Octocrylene: From Sunscreens to the Degradation Pathway during Chlorination Processes: Formation of Byproducts and Their Ecotoxicity Assessment

Antonio Medici 1, Lorenzo Saviano 2, Antonietta Siciliano 2, Giovanni Libralato 2, Marco Guida 2, Lucio Previtera 3, Giovanni Di Fabio 1 and Armando Zarrelli 1,*

Octocrylene (OCT) is a viscous, clear and colorless oil, introduced in commercial sunscreens and anti-aging creams about 15 years ago. It is the 2-ethylhexyl ester of 2-cyano-3,3-diphenylacrylic acid, with the extended conjugation of the acid portion that absorbs UVB and short-wave UVA (ultraviolet) rays, with wavelengths from 280 to 320 nm [1], which promote tanning but also contribute to the onset of sunburn and skin cancer. It is used in various body care products [2,3], in concentrations up to 10%, in order to provide an adequate sun-protection factor or to protect the body care formulations themselves from UV radiation. This filter was recently indicted for the risk of inducing potential adverse effects on the endocrine system [4], as well as having an allergic and/or photoallergic potential [5]. In recent years, there has been an increase in the number of cases of photocontact allergic reactions to octocrylene, which has been referred to as an “emerging allergen”. However, it has the advantage of working in synergy, allowing for wide and beneficial photoprotection; for example, it stabilizes avobenzone (butyl methoxydibenzoylmethane), a molecule present in the UVA filter. The European Chemicals Agency (ECHA) constantly evaluates the safety profile of this filter, like all chemicals used in cosmetics and registered

Abstract: Octocrylene is an organic sunscreen whose main action is to absorb UVB radiation and short UVA wavelengths; it is used in various cosmetic products in order to provide an adequate sun-protection factor or to protect the cosmetic formulations themselves from UV radiation. This filter is believed to be a possible endocrine disruptor and is also questioned due to its allergic and/or photoallergic potential. However, it continues to be widely used, and it has been found in various environments, not least those of swimming pools, where it is evidently released by consumers, to the point that it is now considered an emerging micropollutant. The present investigation presents the possible chemical fate of octocrylene in the typical chlorination conditions of wastewater or swimming pools. A total of 11 disinfection byproducts were identified, and 6 were identified for the first time, and separated by HPLC. These products were identified through careful mass spectrometry studies and 1D and 2D NMR experiments. A formation mechanism has been proposed that justifies the chemical structures of all of the compounds identified. The ecotoxicological assessment of octocrylene and their products was carried out by employing Phaeodactylum tricornutum, Brachionus plicatilis and Aliivibrio fischeri as bioindicators. The ecotoxicity results reveal that toxic byproducts might be generated during the oxidation process, increasing the potential risk to the marine environment.

Keywords: octocrylene; chlorination; hypochlorite; degradation byproducts; water treatment; Aliivibrio fischeri; Phaeodactylum tricornutum; Brachionus plicatilis
under European legislation. Octocrylene has been found in various environments, not least those of swimming pools [6], where it is evidently released by consumers, to the point that it is now considered an emerging micropollutant similar to polyfluoroalkyl substances (PFAS) [7], blue-green algae [8], toxic fungal products [9], hormones [10], psychoactive drugs [11,12], pesticides [13], cosmetics, and industrial additives and drugs [14–18]. These substances, unlike conventional and unconventional pollutants, are still largely unregulated by legislation and are not restricted by maximum permitted values. Furthermore, they are potentially dangerous for the environment and human health, even in an overall context of insufficient data linked to their dangerousness [19–23]. Removal of emerging contaminants from wastewater can be accomplished by ozonation [24], membrane filtration [25], adsorption [26], and, above all, advanced oxidation [27,28].

In this paper, the degradation byproducts (DPs) of OCT were investigated by mimicking the chlorination process normally used in swimming pools to sterilize and disinfect water and to reduce similar emerging pollutants [6,29]. In particular, two different experiments were carried out, one at concentrations of about $10^{-5}$ M, comparable to those at which OCT is present in wastewater, and one at concentrations at least 100 times higher in order to isolate and identify the DPs. The structures of 11 isolated DPs, 6 of which were isolated for the first time, were determined by crossing the data provided by nuclear magnetic resonance (NMR) and those obtained by mass spectrometry (MS), using matrix-assisted laser desorption/ionization as a source and a time-of-flight analyzer (MALDI-TOF) for mass spectroscopy. It was also possible to propose a mechanism of formation that justifies the obtainment of the isolated products.

