

Feature article

Chlorination of UV Filters with Antioxidant Shield in Swimming Pool Waters – Products Identification and Toxicity Assessment

Mojca Bavcon Kralj¹, Albert T. Lebedev² and Polonca Trebše^{1,2*}¹ Faculty of Health Sciences, University of Ljubljana, Ljubljana, Slovenia² Massec, d.o.o. Postojna, Slovenia

* Corresponding author: E-mail: polonca.trebse@zf.uni-lj.si

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Abstract

This work summarizes our research on synthesis, characterization and toxicity of selected UV-A filters and their antioxidant shield in commercial formulation – resveratrol. Benzophenone type of UV filters react under disinfection conditions with chlorine and form different mono- and dichlorinated products, while dibenzoylmethane derivatives, such as avobenzene, react with chlorine and form two main bridge chlorinated products followed by numerous chlorinated species at the advanced stages of the process. Resveratrol showed three main susceptible centers to chlorination, starting from the electrophilic addition to the double bond and continuing with the chlorination of the phenolic moieties. Several experiments conducted under different disinfection conditions (pool/sea water, addition of salts, irradiation) showed basically similar chlorination patterns with some variations in terms of product formation. The results of toxicity assessment using different test organisms (*Vibrio fischeri*, microalgae, daphnids) have shown different sensitivity of testing organisms to the parent UV filters in comparison with chlorinated products as well as different toxicity for specific UV filter in comparison to the others. As the closing loop of all experiments in the laboratory, an up-scaling to the real human skin is presented.

Keywords: UV filters, chlorination, disinfection by-products, toxicity

1. Introduction

Ultraviolet (UV) light, which comes mainly from the sun, causes damage to materials, which are exposed to it. By the name UV, mainly the light with wavelengths of 290–320 nm (UV-B) and 320–400 nm (UV-A) is meant. Photons of UV light cause breakage of covalent bonds and thus induce various oxidation processes, which are mainly chain-radical oxidation with air oxygen. These processes lead to aging and weathering of different construction materials, coatings, plastics, and rubber. Particularly harmful, however, are these processes in biological systems, where they cause damage to skin cells resulting in skin aging processes, various inflammatory processes, and cancer.

To protect against UV irradiation, various substances are used that either reflect or absorb UV light. Compounds, which absorb UV light, are applied in numerous fields. Especially important are these, where the products

are exposed to solar radiation (coating products, plastic products, and cosmetic products). These compounds absorb UV light and are usually called UV filters. As a result of the growing awareness of the harmful exposure to the sun and in order to reduce the risk for skin cancer they are also widely used as personal care products (e.g. sunscreens, shampoos, hair sprays, lipsticks). They protect human body against the harmful effects of sunlight. In addition to inorganic pigments, which reflect UV light in particular, organic compounds, which absorb UV photons, are also used. UV light is of a broad spectral range, 400–290 nm (UV-A and UV-B), therefore no compound can prevent the exposure to the whole spectrum by itself, since the absorption peaks are much narrower. From that reason, a combination of several compounds covering the whole area, is usually applied. Based on the literature survey about the research on the use and effects of old and new formulations, the list of substances permitted by law is regularly updated. The European Union (EU) currently allows 28

organic substances, while some other compounds are allowed in countries around the world, such as Japan and the U.S., where they are treated as biological agents, available without prescription.^{1–3}

The sun protection factor (SPF) depends on the nature and the proportion of UV filter components in the commercial preparation. SPF is an indicator of the effectiveness of a sunscreen. Compounds for protection from the sun are always used in combination, since a single UV filter, which could provide a sufficiently high SPF does not exist. In the final sunscreen products, we observed increased use of inorganic UV filters, especially in sunscreens for children and creams to protect very sensitive skin. The most used is certainly TiO₂. Organic UV filters are somehow less applicable due to potential instability and, therefore, the reduction is SPF. Moreover, due to photosensitivity and the potential synergistic effects, various international health organizations, e.g. U.S. Food and Drug Agency (FDA), limit the combinations of different UV-A and UV-B organic chemical filters.⁴

Organic chemical filters can be divided into two groups, depending on the spectral range covered. The first consists of the UV-A filters, including benzophenone, antranilates and dibenzoylmethanes, the second one, UV-B filters, includes PABA derivatives, salicylate, cinammates and camphor derivatives. As per the European Community, compounds ranked among the organic UV filters for the protection from the sun express characteristics of persistent organic pollutants (POPs). The common characteristic of all these compounds is the presence of aromatic moiety with a side chain, and various degrees of unsaturation.⁵

