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Identification of avobenzone by-products formed by various disinfectants in different types of swimming pool waters

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ABSTRACT

The increased use of sunscreens and other cosmetics containing UV filters causes human and environmental burden. Avobenzone is a widely used UV filter. In its pure form it is known to undergo several transformations including photo-isomerisation, photodegradation, and halogenation. Over 60 disinfection by-products were identified as transformation products of avobenzone in different disinfection reactions of chlorination and bromination in fresh and seawater. Two occasional samples of swimming pool water demonstrated the presence of some of these by-products at noticeable levels as judged by GC-MS peak areas. Although the toxicity of the majority of these products remain unknown, chlorinated phenols and acetophenones are known to be rather toxic. Aquatic bromination of avobenzone resulted in the identification of 33 disinfection by-products (DBPs). Many of them contain bromine in the molecular structure. Addition of copper salt slightly decreases conversion rate simultaneously increasing the levels of major brominated products.

Photostability of 3 commercial sunscreen products (solar protection factor 30) containing avobenzone was studied under different experimental conditions including UVA/UVB, UVC photostimulation and chlorination. The commercial sunscreen products have completely different enhancing and inhibitory effect on avobenzone degradation under UVC light. The complex composition of commercial products caused also a protective shield in case of chlorinated solutions of commercial formulations exposed to chlorine and UVA/UVB light at the same time.

1. Introduction

Swimming is a popular leisure activity, which can provide health benefits for the public. Swimming pools have a wide variety of uses, from leisure and sport in bigger pools to smaller spa pools for improvement of health and less stress (Fakour and Lo, 2018). Due to that reason swimming pool water matrix is quite unique and depends on water matrix, temperature, climate, location and swimming habits (Carter and Joll, 2017). The filling water is commonly disinfected distributed drinking water (freshwater swimming pools), although seawater is sometimes used as well. Among different disinfection approaches, used in pool water, chlorination represents the most popular and economical one (Lakind et al., 2010). Beside natural organic matter (NOM) and human body excretions (sweat, urine, saliva and body cells), personal care products (sunscreens, hair products, lotions/soaps and cosmetics) are also introduced into the swimming pool water and all together contribute to the formation of disinfection by-products (BDPs) (Carter and Joll, 2017; Teo et al., 2015; Manasfi et al., 2016; Zwiener et al., 2007). In general, water disinfection with chlorine-based disinfectants results in the proven formation of more than 700 disinfection by-products (Richardson et al., 2007; Richardson and Temes, 2018) due to the reactions of natural organic substances or other substrates introduced by humans (Weisel et al., 2009) with active chlorine. It should be emphasized that most of the organic chlorine remains in the composition of compounds that have not yet been identified. An even greater problem represents the increased popularity of swimming pools filled with seawater (Manasfi et al, 2017; Luccherra et al., 2007), in which the presence of inorganic salts leads to a significant increase in the range of disinfection byproducts. Moreover, among such products brominated and iodinated compounds appear (Huang et al., 2008;

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Parinet et al., 2012; Manasfi et al., 2016; Font-Ribera et al., 2016). They are usually much more toxic than their chlorinated analogues (Richardson et al, 2007; Plewa et al., 2010; Manasfi et al., 2017).

Latest studies suggest that exposure to the water in swimming pools may result in a variety of health risks, such as increased asthma and allergy, which are associated with microbial and chemical hazards (Bernard et al., 2006; Pasquarella et al., 2013, 2014). Recent studies (Chowdhury et al., 2014; Daiber et al., 2016) have raised concerns on adverse health impacts associated with application of personal care products and disinfection byproducts (DBPs) formed in swimming pools.

Sunscreens containing different protecting UV filters represent an important group of anthropogenic organic compounds appearing in swimming pool and marine bath waters due to human activity. Quite often they are treated as contaminants of emerging concern. Chemical (organic) filters express their UV absorbing characteristics as a function of their individual chemical nature (Shaath, 2010). In order to achieve their prolonged photostability additional stabilizers are added in commercial formulations (Afonso et al., 2014; Vallejo and Gallardo, 2011). On the other hand, the presence of physical (inorganic) filters, like TiO_2 in a nano formulation (20–50 nm) raises questions from two perspectives: the behavior of nano TiO_2 and the fact that as such, TiO_2 much stronger absorbs than scatters UVB/UVA radiation. Organic filters may decompose when exposed to light by direct photolytic reactions or transform in the presence of chlorine and chlorinated medium (water pools, seawater). The formation of halogenated DBPs in chlorinated waters is inevitable, especially when UV filters possess double bonds, phenolic, keto-, or amino moieties (Lebedev, 2007). NIST17 library was used for the identification.