The spatial distribution of OCT is strictly connected to its presence in the marine aquatic environment; this presence is due to the anthropogenic activities responsible for its direct emission. For this reason, an ecotoxicological assessment was carried out with marine aquatic bioindicators such as *Aliivibrio fischeri*, *Phaeodactylum tricornutum* and *Brachionus plicatilis*. Emerging organic pollutants such as UV filters therefore require biological assays capable of detecting potential toxicity from a one-health perspective.

2. Results and Discussion

2.1. Chlorination Experiments

The OCT chlorination experiments were performed in the concentrations in which this micropollutant was detected in swimming pool water [6], of approximately $10^{-5}$ M. Specifically, the solutions of the sunscreen were treated for 10 min with 10% hypochlorite (OCT: hypochlorite molar ratio of 2:1; concn.), under magnetic stirring and at room temperature. Then, the tests were repeated at much higher concentrations of the contaminant (> $10^{-3}$ M), with a much lower ratio of OCT: oxidizing agent (1:20), in order to have sufficiently high quantities of byproducts isolated to proceed with the structural identification.

The course of the reaction was monitored by HPLC, and the DPs obtained were isolated according to Scheme 1, using column chromatography and HPLC and completely characterized using NMR and MS analyses. Finally, DP1–DP11 (Figure 1) were isolated at percentages of 1.12, 2.97, 0.89, 0.91, 1.15, 2.36, 1.08, 1.55, 6.39, 0.59, and 1.19, respectively. The proposed mechanism of their formation from OCT is shown in Figure 2, and DP3–DP5 and DP7–DP9 were isolated for the first time.
Scheme 1. The isolation of the degradation byproducts.

Figure 1. Chemical structures of octocrylene and its degradation byproducts DP1–DP11.
Figure 2. Plausible mechanism for the formation of DP1-DP11.
2.2. Structure Elucidation of Degradation Byproducts DP1–DP9

In the OCT treatment, the concentration of DP1–DP11 reached its maximum after about 2 h, with a degradation of 15% and a transformation of approximately 20%; percentages of byproducts ranged from 0.59% for DP10 to 6.39% for DP9. In a basic environment, OCT can undergo a retro-aldol condensation, which leads to the formation of intermediate I1 and the byproduct DP2. From this and other byproducts, it is usually possible to obtain the byproduct DP11; the intermediate I1, identifiable with 2-ethylhexyl cyanoacetate, can undergo hydrolysis of the ester bond and lead to the formation of the byproduct DP10 and α-cyanoacetic acid, probably contained in the aqueous phase rich in salts and low-molecular-weight compounds (Scheme 1). Finally, the DP10 could decarboxylate to the DP1 byproduct. The byproduct DP10 is also obtained from the hydrolysis of the ester bond of the starting product, together with the byproduct DP8. The latter, by decarboxylation, could provide the intermediate I2, which, by the hydrolysis of the cyano group, provides the intermediate I3. Considering the degradation reaction of OCT in the presence of sodium hypochlorite, it can be assumed that a Weerman degradation takes place that leads to the formation of nitrene I6, through the deprotonation (I4) and chlorination (I5) of the amide nitrogen of the intermediate I3.

The transposition of nitrene I6, or more probably the elimination of HCl from the intermediate I5 with the concomitant transposition of the residue bound to the carbonyl, allows for the obtainment of the isocyanate I7, from which alcoholises of the intermediate I8 are obtained, from which, for the subsequent oxidation, the intermediate I9 is obtained. The hydrolysis of the latter and the subsequent oxidation of the intermediate I10 obtained provides the byproduct DP7. This can react with the DP6 present in the solution and provide the byproduct DP3 and, for subsequent chlorination, create the byproduct DP4. The direct chlorination to the C-2/C-3 carbons of the starting product allows for and explains the obtainment of the byproduct DP5, from which DP9 and DP6 are obtained by the hydrolysis of the ester bond.

2.3. Spectral Data

Octocrylene: 2-Ethylhexyl 2-cyano-3,3-diphenylacrylate. Oily liquid. ¹H- and ¹³C-NMR, see Table S1. MS-TOF (positive ions): m/z calculated for C₂₄H₂₅NO₂ m/z 361.20 [M⁺]; found 362.47 [M + H⁺] (68%).

DP1: Heptane. Identified by comparison with an authentic sample.

DP2: Benzophenone. Identified by comparison with an authentic sample.

