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Evane Thorel, Fanny Clergeaud, Alice M S Rodrigues, Philippe Lebaron, Didier Stien. A comparative metabolomics approach demonstrates that octocrylene accumulates in *Stylophora pistillata* tissues as derivatives and that octocrylene exposure induces mitochondrial dysfunction and cell senescence. *Chemical Research in Toxicology*, In press, 35, pp.2160-2167. 10.1021/acs.chemrestox.2c00248 . hal-03833207

HAL Id: hal-03833207

<https://hal.science/hal-03833207>

Submitted on 28 Oct 2022

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A comparative metabolomics approach demonstrates that octocrylene accumulates in *Stylophora pistillata* tissues as derivatives and that octocrylene exposure induces mitochondrial dysfunction and cell senescence

Evane Thorel¹, Fanny Clergeaud¹, Alice M. S. Rodrigues^{1,2}, Philippe Lebaron¹, Didier Stien^{1,*}

¹Sorbonne Université, CNRS, Laboratoire de Biodiversité et Biotechnologie Microbienne, UAR3579, Observatoire Océanologique, 66650 Banyuls-sur-Mer, France

²Sorbonne Université, CNRS, Fédération de Recherche, Observatoire Océanologique, FR3724, 66650 Banyuls-sur-Mer, France

* didier.stien@cnrs.fr

ABSTRACT

The objective of this work was to study the effect of octocrylene on *Stylophora pistillata* and to compare the metabolomic response of this coral species to that obtained with *Pocillopora damicornis*. When *S. pistillata* coral was exposed to octocrylene, it accumulated octocrylene derivatives similar to *P. damicornis*. Octocrylene-fatty acid conjugates were found, as well as octocrylene heterosides. Furthermore, the tissue concentrations of various acylcarnitines and three sphingoid bases increased significantly. This phenomenon was indicative of mitochondrial dysfunction and the induction of cellular senescence processes in exposed corals. Overall, the responses of the two corals to octocrylene pollution were consistent. The proven impact of octocrylene on a second coral species suggests that potential environmental octocrylene pollution could impact many reef-building species. Furthermore, this work demonstrates that octocrylene may be modified *in vivo* by many organisms and that levels of octocrylene contamination in the food chain have probably been underestimated until now. Massive dataset identifier: MSV000089720.

KEYWORDS

Octocrylene, Coral, *Stylophora pistillata*, *Pocillopora damicornis*, Acylcarnitines, Cellular senescence, Metabolomics

INTRODUCTION

Organic ultraviolet (UV) filters are compounds used, in particular, in personal care products to provide sun protection. Nevertheless, some of these compounds are criticized because of their potential impact on humans and the environment. In humans, it has been shown that lower-molecular weight organic UV filters (< 500 Da) can pass through the skin barrier and may induce a risk for users, a risk that is currently poorly documented.¹ For example, Bury *et al.* demonstrated that octocrylene (OC), ethylhexyl salicylate and homosalate can be transferred to the bloodstream following topical application and that these xenobiotics are metabolized *in vivo*.²⁻⁵ The possibility of these metabolites having an effect in the body is not yet known. In addition, we and others demonstrated that octocrylene is not stable in cosmetic formulations and that its hydrolysis product, benzophenone, should be considered an additional risk factor associated with octocrylene use in cosmetics.⁶⁻⁸ As a precautionary measure, the FDA has recently classified all organic UV filters on the US market as non-GRASE (Generally recognized as safe and effective) pending further investigation.⁹

In the environment, the impact of octocrylene is somewhat unclear, on the one hand, because the results for a species in a laboratory test are not easily transferable to the same species in its natural environment. On the other hand, a single species is not always representative of complex environments where the surrounding taxonomic diversity can be very high.¹⁰ In addition, it is sometimes difficult to assess the concentrations of xenobiotics in the environment, especially since a complete picture of environmental contamination by newly emerging pollutants would require considerable resources. A reasonable approach is to concentrate investigative efforts in relevant places. As far as UV filters are concerned, it is

important to monitor and evaluate their impact on bathing sites since bathing is one of the major causes of contamination of aquatic environments by UV filters. For example, we showed that localized concentrations of benzophenone-3 (or oxybenzone) endangered organisms living in Hanauma Bay and that the main sources of benzophenone-3 were bathers and beach showers from which large quantities of UV filters—including octocrylene—leach into the sea.^{11,12} This situation in Hanauma Bay is not unique, as water column concentrations of octocrylene greater than 1 µg/L and up to 200 µg/L have been described in various bathing areas around the world.^{13–15} Octocrylene, like many other UV filters, is extremely lipophilic and octocrylene concentration can be even higher in sediments, for which contamination data are more sparse. Nonetheless, concentrations of 100 µg/kg to over 1,000 µg/kg are frequently measured.^{16–18}