When exposed to UV radiation, UV filters must be relatively stable. Sunscreen products are used primarily in special conditions, such as swimming in the sea, swimming pools, on the snow, and in the mountains, where a thorough protection is needed. Considering that the 100% stability to UV radiation of UV filters and other added compounds present in SPF is impossible, natural ROS scavengers are usually included in cosmetics formulations. *Trans*-resveratrol (RES) is one of them. With the two phe-

nol moieties in the chemical structure, it shows antioxidant,⁶ anti-inflammatory,⁷ and anti-tumor⁸ properties. Commercially it is often present in cosmetics, nutraceuticals,⁹ and food packaging to increase food stability or/and prevent oxidation.¹⁰ Nevertheless, several recent studies showed that both UV filters and antioxidants are decomposed by light. Mostly, two types of reactions occur: a) direct photolytic reactions, and b) chlorination of aromatic rings or side chains, due to the presence of chlorine and chlorate medium (such as pools, salty seawater).^{11–16}

The main environmental concern of UV filters is related to their high lipophilic character ($\log K_{ow}$ 4–8), relative stability against biological decomposition, and organic carbon distribution coefficient ($\log K_{oc}$ 3–4).¹³ They were found to accumulate in the aquatic environments, mainly soils and sediments and in the food chain. Some of them have been detected in fish in the range of 25–1800 ng/g, and in the fat of human milk in the range of 16–417 ng/g.^{1,2}

When these chemicals are released to the aquatic environment, they can also cause adverse biological effects on aquatic organisms through mechanisms such as toxicity and estrogenic activity. Adverse effects could be expected from original chemicals or their degradation/chlorination intermediates. The existing ecotoxicological data have confirmed their estrogenic hormonal activity and multiple endocrine-disrupting activities such as androgenic, anti-estrogenic, and estrogenic activities.^{17–20}

Many reports have shown that the toxicity of chlorinated organic compounds derived from chlorination processes was higher than that of their parent compounds.^{21–24} In these studies, it was shown that the toxicity might come from some of the chlorinated products. Beside that it was noticed that different effects of benzophenones chlorination processes might be expressed not only in the significant increase of toxicity, but also decrease or it may remain unchanged.²⁵ The toxicity of benzophenone type chlorinated products depends on their molecular structure, i.e., the position, number and type of their substituents, and transformation ratios.²⁵ The transformation activity of precursors presents the intrinsic factor for toxicity changes during chlorination treatment.

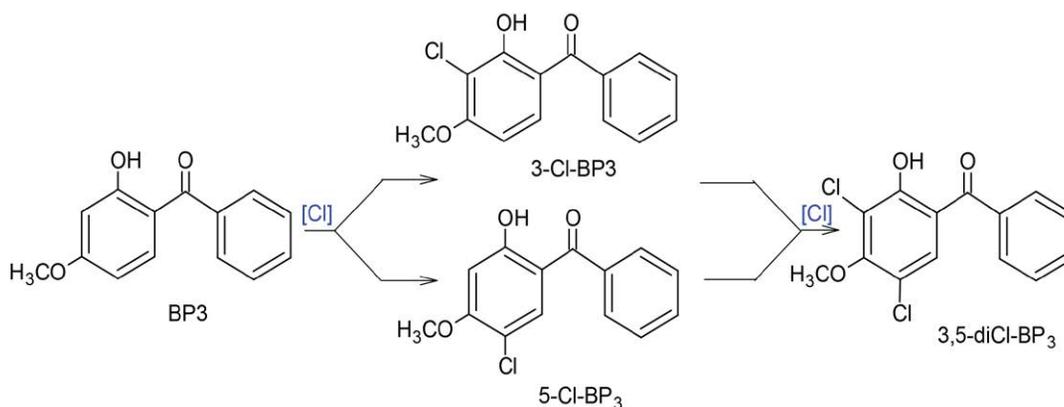


Figure 1. Reaction of BP3 under disinfection conditions.

2.1. Reactions of Benzophenone Type UV Filters under Disinfection Conditions

In the case of benzophenone type of UV filters chlorination according to the literature may occur at the aromatic ring or at the side chain. The formation of halogenated byproducts in chlorinated waters is inevitable, especially when filters contain phenolic rings and/or aromatic amines.^{12,14–16,26–31} Within our studies we have focused on BP3 (2-hydroxy-4-methoxybenzo-phenone), BP4 (2-hydroxy-4-methoxybenzophenone-5-sulfonic acid), and DHHB (hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]-benzoate).