In our research we focused on aquatic halogenation of avobenzone (4-*tert*-butyl-4′-methoxy-dibenzoylmethane), the most common UVA filter (400–320 nm) of this type in the formulation of sunscreens, sold under the trade names Parsol 1789 or Eusolex 9020. It is a constituent of numerous sunscreen products being one of UVA filters permitted to be used by the European Commission on Health (The Encyclopedia of UV Filters, 2007). Avobenzone exists in two tautomeric forms: enol and keto ones. In sunscreen formulations, avobenzone exists predominantly in the enol form, which has a maximum absorption at wavelengths ranging from 350 to 365 nm depending on the solvent used. Its enol form exhibits an excellent UVA absorption (at 357 nm) but its diketo form expresses absorption in the UVC region and thereby ineffective as UVA or UVB filter. In addition, it has been reported to fragment when exposed to UV radiation into reactive species and photo-adducts (Shaath, 2010), triggering photoallergic reactions (Karlsson et al., 2009). Earlier studies (Santos et al., 2013; Crista et al., 2015) have dealt with the aquatic chlorination of avobenzone. Using LC/MS method the formation of two primary by-products in the reaction of avobenzone with sodium hypochlorite in water (a mono- and dichloro- substituted compounds) was demonstrated. The structures of these two primary products were confirmed by liquid chromatography–tandem mass spectrometry (HPLC-MS/MS), NMR spectroscopy and gas chromatography–mass spectrometry (GC/MS) (Kalister et al., 2016). The combined action of active chlorine and UVC radiation results in the formation of several dozens of compounds (including chloroanhydrides and chlorophenols). Some of them are not produced when these two disinfection methods are applied alone (Trebše et al., 2016). The primary chlorination products of avobenzone (2-chloro-1-(4-*tert*-butylphenyl)-3-(4-methoxyphenyl)-1,3-propanedione and 2,2-dichloro-1-(4-*tert*-butylphenyl)-3-(4-methoxyphenyl)-1,3-propanedione) were studied further for degradation at different pH, as well as for photostability (Wang et al., 2017). The chlorination in seawater lead to the formation of a wide range of brominated products (Chugunova et al., 2017). This result forced us to turn to the modeling of the reaction of the aqueous chlorination of avobenzone in the presence of individual inorganic salts, in particular Br^-, I^-, Cu^{2+} and Fe^{3+}. These studies are rather important as, for example, concentration of bromides in freshwater is usually in the range 0.1–1.0 mg/L, exceeding sometimes 2 mg/L in Israel, and reaching 4.13 mg/L in Australia (Pan and Zhang, 2013). Over 30 compounds including numerous halogenated derivatives were identified by GC-HRMS (Detenchuk et al., 2019). Many of them contain bromine and even iodine in the molecular structure. The results allow for the conclusion that aquatic chlorination in the presence of bromides and iodides brings to halogenated products with potentially much higher toxicity than that of the corresponding chlorinated species (Plewa et al., 2004; Plewa et al., 2008; Plewa et al., 2010; Manasfi et al., 2017). Among the most relevant from the environmental point of view one should mention brominated phenols and substituted acetophenones. In the presence of KI even iodinated acetophenones were detected (Detenchuk et al., 2019). Iodinated organic compounds are detected in the environment quite rarely, however there are some publications on the presence of them in DBP mixtures (Krasner et al., 2006; Richardson et al., 2008; Postigo et al., 2017).

Our previous studies on avobenzone were completed with the stability study of three commercial sunscreen products (solar protection factor 30) containing avobenzone under different experimental conditions (UVA/UVB, UVC photostimulation and chlorination) and are presented in this work. Additionally, aquatic bromination of avobenzone with potassium hypobromite as an alternative disinfection agent used worldwide, was conducted to see the difference in the products in comparison with aquatic chlorination. Finally, analysis of selected water samples, taking from swimming pools in Slovenia, filled with fresh and seawater has been performed. The results obtained with real samples were compared with that of the earlier experiments in terms of products formation identified previously as DBPs forming during aquatic chlorination of avobenzone (Kalister et al, 2016; Trebše et al, 2016; Wang et al., 2017; Chugunova et al., 2017; Detenchuk et al., 2019).

2. Experimental

The chemicals in this study were used as purchased:

Avobenzone (1-(4-Methoxyphenyl)-3-(4-*tert*-butylphenyl)propane-1,3-dione) (96% purity) from Fluka, Degussa P25 TiO_2, and acetonitrile was HPLC grade from Sigma. Chlorination was achieved with the use of Oasis® Water Purification Tablets, manufactured in the UK by Hydrachem, containing 17.0 mg sodium dichloro isocyanurate pro tablet per 1 L, generating 10 mg of active chlorine in 1 L of chlorinated water.

Three commercial products, containing avobenzone, named by initials H30, N30, G30 in order to protect their trade marks, were studied. All of them offered a solar protection factor (SPF 30). Their composition of organic and inorganic filters is summarized in Table 1.

Table 1 shows that all three commercial products contained avobenzone, which was the main target compound in our previous research. The complete list of the content of commercial sunscreen products is presented in Supplemental Material in Table S3.

2.1. Preparation of solutions for degradation and chlorination experiments of sunscreen products

Solutions of avobenzone containing sunscreen products were prepared daily fresh. The working solution was prepared by diluting 1 mL of acetonitrile solution of individual sunscreen product (SCP) containing avobenzone in 100 mL flask with deionized water or 100 mL of chlorinated water (10 mg L^{-1} chlorine) in case of chlorination experiments. The acetonitrile solutions of avobenzone in SCP were prepared daily fresh by weighting (30.0 mg–90.0 mg of SCP, depending on the content of avobenzone in the SCP) and diluting it in 10 mL flask with acetonitrile.