For the same reason, it is to be expected that these organic compounds may also accumulate in the food chain, which appears to be the case for benzophenone-3 and octocrylene, especially considering the existence of derivatives resulting from the transformation of octocrylene in marine organisms.¹⁹ In the natural environment, Mitchelmore *et al.* measured water column concentrations of octocrylene below 10 ng/L at a Waikiki beach site for concentrations in coral collected from the same site ranging from 50 to 800 µg/kg, suggesting a bioaccumulation process in wild coral.²⁰ In this work, the contribution of octocrylene-fatty acid conjugates was not measured. In addition to coral, octocrylene has also been detected in significant concentrations in many marine organisms ranging from filtering organisms such as mussels to dolphins.^{21–23} Finally, octocrylene was also detected in human breast milk when mothers used octocrylene-containing cosmetics.²⁴

This is of particular concern, as octocrylene transformation processes may be ubiquitous in the animal kingdom.^{2,3,19}

In our previous work, we demonstrated that when the coral *Pocillopora damicornis* was exposed to octocrylene, the tissue concentration of 15 acylcarnitines increased drastically.^{25,26} The elevation of acylcarnitines in the treated coral suggested that there may be abnormalities in fatty acid metabolism. Acylcarnitines are common biomarkers of cell toxicity, and in many models, including human pathologies, elevated acylcarnitine concentrations have been correlated with mitochondrial dysfunction in which mitochondrial fatty acid β -oxidation and cell bioenergetics were affected.²⁷⁻³⁰ In our experiment, *P. damicornis* metabolome was significantly impacted by octocrylene after only 7 days of exposure to 50 $\mu\text{g/L}$ octocrylene. The highest nontoxic octocrylene concentration (no observed effect concentration, or NOEC) in our assay was 5 $\mu\text{g/L}$, although at this concentration, octocrylene derivatives accumulated in coral. Environmental concentrations higher than 50 $\mu\text{g/L}$ have been previously reported.³¹ Corals are subjected to long-term exposure (from several weeks to months per year) at high octocrylene concentrations due to the continuous release of octocrylene into the environment and because octocrylene and octocrylene derivatives accumulate in coral, as demonstrated by us, Mitchelmore *et al.*, and Tsui *et al.*^{20,23,25} Thus, we are convinced that octocrylene is among the major causes of coral depletion in anthropized and bathing areas. That said, given the species diversity of coral reefs, it is useful to measure the effect of octocrylene on another reef-building species. *Stylophora pistillata* is a scleratinian coral of the family Pocilloporidae that is widely distributed in the tropical and subtropical areas of the Indo-Pacific. Its range extends from Madagascar and the Red Sea in the east to the islands of the western Pacific. The species is

maintained at the research aquarium of the Oceanological Observatory of Banyuls-sur-Mer and has been proven to be perfectly adapted to the conditions of the test developed with *P. damicornis*, in which individual suspended coral nubbins are maintained in 200 mL artificial seawater and exposed to a pollutant for one week. We therefore evaluated the effect of octocrylene exposure on *S. pistillata* using the metabolomic profiling approach developed with *P. damicornis*.

MATERIALS AND METHODS

***Stylophora pistillata*.** Samples of the coral *S. pistillata* were obtained from the Canet-en-Roussillon aquarium “Oniria” and were acclimated for more than two years in tanks at the Banyuls Oceanological Observatory. Corals were maintained in artificial seawater (ASW) prepared with reverse osmosis purified water and Tropic Marin Pro-Reef salts. Salinity was adjusted to 36 g/L, the pH was 8.0, and the temperature was set at 24 °C. All experiments were conducted with the same ASW.