In the case of BP3, its diluted aqueous solutions were treated with NaOCl or trichloroisocyanuric acid (TCCA) in the ratio 1:1 at room temperature and after certain period (up to 24 h) reactions were stopped by addition of Na₂SO₃. Detailed analysis (HPLC-DAD and independent synthesis of products) proved the formation of 5-chloro-2-hydroxy-4-methoxybenzophenone (5Cl-BP3) and 3,5-dichloro-2-hydroxy-4-methoxybenzophenone (3,5-diCl-BP3) with the small amount of 3-chloro derivative (3-chloro-2-hydroxy-4-methoxybenzophenone, 3-Cl-BP3) in the case of BP3 (Figure 1). After 24 h we did not observe the presence of BP3.³¹

Chlorination of BP4 in neutral aqueous environment resulted in the formation of two products, 5-benzoyl-5-chloro-4-hydroxy-2-methoxybenzenesulfonic acid (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.³¹

In the case of DHHB, HPLC-DAD revealed the formation of several products, which were later identified by

LC-MS/MS as 3-chloro DHHB, 3,5-dichloro DHHB, and the product chlorinated at the aromatic ring with substituted ethyl group.³² HPLC-ESI-MS and HPLC-ESI-MS/MS experiments of parent compound DHHB undertaken in the positive mode, together with the accurate mass measurements, revealed the detailed fragmentation pathway, which enabled us to elucidate the structure of chlorinated products. According to HPLC-DAD analysis, three products are formed, two of them already in the early stage of reaction of DHHB with NaOCl; the concentration of both increased with time. The presence of ions of *m/z* 404 and 406 in the ratio 3:1 for P1 and *m/z* 432 and 434 for P2 confirms the presence of one chlorine atom in the molecules. Based on MS² experiments we concluded that product 1 (Ph-Cl-DHHB) lacked an ethyl group and contained a chlorine atom instead, which is either in positions three or five of the aromatic ring. In the case of product 2, chlorination involved phenolic moiety of DHHB. Collision-induced dissociation (CID) conditions confirmed the formation of 3-substituted product, 3-Cl-DHHB. The structural elucidation of the third by-product was possible using the same procedure as with other ones. It represented a product of introduction of two chlorine atoms into positions three and five of the phenolic ring of DHHB (3,5-diCl-DHHB) (Figure 2).

All identified products were also independently synthesized, fully characterized by spectroscopic methods (NMR, IR, MS), and were employed as chromatographic standards.^{31,32}

2.1.1 Chloro-Derivatives of BP3 and BP4 in Swimming Pool Water

Swimming pools water disinfection is required to keep its quality and to prevent public health issues, besides it is also highly regulated.³¹ On the other hand, being aware

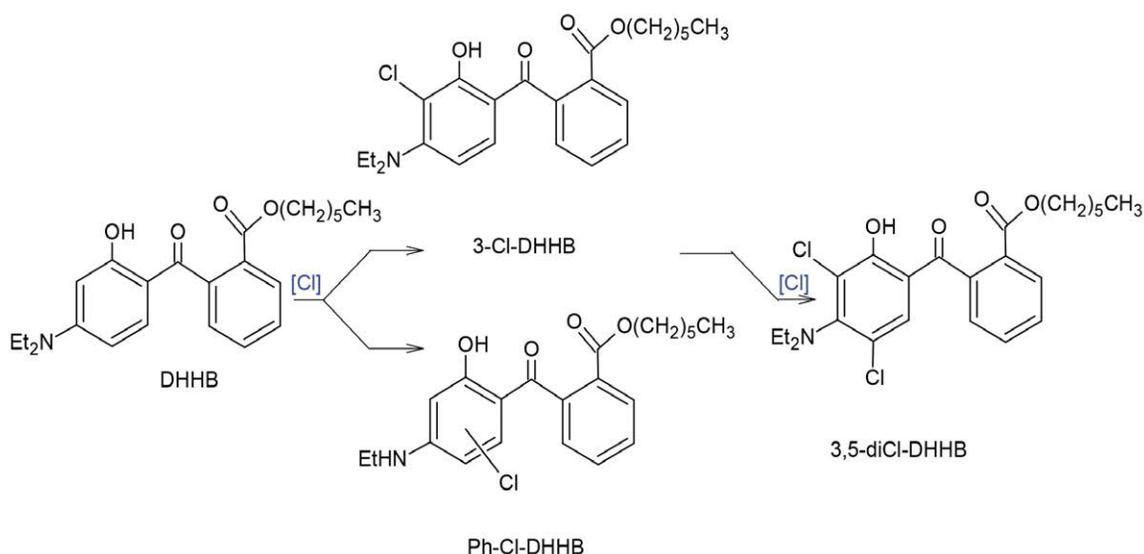


Figure 2. Chlorinated products of DHHB.

of sunburn consequences, people often use SPFs. Therefore, BP3 and BP4 appear in bathing waters as common UV filters. That was the reason to monitor them together with their chloro-derivatives.³¹ In summer season of 2011, we undertook a survey by taking samples from 13 bathing areas in Slovenia (swimming pools with fresh and marine water). The presence of BP3 was reliably confirmed at two locations in swimming pools with fresh water in the concentrations of $0.3 \mu\text{g L}^{-1}$ and $1.7 \mu\text{g L}^{-1}$, respectively. 3,5-diCl-BP3 was found only in one swimming pool ($6.6 \mu\text{g L}^{-1}$).