Chlorination experiments of avobenzone and sunscreens were performed with Oasis® water purification tablets, the only NSF (National Sanitation Foundation) approved tablets, manufactured in the UK (GMP

Table 1
The composition of organic and inorganic filters in SCP: H30, N30, G30.

Organic and inorganic filters	H30	N30	G30
UVB protection - salicylates			
3,3,5-Trimethylcyclohexyl salicylate	**present**	**present**	not present
2- Ethy hexyl salicylate	**present**	**present**	**present**
Ethyl hexyl triazone	not present	not present	**present**
UVA + UVB protection – benzophenones, esters			
Benzophenone-3	**present**	not present	not present
Octocrylene	not present	**present**	**present**
UVA protection - dibenzoyl methanes			
Avobenzone	**(2.03 ± 0.13)%**	**(4.42 ± 0.30)%**	**(3.20 ± 0.18)%**
Inorganic			
TiO_2 (nano)	not present	**present**	**present**

and ISO9001:2000) by Hydrachem. One water purification tablet is used to purify 1 L of water. It contains 17.0 mg of NaDCC (sodium dichloroisocyanurate or sodium triclosene), which generates 10 mg L^{-1} of available chlorine in 1 L of water in a minute. The tablet is effervescent, allowing it to dissolve in less than 1 min, but according to producers' protocol the usage starts 10 min later.

2.2. UVA/UVB and UVC photodegradation experiments

The photo-degradation experiments were performed in a custom-made photoreactor w(meaning that escaping carbon dioxide gas causes the tablet to dissolve quickly)ith 4 UVA (20 W, CLEO) lamps and 2 UVB (20 W, CLEO) lamps for UVA/UVB experiments and with 6 germicidal UVC lamps (20 W, CLEO) for UVC experiments equipped with quartz glass cell with the effective volume of 100 mL. The solutions were irradiated for fixed periods of time (0, 15, 30, and 60 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).

2.3. Bromination experiments

40 mL of phosphate buffer was placed into 100 mL flask where added 0.4 mL of avobenzone solution in acetonitrile (400 μg of avobenzone) and 0.5 mL of KBrO solution (10 equivalents of hypobromite). Reaction mixture was stirred in the dark at the room temperature for 24 h. Then ~2 mg of sodium sulphite was added to quench the excess of active bromine. Aliquot of 10 mL of the reaction mixture was acidified with 10% sulfuric acid to pH 2 and extracted 3 times with 3 mL of dichloromethane. Then NaOH solution (10%) was added to the water samples to pH 11 and dichloromethane (3 mL) extraction was repeated 3 times. The extracts were dried over sodium sulfate, combined, and concentrated to 0.1 mL. Experiment with addition of the copper salt (210 μg of $CuSO_4$) was conducted similarly.

2.4. Freshwater and seawater swimming pool sampling

Sampling has been performed in early June 2019 at aquapark in Slovenia, working whole year, offering a diverse selection of water experiences of indoor and outdoor pools with sea and freshwater. Since we have not been interested in final concentration of THMs, but in the most common avobenzone degradation products, pH, T and conductivity have not been measured, but being obtained from the official institutions. Random samples of 0.5 L have been collected in 2 pools, one filled with freshwater and another with seawater and have been kept in the refrigerator till analysis.

Water samples (0.5 L) were acidified with 10% sulfuric acid to pH 2 and extracted 3 times with 20 mL of dichloromethane. Then NaOH solution (10%) was added to the water samples to pH 11 and dichloromethane (20 mL) extraction was repeated 3 times. The extracts were dried over sodium sulfate, combined, and concentrated to 0.1 mL.

2.5. Analytical procedures

The HPLC analyses of SPCswere carried out on an Agilent 1100 Series chromatograph, coupled with UV–Vis detector. The chromatographic separations were run on a Zorbax C18 column (4.6 mm ID × 250 mm, 5 μm) using a 85:15 mixture of acetonitrile:water. The column temperature was kept at 25 °C with the flow rate of 1.0 mL min^{-1}, injection volume 10 μL and the duration was 25 min with 5 min of post run. Avobenzone was monitored at 350 nm. All the analyses were done in duplicates and are presented as mean values.

2.5.1. GC-MS analysis of pool waters

The GC-MS analysis was carried out with Shimadzu GC-MS QP2010Ultra instrument with autosampler AOC-5000Plus (Shimadzu - CTC Analytics). The following conditions were applied: capillary column DB-17MS, 30 m × 0.25 mm, 0.25 μm; injection volume 1 μL splitless; injector temperature 300 °C; carrier-gas – helium with flow rate 1 mL/min at constant pressure 53.5 kPa. Temperature gradient: 50 °C (3 min) − 20 °C/min − 320 °C (13.5 min); transfer line temperature 300 °C; ion source temperature 230 °C; ionization energy 70 eV; mass range 45–800 Da. Wiley and NIST11 libraries were used for the identification. Perdeuteronaphthalene and perdeuterophenanthrene were used as internal standards for quantification.