Exposure to octocrylene, extraction, metabolomic profiling and analyses. The method of exposure and analysis was described in detail in our previous publications, and is provided in Supporting Information.^{25,26} Briefly, coral nubbins of *S. pistillata* (n = 5) were exposed to 300 and 1000 µg/L octocrylene. After exposition, corals were extracted with methanol/acetonitrile 1:1. Extracts were collected by filtration on cotton, concentrated to dryness and dissolved again in the same solvent at 1 mg/mL. Metabolomic profiles were recorded by UHPLC-Orbitrap-MS (2 µL injected, 2 µg on column). Compound Discoverer software was then used to compare profiles of exposed corals with those of control corals (n

= 5) exposed to only DMSO (0.25% v/v), which we previously showed had no effect on the coral. In the presence of DMSO, corals were healthy after 7 days of exposure, and no difference was detectable in the metabolomic profiles compared to corals in ASW only. Marker metabolites were annotated manually, optionally with the help of Sirius software.³²

RESULTS AND DISCUSSION

The purpose of this work was to study the effect of octocrylene on the coral species *S. pistillata* and to investigate whether the metabolomic response of this second species was comparable to that obtained with *P. damicornis*.²⁵ The coral *S. pistillata* was exposed to octocrylene concentrations of 300 and 1000 µg/L for 7 days with water renewal every 24 h to test for possible accumulation of octocrylene derivatives and to ascertain whether octocrylene could impact the health of the coral by disrupting the metabolism of fatty acids, as observed previously in *P. damicornis*.

Octocrylene derivatives in coral *S. pistillata* exposed to octocrylene (Figures S1-S45). When the species *S. pistillata* was exposed to octocrylene, we established accumulation of octocrylene derivatives. First, eight compounds could be easily annotated either because they were identical to those previously annotated²⁵ or because they were close analogs. These are compounds **1–8**, for which various fatty acids have been grafted onto the octocrylene ethylhexyl chain (Figure 1, Table 1). In mass spectrometry, the ammoniated molecular ions of these compounds fragmented to yield a first daughter ion at m/z 360.1958 corresponding to a protonated dehydro-octocrylene (Figures S1-S2). The location of the double bond on the ethylhexyl chain and, thus, the modification of the ethylhexyl chain by

grafting a fatty acid could then be confirmed by the presence of a second daughter ion at m/z 250.0863 corresponding to the protonated 2-cyano-3,3-diphenylacrylic acid, the fragmentation pattern of which then matched that recorded for octocrylene. This led to the conclusion that the 2-cyano-3,3-diphenylacrylic acid subunit was not modified. It was thus possible to demonstrate that 8 octocrylene derivatives linked to C16:0 and 1, C18:0 and 1, and C22:6 fatty acids were present in coral *S. pistillata* exposed to octocrylene. This result is broadly similar to that obtained by analysis of octocrylene-exposed *P. damicornis*, with apparently slightly more fatty acid diversity detected in *P. damicornis*.

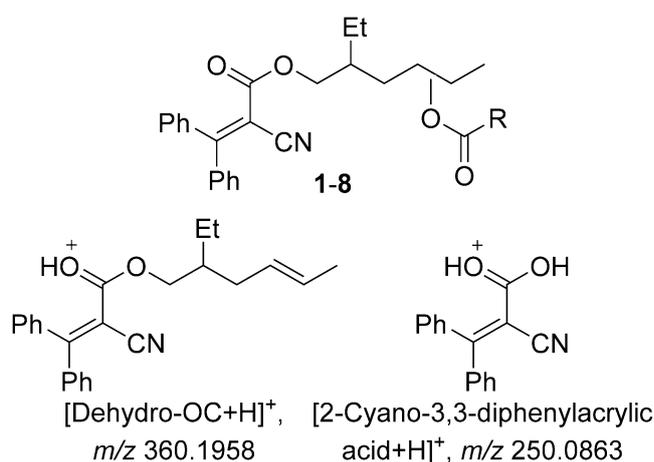


Figure 1. General structure of compounds **1-8** and key daughter ions in collision-induced dissociation spectra of the ammoniated molecular ions