DP3: 2-Ethylhexyl 2,2-diphenylacetate. White powder. ¹H- and ¹³C-NMR, see Table S2. MS-TOF (positive ions): m/z calculated for C₂₂H₂₉O₂ m/z 324.21 [M⁺]; found 325.47 [M + H⁺].

DP4: 2-Ethylhexyl 2-chloro-2,2-diphenylacetate. White powder. ¹H- and ¹³C-NMR, see Table S3. MS-TOF (positive ions): m/z calculated for C₂₂H₂₇ClO₂ m/z 358.17 [M⁺]; found 361.82 [M + H⁺], 359.78 [M + H⁺].

DP5: (2S)-2-Ethylhexyl 2,3-dichloro-2-cyano-3,3-diphenylpropanoate. White powder. ¹H- and ¹³C-NMR, see Table S4. MS-TOF (positive ions): m/z calculated for C₂₄H₂₇Cl₂NO₂ m/z 431.14 [M⁺]; found 436.33 [M + H⁺], 435.36 [M + H⁺], 434.38 [M + H⁺], 433.35 [M + H⁺], 432.36 [M + H⁺].

DP6: 2-Ethylhexan-1-ol. Identified by comparison with an authentic sample.

DP7: 2,2-Diphenylacetic acid. White powder. ¹H- and ¹³C-NMR, see Table S5. MS-TOF (positive ions): m/z calculated for C₁₄H₁₂O₂ m/z 212.08 [M⁺]; found 213.27 [M + H⁺].

DP8: 2-Cyano-3,3-diphenylacrylic acid. White powder. ¹H- and ¹³C-NMR, see Table S6. MS-TOF (positive ions): m/z calculated for C₁₆H₁₁NO₂ m/z 249.08 [M⁺]; found 250.19 [M + H⁺].

DP9: (S)-2,3-dichloro-2-cyano-3,3-diphenylpropanoic acid. White powder. ¹H- and ¹³C-NMR, see Table S7. MS-TOF (positive ions): m/z calculated for C₁₆H₁₁Cl₂NO₂ m/z 319.02 [M⁺]; 324.18 [M + H⁺], 323.16 [M + H⁺], 322.19 [M + H⁺], 321.18 [M + H⁺], 320.21 [M + H⁺].

DP10: 2-ethylhexanoic acid. Identified by comparison with an authentic sample.
2.4. Toxicity Assessment

Octocrylene has a relatively high environmental stability in aquatic environments and is hardly removed from wastewater treatment plants [30,31]. Previous studies showed that OCT was poorly removed from wastewater treatment plants (0–10% degradation in aerobic conditions) [32].

Toxicity data were reported in Figure 3A–C for *P. tricornutum*, *B. plicatilis* and *A. fischeri*, in that order, considering the effect of OCT and its byproducts.

Figure 3. Toxicity data regarding exposure of *P. tricornutum* (A), *B. plicatilis* (B) and *A. fischeri* (C) to OCT and its byproducts (DP1-DP11). Data with different letters (a–d) are significantly different (Tukey post hoc, *p* < 0.05).

Chronic toxicity with *P. tricornutum* was also identified, as 83% of DPs showed growth inhibition effects ranging between 20% and 50% (OCT, DP1, DP3, DP4, DP6, DP7, DP8, DP9, DP10 and DP11). DP5 has no toxicity, while DP2 was the most toxic compound (Figure 3A).

The effects of this UV filter and its chlorinated derivatives on the acute toxicity of *B. plicatilis* change, probably due to the lower sensitivity of the bioindicator. The toxicity
has been evaluated by observing the mortality rate of *B. plicatilis* after 24 h of exposure. As reported in Figure 3B, no significant effect on mortality was observed in rotifers exposed to OCT and its degradation byproducts. In fact, all the investigated samples showed a toxicity ranging between 10% and 32%, with the exception of DP3, which had a residual toxicity of approximately 47%. In the latter case study, DP2, on the other hand, has a toxicity of only 20%, while DP3 appears to be the most toxic degradation byproduct for the aforementioned bioindicator.

The acute toxicity of OCT and its chlorinated derivatives towards *A. fischeri* was shown in Figure 3C. After 30 min of exposure, the bioluminescence inhibition swung between 14% and 90%, except for the parent compound OCT, which was the only compound to exhibit biostimulation behaviour. So, 16% of the tested samples did not exceed 20% of the effect (DP1 and DP3), 33% of the degradation byproducts are included in the range between 26% and 33% of the effect (DP5, DP9, DP10 and DP11), while there was another 33% increase in toxicity up to 60% of the effect (DP4, DP6, DP7 and DP8). DP2 (90% of the effect), once again, appears to be the most toxic product. The toxicity trend observed in *A. fischeri* is in good agreement with our previous results on *P. tricornutum*.