2.5.2. GC-MS analysis of avobenzone aquatic bromination

Pegasus® GC-HRT (LECO Corporation, Saint Joseph, MI, USA) coupled to an Agilent 7890A Gas Chromatograph (Agilent Technologies, Palo Alto, CA, USA) was used. The data were acquired using 10 full (10–800 m/z range) spectra per second in high resolution mode (25,000), with high mass accuracy (< 1 ppm) reliably determining elemental composition of all ions of interest in the mass spectra (Lebedev et al., 2013). Chromatographic separation of the samples was performed using an Rxi-5SilMS 30 m × 0.25 mm, 0.25 μm (Restek Corporation, Bellefonte, PA) column with a constant helium flow of 1 mL/min. All injection volumes were 1 μL, splitless. The injector and the transfer line temperatures were set at 270 °C and 320 °C, respectively. The GC oven program was as follows: 0.5 min isothermal at 50 °C, then 10 °C min^{-1} ramping to 320 °C and 8 min isothermal hold at 320 °C. Perdeuteronaphthalene and perdeuterophenanthrene were used as internal standards for quantification, or quantity estimation depending on the presence of a standard of identified compound. In the latter case the response factor was equal to 1.

2.6. Toxicity measurements

Marine liquid-dried *Vibrio fischeri* NRRL-B-11177 were obtained from the manufacturer (Dr. Lange GmbH, Dusseldorf, Germany). The luminescence of bacteria was measured on a LUMIStox 300

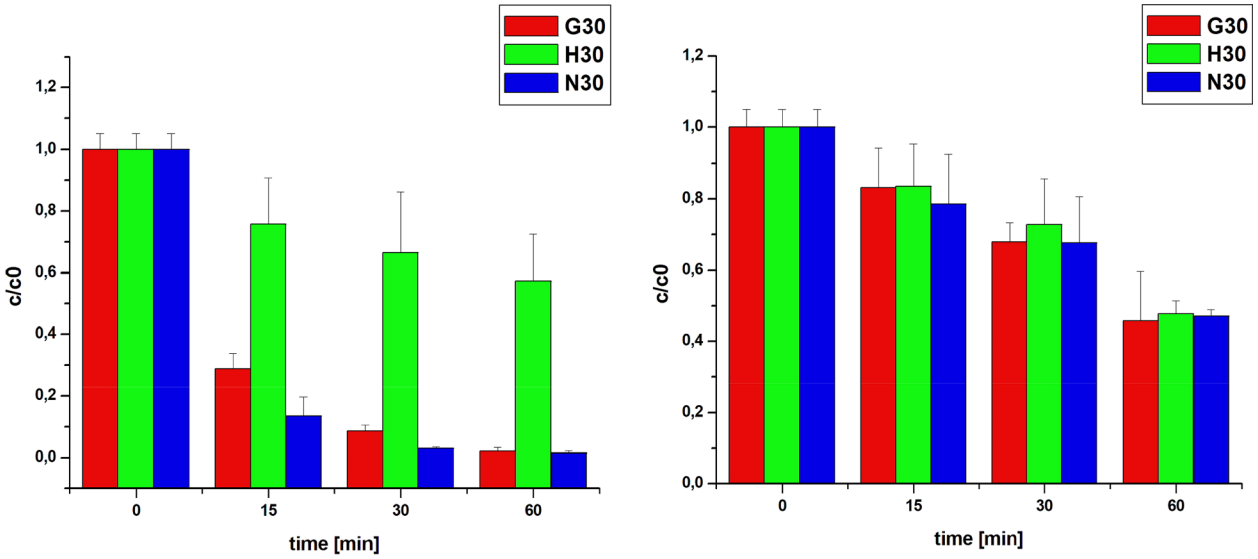


Fig. 1. The degradation of avobenzone in commercial products under UVC light – left, and UVA/UVB light - right.

Table 2
The toxicity of UVA/UVB irradiated solutions of SCPs exposed to chlorination and those without chlorination, expressed as % of inhibition.

G30	**G30-Cl_2**
52,2 ± 1,2	**88,9 ± 0,5**
H30	**H30-Cl_2**
60,6 ± 0,8	**79,7 ± 0,5**
N30	**N30-Cl_2**
34,2 ± 0,6	**92,8 ± 0,1**
Avo	**Avo-Cl_2**
86,6 ± 0,3	**94,3 ± 0,1**
H_2O	**H_2O-Cl_2**
8,1 ± 0,6	35,5 ± 2,5

luminometer (Dr. Lange GmbH, Dusseldorf, Germany) at 15 ± 0.2 °C after 30 min of exposure according to ISO standard (ISO 11348-2, 2007). The results for each concentration were calculated as the percentage inhibition relative to the control.

3. Results and discussion

3.1. Photostability and chlorination of SCPs

As expected, the degradation of avobenzone as a pure substance differs from the degradation of avobenzone in relatively complicated matrix of SCPs. The photochemical behavior of this UV filter has been extensively studied and it has been found that its photostability is highly dependent on the polarity and proticity of the solvent (Afonso et al., 2014). Avobenzone was found to lose absorption efficacy as a result of photoisomerisation from the enol to the keto form and/or photodegradation to form photoproducts that absorb principally in the UVC region, depending on the solvent (Mturi and Martincigh, 2008).