#	t_R (min)	m/z	Ion type ^a	Molecular formula ^b	Annotation ^b	Relative integration compared to [OC+Na] ⁺ ion peak area
1	14.42	710.4182	[M+Na] ⁺	C ₄₆ H ₅₇ NO ₄	C22:6-OC	~10
2	14.49	710.4182	[M+Na] ⁺	C ₄₆ H ₅₇ NO ₄	C22:6-OC*	~10
3	14.65	710.4181	[M+Na] ⁺	C ₄₆ H ₅₇ NO ₄	C22:6-OC	~10
4	15.21	636.4023	[M+Na] ⁺	C ₄₀ H ₅₇ NO ₄	C16:1-OC	~10
5	15.95	638.4181	[M+Na] ⁺	C ₄₀ H ₅₇ NO ₄	C16:0-OC	~100
6	16.02	664.4335	[M+Na] ⁺	C ₄₂ H ₅₉ NO ₄	C18:1-OC	~10
7	16.23	638.4181	[M+Na] ⁺	C ₄₀ H ₅₇ NO ₄	C16:0-OC	~40
8	16.29	664.4337	[M+Na] ⁺	C ₄₂ H ₅₉ NO ₄	C18:1-OC*	~10
9	6.34	562.2410	[M+Na] ⁺	C ₃₀ H ₃₇ NO ₈	C ₆ H ₁₂ O ₆ -OC*	~10
10	6.87	662.2573	[M+Na] ⁺	C ₃₄ H ₄₁ NO ₁₁	C ₁₀ H ₁₅ O ₉ -OC*	~10
11	6.96	662.2574	[M+Na] ⁺	C ₃₄ H ₄₁ NO ₁₁	C ₁₀ H ₁₅ O ₉ -OC*	~10
12	15.52	640.4337	[M+Na] ⁺	C ₄₀ H ₅₉ NO ₄	C16:0-dihydroOC*	~10
13	9.80	431.1980	Frag.	Unk.	Unk.*	~10

^aFrag.: Fragment

^bUnk.: Unknown

*Not previously detected in *P. damicornis*.

Table 1. Analytical data of octocrylene transformation products formed upon exposure of the coral *S. pistillata* to octocrylene

That being said, other octocrylene derivatives that were not detected in *P. damicornis* were found in *S. pistillata* exposed to octocrylene and could be annotated. Three of these derivatives also resulted from the functionalization of the octocrylene ethylhexyl chain. They were therefore structurally comparable to octocrylene-fatty acid conjugates. For compound **9**, for example, it was possible to show that the ion at m/z 562.2410 was a sodium adduct of the empirical formula C₃₀H₃₇NO₈Na⁺ (calcd. 562.2411), corresponding to a compound with 13 degrees of unsaturation. In the MS² spectrum of the ammonium adduct (exp. m/z 557.2858), the presence of the first fragmentation product at m/z 360.1963 corresponding to octocrylene possessing a double bond on its ethylhexyl chain clearly indicated that this compound was an octocrylene derivative functionalized on the ethylhexyl chain with a group lost by β -elimination (Figure S34). The same ions as those formed upon

[OC+NH₄]⁺ fragmentation were found at *m/z* 250.0867 and 232.0759 in the MS² spectrum of compound **9**. The ion at *m/z* 360.1963 was produced by the successive loss of ammonia and a neutral molecule, which was attributable to a hexose (C₆H₁₂O₆). An ion with the formula C₁₄H₂₆O₆Na⁺ (exp. *m/z* 313.1624) was also observed among the daughter ions in the MS² spectrum of [9+Na]⁺ corresponding to the glycosylated ethylhexyl chain that lost the 2-cyano-3,3-diphenylacrylic acid by β-elimination. Thus, compound **9** could be annotated as an octocrylene heteroside without further determination of the sugar configuration or its exact position on the ethylhexyl chain, as in the case of fatty acid conjugates. Compounds **10** and **11** were similarly shown to be octocrylene heterosides, in which a subunit with the gross formula C₁₀H₁₅O₉⁻ was grafted onto the ethylhexyl chain. It was not possible to determine the structure of this substituent more precisely.

Compound **12** was another octocrylene derivative specific to *S. pistillata*. Its molecular formula C₄₀H₅₉NO₄ was deduced from the exact mass of the sodium adduct at *m/z* 640.4337 (calcd. for C₄₀H₅₉NO₄Na⁺ 640.4336). In the MS² spectrum of the sodium adduct, the first neutral loss corresponded to the loss of a palmitic acid molecule (C₁₆:0), yielding the ion at *m/z* 384.1934 (Figure 2). This product then lost a C₈H₁₄ chain corresponding to the ethylhexyl chain possessing one more double bond resulting from the loss of palmitic acid by β-elimination, demonstrating that palmitic acid was indeed attached to the ethylhexyl chain, as with the other octocrylene derivatives. In contrast, the product ion at *m/z* 274.0841 was a sodium adduct of monohydrogenated octocrylene. The hydrogenation of the central double bond within the 2-cyano-3,3-diphenylacrylic acid substructure could be further confirmed by the formation of the fragmentation product at *m/z* 167.0860 corresponding to the diphenylmethylium cation. This product cannot be formed by

fragmentation of the $[\text{OC}+\text{Na}]^+$ ion when the central double bond is present. Taken together, these observations allowed Compound **12** to be annotated as C16:0-dihydroOC.