The discharge into the marine environment of chlorinated sewage effluents containing these degradation byproducts represents the worst-case scenario for environmental safety; indeed, in our study, these byproducts could have negatively influenced the physiology of single-celled organisms such as *A. fischeri* and *P. tricornutum*, but they did not affect rotifers. This study highlighted the concerns and the potential risks from OCT byproducts that may emerge and impact the quality of the marine ecosystem, especially concerning uncontrolled doses.

3. Materials and Methods

3.1. Drug and Reagents

Octocrylene (99%) was purchased from Sigma Aldrich (Milan, Italy). All of the other chemicals and solvents were purchased from Sigma Aldrich (Milan, Italy) and were of HPLC grade and used as received. All of the chemicals were of analytical grade and supplied by Sigma Aldrich.

The toxicity tests were conducted with two combinations. In the first combination, osmotic adjustment solution (OAS) (22 g L\(^{-1}\) NaCl) was used as a control for optimal conditions according to the ISO 11348-3 standard [33]. In the second combination, synthetic sea water was used as the control solution according to the ISO 10,253 standard [34,35]. The synthetic sea water used for analytical procedures comprised the following salts: NaCl (22 g L\(^{-1}\)), MgCl\(_2\)·6H\(_2\)O (9.7 g L\(^{-1}\)), Na\(_2\)SO\(_4\) (3.7 g L\(^{-1}\)), CaCl\(_2\) (1.0 g L\(^{-1}\)), KCl (0.65 g L\(^{-1}\)), NaHCO\(_3\) (0.2 g L\(^{-1}\)) and H\(_3\)BO\(_3\) (0.023 g L\(^{-1}\)).

3.2. Chlorination Reaction

3.2.1. Apparatus and Equipment

Column chromatography (CC) was carried out with Kieselgel 60 (230–400 mesh, Merck, Darmstadt, Germany). HPLC was performed on a Shimadzu LC-8A system using a Shimadzu SPD-10A VP UV-VIS detector (Shimadzu, Milan, Italy). Preparative HPLC was performed using an RP Gemini C18-110A preparative column (10 \(\mu\)m particle size, 250 mm × 21.20 mm i.d. Phenomenex, Bologna, Italy) with a flow rate of 8.0 mL/min. The \(^1\)H- and \(^13\)C-NMR spectra were recorded with an NMR spectrometer operated at 400 MHz and at 25 °C (Bruker DRX, Bruker Avance) and referenced in ppm to the residual solvent signals (CDCl\(_3\), at \(\delta_H\) 7.27 and \(\delta_C\) 77.0; and CD\(_2\)OD, at \(\delta_H\) 3.30 and \(\delta_C\) 49.0). The proton-detected heteronuclear correlations were measured using a gradient heteronuclear single-quantum coherence (HSQC) experiment, optimized for \(^1\)J\(_{HC}\) = 155 Hz, and a gradient heteronuclear multiple bond coherence (HMBC) experiment, optimized for \(^3\)J\(_{HC}\) = 8 Hz. The MALDI-TOF mass spectrometric analyses were performed on a Voyager-De Pro MALDI mass-spectrometer (PerSeptive Biosystems, Framingham, MA, USA). The samples were
lyophilized using a Lyovaport™-200 (Buchi, Cornaredo (MI), Italy), with a compressor with cooling capacity: 1.97 kW for 50 Hz and minimum condenser temperature: −55 °C.

3.2.2. Chlorination Experiments

A 10⁻⁵ M OCT solution was treated for 10 min with 10% hypochlorite (molar ratio OCT/HClO 2:1 concentration, spectroscopically determined λ max 292 nm, ε 350 dm³/mol cm) at room temperature [36]. The presence of OCT was quantified using a Lambda 12 UV-Vis spectrophotometer (Perkin Elmer, USA). Absorbance peaks were determined at 310 nm. The absorbance values were converted into a concentration using a calibration curve prepared from standard solutions with known OCT concentrations. DP1–DP11 were isolated from the methylene chloride extract of the aqueous solution (Scheme 1 and Figure 1) and identified by comparing their retention times with those of commercially available standard compounds, or isolated by performing preparative experiments with a solution of OCT at a concentration higher than 10⁻³ M and treated with 6% hypochlorite at room temperature for 2 h. The pH of the solution, measured and recorded continuously using a pH-meter, increased immediately from the initial pH of 8.0 to 10.8, and the pH remained at this value during the reaction. An aliquot of the solution was taken every 15 min, quenched by sodium thiosulphate excess, filtered, and dried by lyophilisation, and the residue was dissolved in a saturated sodium bicarbonate solution and extracted with ethyl acetate. The course of the reaction was monitored using HPLC. The DPs obtained were isolated using CC and HPLC and were completely characterized using NMR and MS analyses.