As it is evident from Fig. 1, the commercial products have completely different attitude to protect or promote the degradation of avobenzone. The UVC light was more efficient in removing avobenzone in commercial product G30 and N30, whereas it was not very efficient in H30. On the other hand, in case of UVA/UVB photocatalysis the degradation of avobenzone is neither stimulated nor inhibited by the presence of complicated matrices' substances of commercial products.

Moreover, chlorination in the dark dramatically effected the concentration of pure avobenzone in solutions as it was immediately chlorinated. On the contrary it remained in all commercial products (G30 (98,5 ± 3,7) %; H30 (99,2 ± 0,4) %; N30 (99,0 ± 0,2) of remaining avobenzone), being not affected by chlorine attack. Even more interesting observation involves the fact, that commercial products exposed simultaneously to chlorination and UVA/UVB light preserved avobenzone very efficiently (G30 (85,6 ± 1,9) %; H30 (85,6 ± 1,9) %; N30 (88,4 ± 0,8) % of remaining avobenzone). The reasons for such behavior probably involve different effects coming from complex matrix of commercial products. For example, some matrix species may be much more reactive than avobenzone, leading to the elimination of active chlorine due to competitive processes (see Supplement Material, Table S3).

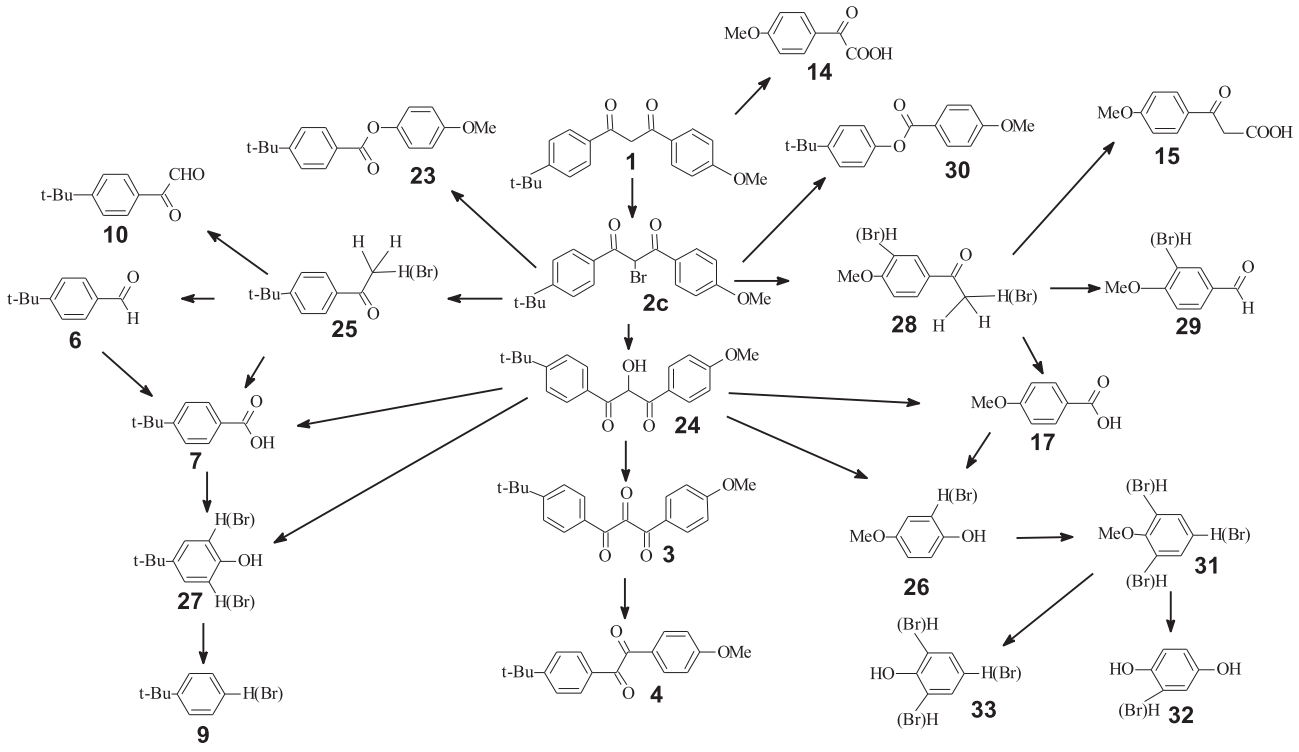
The inhibited luminescence of bacteria *Vibrio fischeri* is an efficient tool to predict toxicity of the samples. For this purpose, the solutions of SCPs exposed to chlorination and those without chlorination were irradiated for one hour with UVA/UVB light and afterwards tested with bacteria *Vibrio fischeri*. Table 2 shows, that the chlorination (pure as well as in SCPs) increased the toxicity in all cases from 19,1% (H-30) and 36,7% (G-30) to 58,6% (N30).