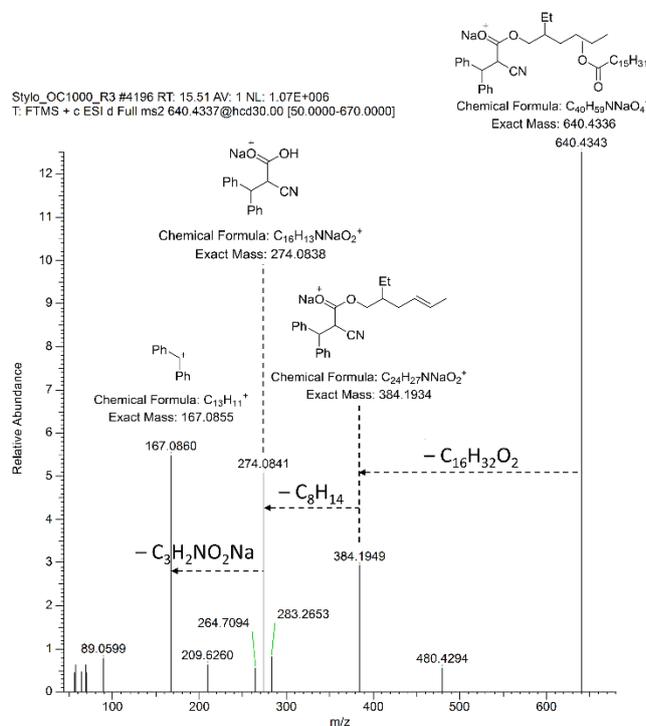


Figure 2. Collision-induced dissociation spectrum of compound **12** sodium adduct at m/z 640.4337, and annotation of the main daughter ions and neutral losses.

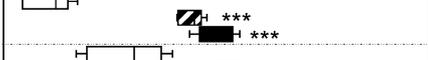
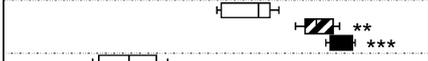
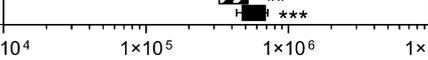
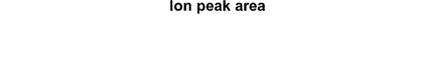
Finally, a compound represented in the differential metabolomic analysis by a dicharged ion at m/z 431.1980 could not be annotated. This ion might be a fragment of a high-molecular weight compound. However, the fragment at m/z 232.0768 that should correspond to the acylium cation produced from the fragmentation of an octocrylene derivative was found in the MS^2 spectrum of the ion at m/z 431.1980, indicating that compound **13** was probably also an octocrylene derivative (Figure S49).

Although some octocrylene derivatives were species-specific, a pool of octocrylene-fatty acid conjugates common or similar to those already characterized in *P. damicornis* exposed to octocrylene was found in *S. pistillata*. We measured the relative integration of the ions listed in Table 1 for compounds **1–13** and compared their peak areas with the peak area of $[\text{OC}+\text{Na}]^+$; the area of the octocrylene derivative sodium adducts was 1.5 to 90 larger than the area of the octocrylene sodium adduct (Table 1). The relative peak area of the ion $[\text{5}+\text{Na}]^+$ was nearly 100 times larger than that of $[\text{OC}+\text{Na}]^+$, indicating that the contribution of octocrylene derivatives in general and compound **5** in particular should not be neglected in studies aimed at estimating the degree of octocrylene contamination in wild corals. The fact that identical and structurally related compounds have been described in the marine worm *Hediste diversicolor* and in humans suggests that the mechanism by which octocrylene is transformed, in this case by coral holobiont, may be widespread in the living world and that contamination rates of living organisms, from coral to dolphins and humans, may have been largely underestimated until now.^{2,3,19}

