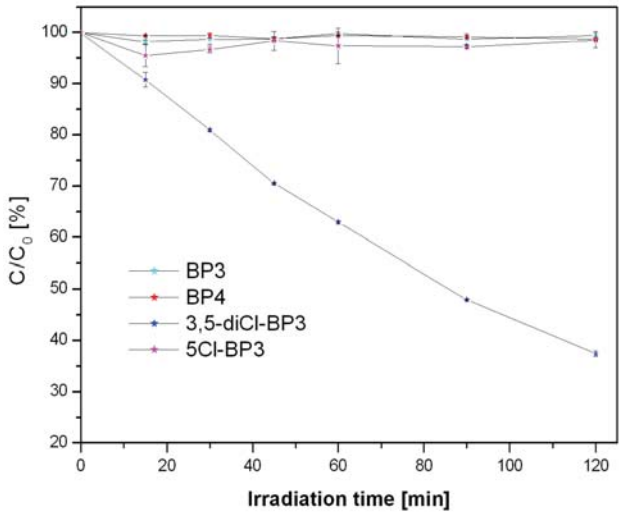

Figure 2. Photostability of BP3, 5-chloro and 3,5-dichloro derivatives of **BP3** and **BP4** under UV-A irradiation.

3. 3. Photostability of Chlorinated Products of BP3 and BP4

Photostability experiments of BP3, BP4 and their chlorinated products (3Cl-BP3, 5Cl-BP3, 3,5-diCl-BP3) in water revealed different stability of each compound in the presence of the UV-A light after 120 minutes of exposure. It can be seen from Figure 2, the case of parent compounds, BP3 and BP4, as well as of 5-Cl-BP3, within 120 minutes of irradiation time, less than 5% of initial concentration disappeared. In the case of environmentally less important 3-Cl BP3, less than 10% of initial concentration degraded. These results indicate that photodegradation rate of BP3, BP4, 3-Cl-BP3 and 5-Cl-BP3 using UVA treatment is very low. In contrast to them, the less stable compound appeared to be the 3,5-diCl-BP3 with the degradation of more than 40% of initial compound. Degradation products formed were not identified.

3. 4. Toxicity Data

The results of toxicity tests with *Vibrio fischeri* testing BP3 and BP4 are given in Table 1.

Table 1. 30 min IC (inhibitory concentrations) values obtained for *Vibrio fischeri* for BP3 and BP4

UV filter	BP3	BP4
30 min IC_{20} (mg/L)	33.2	67.3
30 min IC_{50} (mg/L)	151	301
(95% confidence interval)	(150–152)	(300–302)
30 min IC_{80} (mg/L)	268	1350

BP3 and BP4 were found to be slightly harmful to the bacteria *Vibrio fischeri*. The 50% inhibition of luminescence was detected at 301 mg/L of BP4 after 30 min of exposure. The reported 16h EC_{50} values were 210 and 250 mg/L obtained for BP4 using *Pseudomonas putida* as a test organism, which confirmed very low toxicity of BP4 to the bacteria.[22] Due to low solubility of BP3 in water, a stock solution was prepared in acetone.[19] When organic solvent was used to prepare a stock solution, toxicity of a solvent at the highest tested concentration (a negative control) was determined in each experiment. BP3 was nontoxic to bacteria at lower concentrations, however at higher concentrations (> 150 mg/L) significant precipitation of BP3 was observed.

A solubility of 5Cl-BP3 in organic solvents i.e. acetone or ethanol, is lower than that of the parent BP3 UV filter. A stock solution of 5Cl-BP3 was prepared in DMSO in a concentration of 75 g L^{-1}. After tested samples with different concentrations were prepared, 5Cl-BP3 precipitated in the samples with concentrations higher than 50 mg/L. However, concentrations up to 50 mg/L of 5Cl-BP3 were nontoxic to luminescence of bacteria.

3. 5. Determination of Chloro-Derivatives of BP3 and BP4 in Bathing Water

Analysis of water samples taken from 13 bathing areas, swimming pools with fresh and marine water, indicated the presence of BP3 UV filter at two locations in the concentrations of 0.3 μg L^{-1} and 1.7 μg L^{-1}. In both cases swimming pools were filled with freshwater. Presence of 3,5-diCl-BP3 was found at one location in the concentration of 6.6 μg L^{-1}. A 5Cl-BP3 was found at another one

but the concentration was under the limit of quantification.

Disinfection of water in swimming pools using various chlorination agents is a common tool for keeping high water quality. To protect people against sunburn more and more protecting agents are used and BP3 and BP4 are two of them used and have been already found in swimming pool water.[18] It is of great importance to be aware, that BP3 and BP4 react with chlorination agents and mainly 5-chloro and 3,5-dichloro derivatives are formed. For that reason, not only the quality control of swimming pool waters in general, as it is prescribed by the legislation, but also the control of these compounds (parent compounds and chlorinated products) in swimming pool waters should be taken into consideration. It should be also stressed that with this reaction probably their photo-protective properties are altered and an additional harmful compounds might be formed.

4. Conclusions

In this study we pointed out the importance of transformations processes, which may occur under disinfection conditions in swimming waters. Important fact, which was confirmed is, that chloro-derivatives in water seem to be less stable than parent compounds and the toxicity of chlorinated compounds tested by *Vibrio fischeri* was found to be in the same range as that of the starting compounds.

What is more concerning is the fact that chlorinated products are formed very fast and some of them were found in swimming pool waters during summer season when a high number of people visit and enjoy this areas. Additional research should be conducted on toxicity assessment of chlorinated products and determination of compounds formed by the degradation of them when exposed to the natural sunlight. On this basis, additional parameters should probably be included in regular monitoring programme of swimming pool waters.

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Povzetek

V članku so predstavljeni rezultati raziskave reakcij dveh najbolj uporabljanih UV filtrov, benzofenona-3 (BP3) in benzofenona-4 (BP4) z reagenti za dezinfekcijo vode, natrijevim hipokloritom (NaClO) in trikloroizocianurno kislino (TCCA). S HPLC in UV-Vis spektrofotometrijo smo ugotovili nastanek dveh kloriranih produktov, 5-kloro-2-hidroksi-4-metoksibenzofenona in 3,5-dikloro-2-hidroksi-4-metoksibenzofenona, kar je bilo potrjeno z neodvisno pripravljenimi standardi. Študij fotostabilnosti je pokazal nižjo stabilnost 3,5-dikloro-derivata v primerjavi z izhodnimi spojinami in monokloro-derivatom. Strupenost kloro-derivatov za bakterije *Vibrio fischeri* je primerljiva z nekloriranimi izhodnimi spojinami. Začetne študije prisotnosti teh spojin v kopaliških vodah so potrdile prisotnost BP3 in njegovih kloriranih derivatov.