These results clearly demonstrate that the toxicity of an array of disinfection byproducts is higher than that of the original molecule of UV-filter. Although more than 30 products of avobenzone aquatic chlorination were identified, unfortunately, there is no information on the toxicities of the overwhelming majority of these DBPs. The mixture toxicity depends on combination of several byproducts formed. Since similar molecules are likely to have similar properties and bind to the same group of proteins, chemical similarity is often a good guide to the biological action of an organic molecule (Keiser et al., 2007). Therefore, one can suppose that chlorinated acetophenones and phenols substituted in benzene ring should possess some toxicity similar to their unsubstituted analogs. It is worth reminding that α-chloroacetophenone is a warfare agent known since XIX century (Graebe, 1871).

3.2. Bromination of avobenzone

Bromination becomes more and more popular all over the world as bromine possesses wide range of activity against microorganisms, viruses, bacteria, fungi, and more efficient against algae. Sometimes bromides are added into the water before disinfection with active chlorine on purpose to increase the degree of water purification generating additional powerful reagent. At the same time HBrO is 25 times more active than HClO in producing halogenated species (Myllykangas, 2004; Pan and Zhang, 2013).

Scheme 1 illustrates the principal pathways of transformation of



Scheme 1. Principal pathways of aquatic bromination of avobenzone.

avobenzone in conditions of aquatic bromination. It is quite similar to that of aquatic chlorination (Trebše et al, 2016; Detenchuk et al., 2019). However, brominated compounds are formed in that case. Their toxicity is supposed to be higher than that of the analogous chlorinated species. The reaction starts with the substitution of hydrogen atoms of methylene group for bromine atoms, followed by hydrolysis and formation of the tricarbonyl compound. Transformation of these species defines the formation of all the secondary products belonging to two series (due to two different substitutes in the aromatic rings) of acetophenones, benzoic acids, benzaldehydes and phenols. In our earlier publications on avobenzone aquatic chlorination (Kalister et al., 2016; Trebše et al., 2016; Wang et al., 2017; Chugunova et al., 2017; Detenchuk et al., 2019) we demonstrated that the chlorination starts exclusively with the electrophilic addition to the double bond of the enol form of the molecule. The alternative isomeric products with halogen in the aromatic ring of avobenzone were never detected. A few halogenated ring products were formed at the advanced stages of transformation, e.g. at the stage of the phenols. In the case of bromination nothing has changed. However, quite a number of brominated aromatic species were detected. They were also formed in the reaction of electrophilic substitution in the aromatic ring of the corresponding phenols, as well as aldehydes and acetophenones (compounds 28 and 29) in the case of the strong electronodonating methoxy group. It is also worth mentioning bromination of intermediate anisole with formation of tribromoanisole (compound 31) followed by its hydrolysis with formation of the corresponding tribromophenol (compound 33). The addition of copper salt into the reaction mixture brought to significant increase (5–10 times) of the yields of the brominated products, catalyzing the reaction or reacting photochemically through an electron transfer process (Sykora, 1997).

Surely bulky *tert*-butyl moiety did not promote formation of the corresponding brominated into the ring products. Only 4-*tert*-butylphenol, which was not detected itself, being reactive, produced corresponding 2,6-dibromo-4-*tert*-butylphenol (compound 27).

Another important issue involves formation of bromoform during avobenzone aquatic bromination. Haloforms are well known final products of aquatic chlorination/bromination of organic substrates. However, it forms at the very late stages of reaction due to haloform reaction. In our earlier studies of avobenzone chlorination we saw sometimes only traces of chloroform. Nevertheless, aquatic bromination resulted in formation of bromoform (97 ng). Even more spectacular was the 65-fold increase of the bromoform level during bromination in the presence of copper sulphate (6400 ng). After 24 h of bromination bromoform becomes the major product of the reaction. Taking into account the known high toxicity of bromoform this result arises serious concern on the application of bromination for the water disinfection.

3.3. Analysis of pool water

Finally, we decided to check what disinfection byproducts of avobenzone may be detected in ordinary swimming pool in regular conditions of its exploitation. For that purpose, two pools (with fresh and seawater) were selected. Two samples of the fresh and seawater, taken in early summer 2019, demonstrated the presence of several compounds detected earlier in laboratory experiments as byproducts of avobenzone aquatic chlorination. The detected compounds and their levels in both pools are summarized in Table 3.

Although avobenzone itself was not detected, and all the compounds listed in Table 3 may proceed from various natural or anthropogenic molecules, avobenzone is a possible precursor, while the

Table 3
The levels of the avobenzone aquatic chlorination disinfection byproducts in the swimming-pool water samples (μg/L).