Increased acylcarnitine concentrations in coral *S. pistillata* exposed to octocrylene. One of the key objectives of this work was to examine the effect of octocrylene contamination on acylcarnitine concentrations in the coral *S. pistillata*. Indeed, when *P. damicornis* was exposed to octocrylene, it was shown that the relative concentration of 16 acylcarnitines increased significantly, with a concentration as low as 50 $\mu\text{g}/\text{L}$ octocrylene in sea water.²⁵ Similarly, comparative metabolomic analysis showed that the relative concentration of 8 acylcarnitines increased significantly when *S. pistillata* was exposed to 300 or 1000 $\mu\text{g}/\text{L}$ octocrylene (Table 2). The observed fragmentation pattern characteristic of acylcarnitines enabled facile annotation of these features. In particular, the loss of trimethylamine from

the protonated molecular ion and formation of two products at m/z 85.0284 and 60.0808 corresponding to the carboxyallylium and trimethylammonium cations, respectively, were observed (Figures S50–S77). In addition, the fatty acid side chain was also observed in the fragmentation pattern, both by the loss of a neutral fragment by β -elimination from the protonated molecular ion and by the presence of the fatty acid-derived acylium cation. Eventually, the annotation of compounds **14–16** and **19** could be corroborated by analysis of commercial standards.

Table 2. Analytical data for acylcarnitines, whose concentration increased upon exposure of *S. pistillata* coral to octocrylene. ** $p < 0.01$, *** $p < 0.001$.

#	t_R (min)	m/z	Ion type	Molecular formula	Annotation	Protonated mole- cular ion peak area (Log ₁₀ scale)	Legend
14	4.16	232.1542	[M+H] ⁺	C ₁₁ H ₂₁ NO ₄	C4:0-Carnitine		□ DMSO ▨ OC300 ■ OC1000
15	4.79	260.1856	[M+H] ⁺	C ₁₃ H ₂₅ NO ₄	C6:0-Carnitine		
16	6.51	372.3109	[M+H] ⁺	C ₂₁ H ₄₁ NO ₄	C14:0-Carnitine		
17	6.59	446.3264	[M+H] ⁺	C ₂₇ H ₄₃ NO ₄	C20:5-Carnitine		
18	6.67	398.3264	[M+H] ⁺	C ₂₃ H ₄₃ NO ₄	C16:1-Carnitine		
19	7.03	400.3422	[M+H] ⁺	C ₂₃ H ₄₅ NO ₄	C16:0-Carnitine		
20	7.17	426.3577	[M+H] ⁺	C ₂₅ H ₄₇ NO ₄	C18:1-Carnitine		
21	7.60	428.3735	[M+H] ⁺	C ₂₅ H ₄₉ NO ₄	C18:0-Carnitine		

Palmitoylcarnitine (**19**) was the major analog detected. This is consistent with the fact that C16:0-OC was also the major octocrylene-fatty acid conjugate. The relative concentrations of acylcarnitines increased on average by a factor of 4 at 300 $\mu\text{g/L}$ octocrylene and by a factor of 6 at 1000 $\mu\text{g/L}$ octocrylene (Table S1). This increase was always statistically

significant. As in the case of the coral species *P. damicornis* exposed to octocrylene, the large and significant increase in relative acylcarnitine concentrations in *S. pistillata* suggests an abnormal metabolism of fatty acids. In many pathologies, an elevated relative concentration of acylcarnitines has been correlated with mitochondrial dysfunction, in which mitochondrial β -oxidation of fatty acids and cellular bioenergetics are affected.^{27–30} It can be concluded from this experiment that the responses of the two corals studied are analogous, indicating that octocrylene can presumably impact several coral reefs-building species.

Other metabolites that increase in concentration upon exposure to octocrylene. A number of other holobiont metabolite concentrations also increased upon exposure of *S. pistillata* to octocrylene (Table 3). Compounds **22** and **23** were annotated as lysophosphatidylethanolamines based on their molecular formula and fragmentation spectra (Figures S78–S84). The structure of compound **23** could be confirmed by comparison with an analytical standard. These two compounds were described in our previous article as markers of inflammation in coral, as they are produced from phosphatidylethanolamines by hydrolysis of ω 3-polyunsaturated fatty acids initially attached at the *sn*-2 position, which then participate in the synthesis of anti-inflammatory eicosanoids.²⁶

Table 3. Other coral metabolites that increased in concentration upon exposure of *S. pistillata* to octocrylene.