2. 2. Reactions of Dibenzoylmethane Type of UV Filters under Disinfection Conditions

Besides BP3, BP4, DHHB, avobenzene (4-*tert*-butyl-4'-methoxydibenzoylmethane) is also often present in SPFs. It is an UV-A filter, sold under the trade names Parsol 1789 or Eusolex 9020. It may exist in two tautomeric forms, enol and keto form, but in sunscreen formulations,

avobenzene exists predominantly in the enol one. In the case of avobenzene, we performed several studies under various disinfection conditions and in different matrices. We were able to perform the detailed study to identify products formed under specific conditions. Experimental details with DBPs formed are collected in Table 1.

LC-MS was studied by Santos et al., 2012¹³ who reported mono- and dichloro derivatives of avobenzene as primary products of its aqueous chlorination (Figure 3). Since methoxy group is one of the most powerful electron-donating substituents, a logical conclusion was made by Crista et al., 2015¹⁵ that chlorine occupied *ortho* position of the ring to the methoxy group. Nevertheless, detailed study of that reaction with GC-HRMS³³ showed that both aromatic rings did not contain chlorine atoms. Therefore, aqueous chlorination reaction involves double bond of the enol form of avobenzene rather than the activated benzene ring. The primary products 1-(*tert*-butyl)-2-chloro-3-(4-methoxyphenyl)-1,3-dione and 1-(*tert*-butyl)-2,2-dichloro-3-(4-methoxyphenyl)propan-1,3-dione



Figure 3. Chemical structures of main avobenzene chlorinated products (monochloro avobenzene – left and dichloro avobenzene – right).

Table 1. Sum-up of our research (experimental conditions, formation of chlorination products, references).

Disinfectant / medium	Reaction conditions	DBPs	Reference
NaOCl (0 to 2.5 eq) / distilled water	Room temperature – RT, 1 h	monochloro avobenzene dichloroavobenzene <i>p</i> -methoxychloroacetophenone	<i>Journal of Analytical Chemistry</i> ³⁵
–	UV-C, 1–4 h	4-methoxy-substituted benzaldehyde, benzoic acid, and phenol 4- <i>tert</i> -butyl substituted benzaldehyde, benzoic acid, phenol	<i>Water Research</i> ³⁴
NaOCl (2 and 20 eq) / distilled water	Experiment in the dark, RT, 30 min	beside previously mentioned mono- and dichloro avobenzene, substituted benzaldehydes, benzoic acid, phenols	<i>Water Research</i> ³⁴
NaOCl (2 and 20 eq) / distilled water	UV-C, 30 min	25 disinfection by-products; among them substituted benzaldehydes, benzoic acid, phenols, additionally chlorophenols, chloroanhydrides	<i>Water Research</i> ³⁴
NaOCl (20 eq) / distilled water	addition of inorganic salts (Br ⁻ , I ⁻ , Cu ²⁺ , Fe ³⁺)	brominated and iodinated products, such as brominated phenols and acetophenones (even iodinated)	<i>Journal of Analytical Chemistry</i> ³⁵
KOBr (10 eq) / water	RT, 24 h	several brominated products, including bromoanisol and tribromophenol	<i>Environment International</i> ³⁶
KOBr (20 eq) / water	addition of Cu ²⁺	significant increase of the yields of brominated compounds	<i>Environment International</i> ³⁶
NaOCl (20 eq) / sea water		40 compounds, including numerous brominated derivatives	<i>Journal of Analytical Chemistry</i> ⁴⁰