	Rt, min	Freshwater	Seawater
p-*tert*-Butylphenol	9.437	4.1	4.2
4-Methoxybenzaldehyde	9.774	8.0	1.4
p-*tert*-Butylacetophenone	10.420	0.28	–
4-Methoxyacetophenone	10.502	0.8	1.2
4-Methoxy benzoylchloride	10.554	–	1.1
p-*tert*-Butyl benzoic acid	10.830	2.4	80
4-*tert*-Butyl-α,α-dichloroacetophenone	12.456	–	0.27

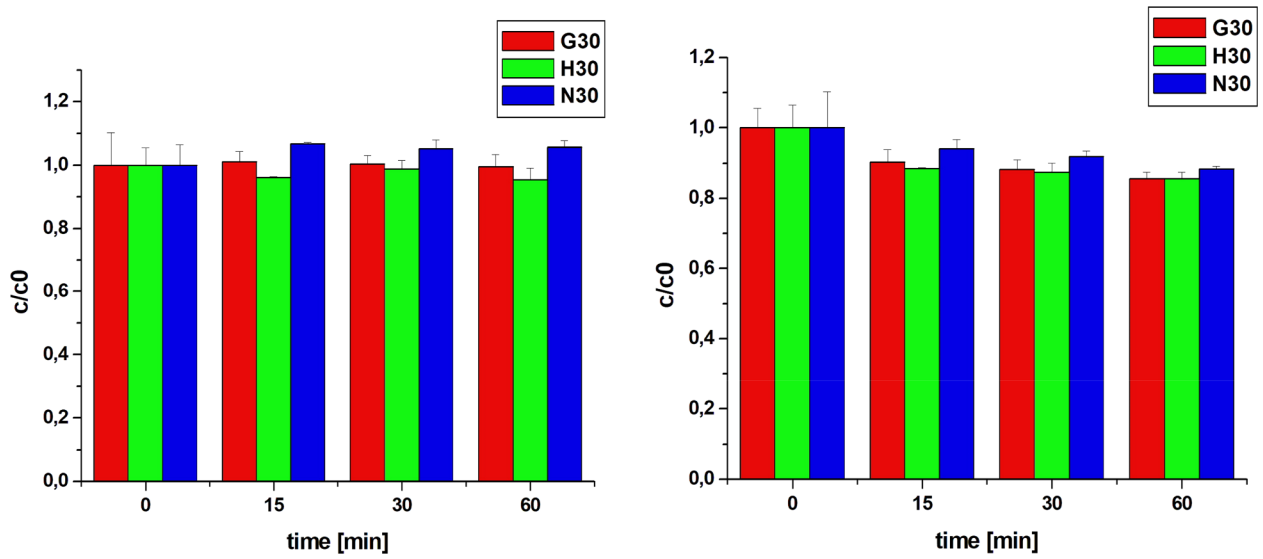


Fig. 2. The chlorination of avobenzone in commercial products (left - dark experiment; right - experiment under UVA/UVB light).

process of its transformation reaches rather advanced stages in the conditions of the swimming pools exploitation. Anyway, possible presence of avobenzone will definitely result in the increase of the levels of these compounds in the pool water. By the way, the traces of less reactive UV-filter octocrylene were detected in the pool water. *Tert*-butylbenzoic acid, being the major component in the swimming pool water (Table 3), was also the major product in laboratory experiments on chlorination in seawater (Chugunova et al, 2017) and quite prominent in the freshwater (Detenchuk et al., 2019). It is worth mentioning that that compound is rather stable and may be accumulated in the aquatic chlorination reaction. Substituted acetophenones often dominated in the laboratory experiments. They were present in the swimming pool water (Fig. 2). Their levels were not high as they represent an intermediate group of DBPs, transforming further with formation of the corresponding acids and phenols. By the way the absence of methoxybenzoic acid among the detected product may be rationalized by the high tendency of methoxy group in activating benzene ring for electrophilic substitution reactions. Since as was already mentioned above we found advanced transformation products in the swimming pool samples, methoxybenzoic acid could react further with the opening of the aromatic ring and formation of highly polar compounds not amenable to GC-MS analysis.

Table 1S in Supplement material contains the list of DBPs of avobenzone chlorination and bromination in various conditions used in our study. The compounds are divided into three groups depending on the reliability of their identification. Group 1 involves identified compounds for which standards were available. These compounds were also quantified using internal standard method with preliminary establishment of the response factors. Group 2 contains compounds which spectra were in the NIST17 library or our private library of mass spectra with accurate masses. The spectrum of the most appropriate hit (not necessarily the first one) was further checked manually using the known fragmentation behavior of organic compounds under electron ionization conditions (Turecek and McLafferty, 1993; Lebedev, 2015). The correctness of fragment ions assignment was confirmed by accurate mass measurements allowing getting their elemental composition. Group 3 contains compounds for which there was no an appropriate mass spectrum in the library. Then manual interpretation was used (Turecek and McLafferty, 1993; Lebedev, 2015). Besides the known rules of fragmentation, we used templates based on the fragmentation pattern of related compounds with confirmed structure, e.g. original avobenzone and its mono- and dihalogenated derivatives. The levels of compounds from groups 2 and 3 were estimated using internal standard method and considering response factor equal to 1. Fig. 1S and the following explanation give an example of the procedure.