# ^a	t _R (min)	m/z	Ion type	Molecular formula	Annotation ^b	Cmpd. class ^b
22	7.52	438.2979	[M+H] ⁺	C ₂₁ H ₄₄ NO ₆ P	1- <i>O</i> -Hexadec-1'-enyl- <i>sn</i> -glycero-3-PE	LPE
23	8.12	482.3243	[M+H] ⁺	C ₂₃ H ₄₈ NO ₇ P	1- <i>O</i> -Octadecanoyl- <i>sn</i> -glycero-3-PE	LPE
24	7.07	316.3211	[M+H] ⁺	C ₁₉ H ₄₁ NO ₂	2-Amino-1,3-nonadecanediol	SB
25	7.65	344.3525	[M+H] ⁺	C ₂₁ H ₄₅ NO ₂	2-Amino-1,3-heneicosanediol	SB
26	7.98	358.3681	[M+H] ⁺	C ₂₂ H ₄₇ NO ₂	2-Amino-1,3-docosanediol	SB
27	15.72	917.5787	[M+H] ⁺	C ₅₆ H ₇₆ N ₄ O ₇	10 <i>R</i> -(methyldioxy)-phaeophytin a	Phaeophytin
28	9.26	598.5043	[M+H] ⁺	C ₃₅ H ₆₇ NO ₆	Unk.	Unk.
29	9.91	626.5357	[M+H] ⁺	C ₃₇ H ₇₁ NO ₆	Unk.	Unk.

^aConcentration increase was significant ($p < 0.05$) for all compounds (**22–29**) at both 300 and 1000 µg/L octocrylene. See Table S2.

^bUnk.: Unknown; PE: phosphoethanolamine; LPE: lysophosphatidylethanolamine; SB: sphingoid base.

Compounds **24–26** were annotated as sphingoid bases of varying length (Figures S85–S93).

For example, **24** was characterized by a protonated molecular ion at m/z 316.3211

corresponding to the formula C₁₉H₄₂NO₂⁺ (calcd. 316.3210). This compound had no degree

of unsaturation, which narrowed the possible structures. The only two known natural

compounds with this formula are 2-amino-1,3-nonadecanediol (or C19-dihydrosphingosine

or d19:0) and a branched analog, 2-amino-16-methyl-1,3-octadecanediol (or ai-d19:0). In

the protonated molecular ion fragmentation spectrum, a C₃H₁₀NO₂⁺ ion and its dehydrated analogue were present at m/z 92.0707 (calcd. 92.0706) and m/z 74.0601 (100%, calcd.

74.0600), respectively, supporting the annotation of **24** as one of two known natural

sphingoid bases. Compound d19:0 has been described in mammalian cell lines or as

sphingolipid constituents of the parasites *Schistosoma mansoni* and *Metroliasthes*

coturnix.^{33–36} The branched isomer ai-d19:0 has been described as a constituent of

sphingolipids from the snail *Euhadra hickonis* and the guinea pig Harder's gland.^{37,38} There

was no clear taxonomic indication in favor of either isomer, which was acceptable in the

context of our study. The choice to annotate **24** as the linear isomer was arbitrary.

Regardless of the exact structure of these 3 sphingoid bases, it is especially noteworthy that their relative concentrations are 3 to 16 times higher in *S. pistillata* exposed to octocrylene, even at 300 µg/L octocrylene. Furthermore, note that sphingoid bases **24** and **25** were present in *P. damicornis* and that their relative concentrations also increased 2-3 times upon octocrylene exposure.

Converging evidence has shown that sphingolipids are involved in the regulation of apoptosis.³⁹ In addition to their function as energy sources, mitochondria also play a key role in many other cellular processes, such as cell cycle control, apoptosis and regulation of cellular metabolism. As such, possible mitochondrial dysfunction can be considered a feature of cellular senescence.^{40,41} The high concentrations of sphingoid bases in both coral species exposed to octocrylene indicate that this xenobiotic might have stimulated cellular senescence. The increase in the relative concentration of various acylcarnitines and the three sphingoid bases was therefore most likely correlative and may indicate the initiation of cellular senescence processes in octocrylene-exposed corals.