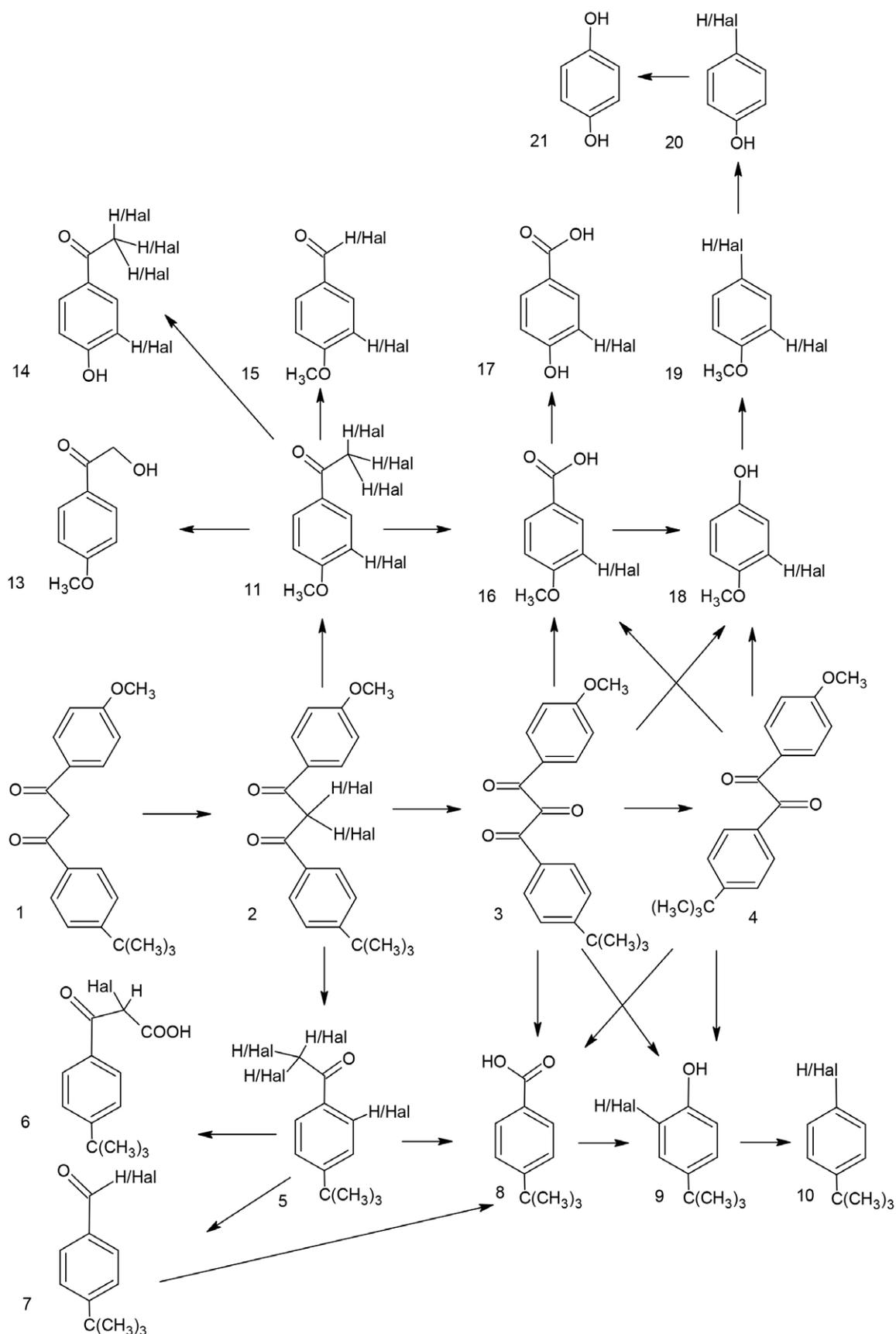


Figure 4. The principal pathways of disinfection process of avobenzone.

were specially synthesized by us.³⁴ Their mass spectra and retention times repeated those observed during aqueous chlorination of avobenzene. Same reaction with the double bond took place in conditions of aqueous bromination.^{35,36}

Then halogen atoms were substituted for oxygen. Further rupture of C-C bonds brought on most various monoaromatic compounds. Figure 4 summarizes the main pathways of the aqueous bromination of avobenzene. Over one hundred DBPs, including substituted aldehydes, acetophenones, acids, and phenols were identified.

Advanced stages of aqueous chlorination and bromination of avobenzene in the fresh and sea water, as well as with the addition of inorganic cations (Cu^{2+} and Fe^{3+}) and anions (Br^- and I^-) to the tap water were studied in detail.^{34–36,40} The experimental conditions dramatically influenced the range and levels of the reaction products. For example, addition of copper ions under aqueous bromination conditions resulted in 100-fold increase in the bromoform yield.³⁵ Although iodinated organic species easily lose iodine in aqueous chlorination, being substituted by chlorine,⁴⁰ two avobenzene iodinated products were still detected upon the addition of iodide anions to the reaction mixture.^{35,36} Iodides and iron ions also accelerated the aqueous chlorination reaction.³⁵

2. 2. 1 Presence of Avobenzene in Swimming Waters

Although avobenzene itself was not detected in bathing waters, it was a precursor of some products. *Tert*-butyl-benzoic acid, being the major product of avobenzene aqueous chlorination in seawater⁴⁰ and freshwater³⁶ in laboratory experiments, appeared to be the major component among the targeted DBPs in the swimming pool water as well. Being rather stable, it may be accumulated in the environment. Acetophenones, being well represented in the laboratory experiments, were also detected in the real bathing waters. Their levels were not high as they are intermediates ending up in acids and phenols.