Summarizing the overall results on the avobenzone aquatic chlorination and bromination the following issues should be emphasized. Over 60 DBP were identified in the aquatic halogenation experiments carried out in distilled and seawater as well as with addition of Br^-, I^-, Fe^{3+} and Cu^{2+} ions (Fig. 3). Unfortunately, only few of these compounds were studied earlier by toxicologists. For the vast majority of new DBPs the type and value of their toxic effect remains unknown. The drastic changes in the structure and levels of DBPs depending on the addition of each particular inorganic ion demonstrate the complexity of the mechanism of aquatic chlorination of avobenzone. For example, addition of Cu^{2+} ions into the aquatic bromination reaction mixture brought to 65-fold increase of bromoform level, somehow catalyzing the reactions or triggering photochemical electron transfer process (Sykora, 1997), while addition of Fe^{3+} into the aquatic chlorination reaction mixture brought to very high levels of potentially toxic α,α-dichlorinated acetophenones (Detenchuk et al., 2019). Addition of KBr

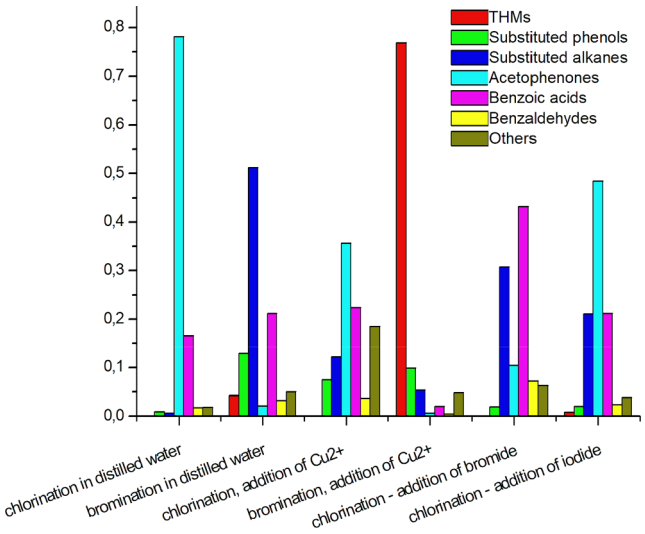


Fig. 3. Distribution of different group of DBPs in chlorination and bromination experiments after 1 h (1 - chlorination in distilled water, 2 - bromination in distilled water, 3 – chlorination, addition of Cu^{2+} - chlorination, 4 - bromination, addition of Cu^{2+}, 5 - chlorination - addition of bromide, 6 - chlorination - addition of iodide), expressed as the percentage of specific group of compounds.

to the chlorination reaction mixture resulted in formation of a number of brominated compounds with toxicities generally higher than that of the corresponding chlorinated ones (Plewa et al., 2010). Although there were no high levels of iodinated products in the reaction with addition of KI, the levels of chlorinated products were notably higher in comparison with the chlorination without KI. Moreover, two α-iodinated acetophenones were detected earlier in our experiments and in other studies (Detenchuk et al., 2018; Bichsel and von Gunten, 1999). It is worth mentioning that inorganic salts also brought to some decrease of the conversion rate of the initial avobenzone. This effect may involve participation of active chlorine in side reactions (e.g. bromate and iodate formation). The number of possible reactions and corresponding products increases when seawater is used. The effect should involve the presence of numerous inorganic ions and organic compounds influencing the principal reaction of avobenzone aquatic chlorination by catalyzing certain stages of the overall process or inhibiting them due to competitive reactions of active chlorine. The results obtained allow proposing that new products and peculiarities may be found conducting reaction of aquatic chlorination at different pH values, temperatures or solar conditions (in the case of open swimming pools). The further study should also aim the toxicity evaluation of the identified DBPs and carrying on thorough quantitative experiments finding the ways to minimize their formation in reaction of avobenzone with active chlorine.

4. Conclusion

Although avobenzone is stable in organic solvents UV-irradiation and/or the presence of disinfectants leads to the formation of the two pairs of the corresponding substituted benzoic aldehydes and acids, as well as phenols and acetophenones. Over 60 disinfection by-products were identified as transformation products of avobenzone in different disinfection reactions of chlorination and bromination in fresh and seawater. Two occasional samples of swimming pool water demonstrated the presence of some of these byproducts at noticeable levels as judged by GC-MS peak areas. Although the toxicities of the majority of these products remain unknown substituted chlorinated phenols and acetophenones are known to be rather toxic. Aquatic bromination of avobenzone brings to the similar array of products. However, these are brominated products, which toxicity is supposed to be higher than that of their chlorinated analogues. Moreover, very high levels of bromoform during that reaction worth special attention. The assortment of DBPs of avobenzone significantly depends on the composition of the inorganic salts of water, while the levels of DBPs may vary by orders of magnitude. These facts prove very complex mechanism of aquatic chlorination of that compound bringing to the results, which are rather difficult to predict. The assortment and levels of the byproducts depend not just on the concentration of active chlorine, but also on the type of disinfecting agent, ratio substrate/active chlorine, pH, temperature, reaction time, and composition of water, including both organic and inorganic substituents. Since the toxicity of the chlorinated avobenzone and its commercially available formulations is always higher than that of the original avobenzone toxicological studies of the individual disinfection byproducts should be carried out.

CRediT authorship contribution statement

Albert T. Lebedev: Methodology, Software, Validation, Resources, Writing - review & editing, Supervision, Funding acquisition. **M. Bavcon Kralj:** Methodology, Writing - original draft, Visualization. **Olga V. Polyakova:** Investigation, Data curation. **Elena A. Detenchuk:** Investigation. **Sergey A. Pokryshkin:** Formal analysis. **P. Trebše:** Conceptualization, Methodology, Resources, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105495.

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