Finally, the last compound (**27**) was annotated putatively as 10*R*-(methyldioxy)-phaeophytin a (Figures S94–S97). The formula of this compound was C₅₆H₇₆N₄O₇, as demonstrated by the mass-to-charge ratio of the protonated molecular ion at *m/z* 917.5787 (calcd. 917.5787).

This annotation was based on the most likely proposal according to SIRIUS with CSI:FingerID software.^{32,42} Annotation as a pheophytin derivative was supported by the characteristic fragmentation of the phytol chain resulting in a neutral loss of 278.2974 Da in MS² (Figure S96). This compound was more than 10 times more concentrated in the exposed coral

extracts. 10R-(Methyldioxy)-phaeophytin a is a peroxidized form of pheophytin a, but the functional significance for accumulation of this compound is unclear based on current knowledge in the literature.

Conclusion

Overall, this work demonstrates that the response of coral *S. pistillata* exposed to octocrylene is similar to that previously reported for *P. damicornis*. In both species, it has been shown that exposure to octocrylene results in the accumulation of octocrylene derivatives, and triggers an increase in the concentration of several metabolites which are indicative of mitochondrial dysfunction and the induction of cellular senescence.

Several recent studies, including ours, have shown that, for various reasons, octocrylene is harmful to health and the environment.^{6-8,25,43-47} It seems that many cosmetic companies are now formulating their products without octocrylene for the benefit of their customers and the preservation of coral reefs. The proven impact of octocrylene on a second coral species *S. pistillata* suggests that potential environmental pollution with octocrylene can impact many reef-building species. Furthermore, this work demonstrates that levels of octocrylene contamination in the food chain from basal organisms to large predators and humans have probably been underestimated until now due to the variety of octocrylene derivatives that accumulate in corals in response to octocrylene exposure. C16:0-OC was the major compound in both corals, and the quantification of this compound could be a good proxy to estimate the rate of octocrylene transformation and the actual degree of contamination of corals in natural environments. Finally, there is no standardized test to

date for assessing xenobiotic toxicity to corals. The metabolomic approach developed by our group is robust while current simpler approaches are reductive. For example, the measurement of bleaching considers only one aspect of toxicity which is the disruption of the coral–Symbiodiniaceae symbiotic relationship. Our group has shown that when corals are exposed to octocrylene for one week, octocrylene does not cause bleaching, whereas significant deleterious effects, not on the symbiont but on the coral animal itself, were recorded. We therefore consider it crucial to develop new, simple, nonreductive approaches to assess xenobiotic toxicity to coral. These new approaches must propose solutions to evaluate not only water-soluble compounds but also lipophilic compounds, as a large part of UV filters are almost insoluble in water. The experimental protocol developed in our work is designed to address this issue. However, indicators that are easily accessible to laboratories accredited in the context of national and international regulations and that would perfectly correlate with stress markers detected today by metabolomic analysis remain to be discovered.

Associated Content

Supporting Information

The Supporting Information is available free of charge at

Detailed experimental section, relative concentration data for all acylcarnitines and other coral metabolites, general fragmentation scheme of compounds 1-8 ammoniated and sodiated adducts, proposed structures (if any) and MS and MS² spectra for all compounds described in the present article.

Author Contributions

F.C., E.T., A.M.S.R, D.S.: investigation, review, and editing; D.S.: formal analysis, writing of the original draft, and editing; and P.L., D.S.: conceptualization, editing. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Acknowledgments

This work benefited from a grant from the Agence Nationale de la Recherche (ANR) (SPOC, ANR-21-CE34-0027-01).

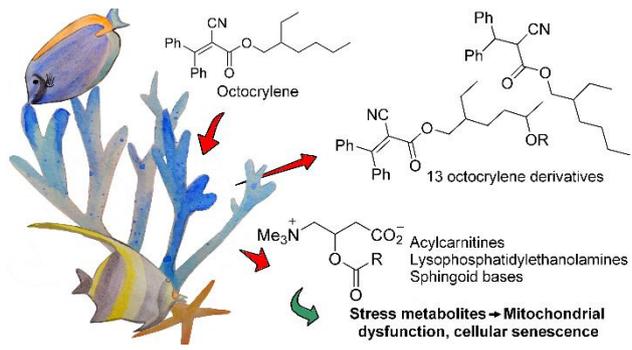
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