3.2.3. Chlorination Procedure and Product Isolation

Octocrylene (607 mg, 1.68 mmol) was dissolved in 22 mL of acetonitrile, and the solution was diluted with water until a final volume of 0.9 L was reached. A sodium hypochlorite solution (approximately 6% active chlorine, molar ratio OCT/HClO 1:20; concentration spectroscopically determined at λ max of 292 nm, ε 350 dm³/mol cm) was added drop by drop to this solution under magnetic stirring at room temperature. The reaction was stopped after 2 h with an excess of sodium thiosulphate and concentrated by lyophilisation. The reaction was stopped after 2 h with an excess of sodium thiosulphate and concentrated by lyophilisation. The residue was dissolved in water and pH-adjusted to 5.0, and this solution was extracted using methylene chloride. The crude organic fraction (835 mg) was chromatographed on silica gel CC, eluted with a gradient of chloroform:methanol (99:1 to 10:90, v/v) to yield 9 fractions. The fraction Fr. 2 (62 mg), eluted with chloroform:methanol (97:3), was chromatographed on silica gel CC, eluted with a gradient of petroleum ether:acetone (98:2 to 90:10, v/v) to yield DP1 (27 mg). The fraction Fr. 4 (49 mg), eluted with chloroform:methanol (90:10), was chromatographed on silica gel CC, eluted with a gradient of petroleum ether:acetone (90:10 to 50:50, v/v) to yield DP2 (29 mg). The fraction Fr. 7 (58 mg), eluted with chloroform:methanol (60:40), was separated by semipreparative HPLC using a reversed-phase column Phenomenex Gemini 10 µm 110 Å C18 (250 × 21.20 mm) and eluted with a gradient of CH₃COONH₄ (A, pH 4.0; 10 mM) and methanol (B), starting with 30% B for 5 min and followed by the installation of a gradient to obtain 100% B over 30 min, at a solvent flow rate of 8 mL/min to yield DP3 (7 mg) and DP4 (3 mg). The fraction Fr. 8 (405 mg), eluted with chloroform:methanol (80:20), was chromatographed on silica gel CC, eluted with a gradient of methylene chloride:methanol (90:10 to 0:100, v/v) to yield 9 fractions. The fraction Fr. 8.2 (61 mg), eluted with methylene chloride:methanol (90:10), was chromatographed on TLC, eluted with chloroform:methanol (80:20), to yield DP5 (47 mg). The fraction Fr. 8.4 (131 mg), eluted with methylene chloride:methanol (80:20), was chromatographed on TLC, eluted with chloroform:methanol (70:30), to yield DP9 (89 mg). The fraction Fr. 8.6 (43 mg), eluted with methylene chloride:methanol (65:35), was separated by analytical HPLC using a reversed-phase column Phenomenex Gemini 10 µm 110 Å C18 (250 × 21.20 mm) and eluted with a gradient of CH₃COONH₄ (A, pH 4.0; 10 mM) and methanol (B), starting with 30% B for 5 min and followed by the installation of a gradient...
to obtain 100% B over 30 min, and eluted again with the same mixture for another 10 min, at a solvent flow rate of 8 mL/min to yield DP8 (20 mg). The fraction Fr. 8.8 (53 mg), eluted with methylene chloride:methanol (60:40), was chromatographed on TLC and eluted with petroleum ether:acetone (65:35), to yield DP7 (20 mg).

The fraction Fr. 9 (31 mg), eluted with chloroform:methanol (90:10), was separated by analytical HPLC using a reversed-phase column Phenomenex Kromasil 10 µm 100 Å C18 (250 × 10.00 mm) and eluted with a gradient of CH$_3$COONH$_4$ (A, pH 4.0; 10 mM) and acetonitrile (B), starting with 20% B for 5 min and followed by the installation of a gradient to obtain 100% B over 30 min, at a solvent flow rate of 4 mL/min to give 3 fractions. The fraction 9.1 was purified by HPLC using a column Discovery RP-amide C16 (150 × 4.6 mm), 5 µm, and eluted with 0.1% TFA in acetonitrile:water (25:75), at a solvent flow rate of 0.8 mL/min to give DP11 (2 mg). The fractions Fr. 9.2 and Fr. 9.3 were identified as DP6 (5 mg) and DP10 (2 mg), respectively.