2. 3. Reactions of Resveratrol under Disinfection Conditions

Resveratrol, an antioxidant usually added to sunscreen formulations to prevent oxidation of the UV filters, rapidly reacts with aqueous chlorine both in pure form and in commercial formulations. In the laboratory experiment it disappeared promptly, while 82 transformation products were tentatively identified.³⁷ GC-MS enabled identifying 95% of semi-volatile resveratrol transformation products, the others were established by UPLC-MS. Unfortunately, toxicity of only few of them is known, all the others are still not classified. There are several principal pathways of transformation, including DBPs coming from the addition to and the rupture of the central double bond,

as well as numerous products of electrophilic substitution in the activated phenolic rings.

In the primary reactions the number of carbon atoms remained equal – fourteen.³⁷ The first one involved electrophilic addition to the central double bond connecting the two phenol moieties, which resulted in a bunch of transformation products (positional isomers) including hydroxylated or/and chlorinated resveratrol. However, only dichloro resveratrol was reliably identified in the reaction mixture. Since the double bond represents an extremely reactive moiety in aquatic chlorination³⁸ (Figure 5, reactive center 1), the forming compounds coming from dichloro resveratrol immediately react further by the mechanism of electrophilic substitution in the aromatic ring or with the cleavage of the central aliphatic C–C bond.

The cleavage ended up in transformation products with one benzene ring in the molecule (hydroxybenzaldehyde, mono- and dichloro- hydroxybenzaldehyde, dihydroxybenzaldehyde and their derivatives with chlorine atoms on the ring, hydroquinone, chloro- and dichlorohydroquinone, phenol, and chlorophenols). Not to forget that chlorophenols were included in the list of priority pollutants of US EPA already in 1970.

The second pathway of transformation of resveratrol involved electrophilic substitutions in the aromatic ring. Benzene rings in resveratrol are highly reactive. They have activating *ortho-para*-directing hydroxyl groups in both rings. In the diol ring all positions are very reactive, although the most reactive one is between two hydroxyls (Figure 5, reactive center 2). The main semi-volatile products identified by GC-MS were mono-, di-, and trichloro-substituted resveratrols (two isomeric monochloro- derivatives, one dichloro- derivative, and one trichloro-). A tetrachloro- derivative as well as di-, tri-, tetra-, etc. chlorinated/hydroxylated compounds were identified by UPLC-MS (LC-MS/MS) due to lower volatility. Unfortunately, neither EI nor ESI-MS/MS enabled establishing the exact positions of these groups in the molecules.

Cyclization by *ortho*-positions of the resveratrol aromatic rings may be considered as the third transformation pathway. This cyclization generated phenanthrene-like molecules, which reacted further, similarly as in chlorination of orcinol.³⁹ After that, some products of substitutions of hydrogens for chlorines and several dicarbonyl products could be formed due to haloform reaction. The most environmentally problematic in the aqueous resveratrol chlorination was the formation of biphenyl-like molecules. Being prohibited for the last 30–40 years, they are highly toxic and unfortunately persistent in the environment.

In summary, it is worth mentioning that only few of 82 identified compounds have the toxicological data available, among them: chlorophenols and hydroxylated polychlorinated biphenyls. It is possible only to predict the toxicity of the others from the similarity to the classified compounds. However, they are too numerous and may be

represented by various isomers. Moreover, they are commercially unavailable and have to be synthesized before.

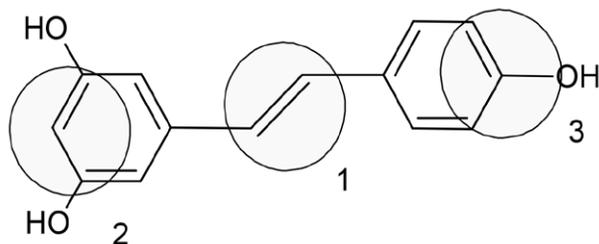


Figure 5. Chemical structure of resveratrol and its reactive centers.

3. Stability of Chlorinated Products

Photostability of chlorinated benzophenones and dibenzoylmethanes (3-chloro, 5-chloro, and 3,5-dichloro products) has been performed in a custom-made photoreactor with six UV-A lamps as already described.²⁸ The experiment revealed different photostability of each compound in the presence of UV-A light after 120 min of irradiation.

In the case of benzophenones, parent benzophenone, 3-chloro, as well as 5-chloro derivatives showed high stability toward UV-A irradiation, while 3,5-dichloro product degraded more than 40% within 120 min of UV-A irradiation.²⁸

In the case of avobenzone, dichloroavobenzone exhibited the lowest UV-A stability with a half-life of 22.4 min \pm 0.7 min, while avobenzone and chloro avobenzone were much more stable (half-lives 126 \pm 16 min and 128 \pm 25 min, respectively). Additional experiments were performed to study pH stability, as well as removal capacity (using TiO₂/UV-A). They have shown higher stability at neutral pH for all three compounds, whereas the least stable was dichloro avobenzone (half-life 14.1 \pm 0.6 min) under photocatalytic conditions.⁴¹

Our studies on chlorination have been completed with the stability study of three commercial sunscreen products (SPF 30) containing avobenzone under different experimental conditions (UV-A/UV-B, UV-C photostimulation and chlorination). As it was predicted, the degradation of avobenzone as a single compound differs from the degradation of avobenzone in relatively complicated matrix of SCPs. It was shown that commercial products had completely different attitude to protect or promote the degradation of avobenzone when it was treated as analytical standard or as an ingredient in different sunscreens.³⁶

4. Toxicity of Selected UV Filters And Their Chlorination Products

All our studies have been combined with toxicity experiments. The toxicity of selected UV filters and their

chlorinated by-products was tested with different test organisms (luminescent bacteria *Vibrio fischeri* (LUMISTox, Dr. LANGE), green algae *Pseudokirchneriella subcapitata*, or *Daphnia magna* Straus) based on standard ISO guidelines.^{42–44}

The results of toxicity monitoring revealed slightly increased toxicity of BP3 and BP4 to bacteria *Vibrio fischeri*. 30 min IC (inhibitory concentrations) obtained for *Vibrio fischeri* were as follows: IC₂₀ for BP3 was 33.2 mg/L and 67.3 mg/L for BP4. The 50% inhibition of luminescence was detected at 301 mg/L of BP4 after 30 min of exposure. The reported 16h-EC₅₀ 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.³¹ In the case of BP3 and 5-chloro BP3, we had to face with its very low solubility, and from that reason stock solution was prepared in acetone or DMSO. Results revealed BP3 was non-toxic to bacteria at lower concentrations, and in the case of 5-chloro BP3 the concentrations up to 50 mg/L were non-toxic to bacteria.

Toxicity of chlorinated compounds of DHHB tested by marine bacteria *Vibrio fischeri* was found to be in the similar range as that of the starting UV filters.³² This fact we explained by low transformation ratios of parent compounds and similar toxicity level of chlorinated products compared with their parent compounds. Microalgae *Desmodesmus subspicatus* were more sensitive to DHHB than to its chlorinated by-products.³² Contrary, crustaceans *Daphnia magna* were affected more by DHHB's chlorinated products. The toxicity of chlorinated DHHB by-product (i.e. 3-chloro DHHB) was significantly higher compared to DHHB when tested on *D. magna*. Similarly, significant toxicity elevation has been shown in the case of BP4 chlorination products in the experiment with *Phospobacterium phosphoricum*.⁴⁵ Such toxicity changes could be explained by the nature of substituent or by the reactivity of the molecule. According to literature,⁴⁵ the toxicity of benzophenone type UV filters, in general, decreases after the chlorination process.

Toxicity assessment of sunscreens containing avobenzone within chlorination of photodegradation experiments have been performed using marine bacteria *Vibrio fischeri*. It has been shown that within chlorination of avobenzone alone, as well as in sunscreens, in all cases the toxicity increased. We assumed that more toxic products than the original molecule are formed.³⁶

The results of toxicity measurements on resveratrol and a sunscreen containing it showed no inhibition effect on *V. fischeri* at the beginning and after 120 min of exposure, whereas it was significantly higher already at the beginning of chlorination experiment and remained almost the same throughout the whole experiment. Active chlorine reacted immediately with resveratrol, no matter was it present as a pure substance or a component of the sunscreen.³⁷

5. Experiments of Chlorination of Benzophenone and Resveratrol on Human Skin

For this study,⁴⁶ a controlled clinical trial was conducted on 38 volunteers (age: 20–60; female: 28; male: 10) to whom an area of the forearm was irradiated with an UV-B light. To conduct the study, the consent of Slovenian National Medical Ethics Committee was obtained (16 May 2018, No. 0120-368/2017/5), as well as written consents from all volunteers. The clinical study was done at the Faculty of Health Sciences, at the University of Ljubljana, in summer 2019 (from June to August). The investigation was oriented to understand the photoprotection role of UV filter (BP3) and two antioxidants (*trans*-resveratrol and β -carotene) under various conditions (including disinfection conditions) on human skin.