3.3. Ecotoxicity Data

The toxicity of OCT and its degradation byproducts was assessed regarding the following organisms: A. fischeri, P. tricornutum and B. plicatilis. A Microtox® acute ecotoxicity test was performed using the marine bioluminescent bacteria A. fischeri (NRRL-B-11177) to assess the toxicity of OCT and DPs. The bacteria were supplied in a freeze-dried form by Aqua Science LLC (Newark, Delaware, USA) and were stored at −20 °C to preserve their microbial activity. The acute toxicity endpoint was determined after 30 min of exposure according to ISO 11348-3 [33].

An algal growth-inhibition test was performed using benthic diatom P. tricornutum. The algal culture was kept at 20 ± 2 °C and 6000–10,000 Lux light, to obtain a cellular density of 10$^6$ cells/mL. Inocula were taken from pre-cultures set up three days before the experiment to adjust the initial cell density to approximately 10$^4$ cells/mL [35]. The test was carried out and miniaturized for 24-well sterile polystyrene micro-plates. The growth inhibition rate was calculated after 72 h exposure using a UV–Vis spectrophotometer (Hach Lange DR5000) and a 5 cm cuvette.

The acute toxicity test with estuarine rotifer B. plicatilis was performed according to the standard procedure of Rotoxkit M® using certified dehydrated cysts (MicroBioTests Inc.). The test was conducted in multiwell plates with 300 µL per well. Six wells with ten rotifers each were filled to assess the toxicity of the parent compound and of its 11 DPs. Incubation was carried out for 48 h, at 25 °C, in darkness. The number of dead rotifers after the exposure period was observed under a stereomicroscope (LEICA EZ4-HD). The significance of the differences between the mean values of the different tests and controls was verified using Addinsoft XLSTAT (2016.02.27444 Version) by analysis of variance (ANOVA) with a 0.05 significance level. In addition, the post-hoc analyses were carried out with Tukey’s test.

4. Conclusions

This paper investigated the fate, following degradation treatment by chlorination, of one of the most widely used sunscreens, namely, octocrylene, conventionally considered an emerging micropollutant. The reaction was carried out by simulating the disinfection treatment employed in swimming pool waters, using excess sodium hypochlorite. After the chlorination treatment, chromatographic techniques were used to isolate eleven degradation byproducts, which were fully characterized by MS and NMR analyses and via comparison with a commercial standard. Four of them were isolated for the first time. Compared to the initial quantity considered, OCT was recovered unchanged for 45% and transformed into the corresponding byproducts for 20%. A possible mechanism for the degradation of OCT and its degradation byproducts has been hypothesized. Half of the investigated DPs possessed anywhere from slightly to highly toxic effects. Thus, acute toxicity evaluation demonstrated that the presence of OCT in the water distribution system might pose a more significant threat to safety and quality of the water and the environment.
In fact, if on the one hand the disinfection process involves the partial degradation of OCT, it is also true, however, that it involves the formation of degradation byproducts, which are in some cases even more toxic than the starting product.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules27165286/s1, Table S1: 1H, 13C and 2D NMR data of octocrylene in CDCl3; Table S2: 1H, 13C and 2D NMR data of DP2 in CDCl3; Table S3: 1H, 13C and 2D NMR data of DP3 in CDCl3; Table S4: 1H, 13C and 2D NMR data of DP4 in CDCl3; Table S5: 1H, 13C and 2D NMR data of DP5 in CDCl3; Table S6: 1H, 13C and 2D NMR data of DP7 in CDCl3; Table S7: 1H, 13C and 2D NMR data of DP8 in CDCl3; Table S8: 1H, 13C and 2D NMR data of DP9 in CDCl3.

**Author Contributions:** A.M. performed the chlorination experiments; A.S., L.S., G.L. and M.G. performed the acute and chronic toxicity tests; L.P. and G.D.F. performed supervision and writing—review and editing; and A.Z. designed the research study, performed supervision and writing—original draft preparation and and wrote the last version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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**Sample Availability:** Samples of the compounds are not available from the authors.

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