For this application, a portable colorimeter (Konica Minolta Chroma Meters CR-410 [Tokyo, Japan]) was used to measure skin redness using the guidelines for skin color measurement and erythema.⁴⁷ The skin squares 3×3 cm (9 cm^2) were irradiated over different periods (the 1st square uncovered for the entire irradiation period of 8 min, the 2nd for 6 min, and the 3rd for 4 min). Other squares served to check the effectiveness of the UV filter in the presence of antioxidants and disinfectants.

Descriptive statistics (arithmetic means, standard deviation, *t*-test, one-way repeated-measures ANOVA, Mauchly's test, etc.), were used to describe the skin colours' differences between trials.

The role of antioxidants in sunscreens has previously been reported in a study by Gaspar and Campos,⁴⁸ including the combinations of UV filters and vitamins A, C, and E, where the presence of vitamins reduced the skin irritation. Their results are in accordance with the results obtained in our study where we demonstrated the formation of several chlorinated products (5-Cl-BP3, and 3,5-di-Cl-BP3); however, their effect on skin was not tested at that point. Moreover, the formed benzophenone-3 chlorination products were photostable (more than 95% of the initial concentration) during the irradiation periods. The protective role of antioxidants in disinfection conditions was expected also in case of resveratrol and its 82 identified transformation products. In fact, the results proved the resveratrol's protective role and its high potential for acting as a scavenger of reactive oxygen species (ROS) in sunscreens. The addition of antioxidant molecules is beneficial for UV filters by protecting against UV degradation/disinfection processes and other *in vivo* skin effects.⁴⁹

In summary, this clinical study showed that formulations containing antioxidants were more efficient in skin protection than solely UV filters, since they helped to reduce the skin redness. Despite the formation of chlorinated products of BP3 in the presence of chlorinated water, the photoprotection was still effective.

6. Conclusions

In our studies we pointed out the importance of identification of chlorinated products, formed in the transformation processes under disinfection conditions in swimming pool waters. Chlorinated products are a very diverse group of compounds. Usually within disinfection processes they are formed very fast. It is highly important to identify them, characterize and then perform toxicity studies since their effects on humans are in many cases still unknown. Mass spectrometry (MS) has proven once again to be the most powerful analytical tool to study environmental issues. Because of its unsurpassed sensitivity, selectivity, and ability to handle complex mixtures of the most various compounds, it is used both in controlling the levels of targeted toxicants in the environment and in research dealing with the discovery of new natural and anthropogenic compounds.⁵⁰

MS is used as a principal method to determine and to quantify disinfection (chlorination) by-products (DBP). Currently, due to applications of both liquid chromatography (LC-MS) and gas chromatography (GC-MS) techniques approximately 700 disinfection by-products are officially listed.⁵¹

In addition, comparative toxicity studies should be performed for all combinations of parent compounds, as well as for chlorinated products. Our data demonstrated that the toxic potential of benzophenone-like UV filters is related to differences between the type of tested UV filter, the modified effects after chlorination (modification of molecular structure), and species-specific effects (type of organism).

At the end, the closing loop of all efforts of chlorination experiments was the clinical trial, where we have, thanks to volunteers, tested in real environment the photoprotective role of complex mixtures of UV filter, antioxidants during the chlorination process, mimicking in the laboratory the real swimming pool situations.

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Povzetek

Članek povzema raziskave naše skupine o sintezi, karakterizaciji in toksičnosti izbranih UV-A filtrov in ter vlogo antioksidanta resveratrola kot dodatka v kremah za zaščito pred soncem. UV filtri benzofenonskega tipa reagirajo pod dezinfekcijskimi pogoji s klorom, pri čemer se tvorijo mono- in diklorirani produkti. Derivati dibenzoilmetana, kot je avobenzon, pa reagirajo s klorom tako, da najprej reagira metilenska skupina avobenzona, pri čemer se tvori dva glavna klorirana produkta, v nadaljevanju procesa pa sledi nastanek številnih kloriranih produktov. Resveratrol vsebuje tri skupine, na katerih poteče kloriranje, začenši z elektrofilno adicijo na dvojno vez ter s kloriranjem fenolnih delov. Več poskusov, izvedenih v različnih pogojih dezinfekcije (bazen/morska voda, dodajanje soli, obsevanje s svetlobo), je pokazalo podobne vzorce kloriranja z razlikami pri številu in tipu produktov. Rezultati ugotavljanja toksičnosti z uporabo različnih testnih organizmov (*Vibrio fischeri*, mikroalge, vodne bolhe) so pokazali različno občutljivost testnih organizmov na osnovne UV filtre v primerjavi s kloriranimi produkti ter različno toksičnost posameznih UV filtrov. Nadgradnjo vseh laboratorijskih poskusov predstavlja študija izpostavljenosti pogojem kloriranja in obsevanja, ki je bila izvedena na človeški koži.



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