

ECOTOXICOLOGY

Conversion of oxybenzone sunscreen to phototoxic glucoside conjugates by sea anemones and corals

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The reported toxicity of oxybenzone-based sunscreens to corals has raised concerns about the impacts of ecotourist-shed sunscreens on corals already weakened by global stressors. However, oxybenzone's toxicity mechanism(s) are not understood, hampering development of safer sunscreens. We found that oxybenzone caused high mortality of a sea anemone under simulated sunlight including ultraviolet (UV) radiation (290 to 370 nanometers). Although oxybenzone itself protected against UV-induced photo-oxidation, both the anemone and a mushroom coral formed oxybenzone–glucoside conjugates that were strong photo-oxidants. Algal symbionts sequestered these conjugates, and mortality correlated with conjugate concentrations in animal cytoplasm. Higher mortality in anemones that lacked symbionts suggests an enhanced risk from oxybenzone to corals bleached by rising temperatures. Because many commercial sunscreens contain structurally related chemicals, understanding metabolite phototoxicity should facilitate the development of coral-safe products.

Most of the world's coral reefs are endangered (1, 2). Although much of the threat is due to global factors, including increasing sea temperatures (3), coral declines can be exacerbated by local, anthropogenic factors. Much recent concern has focused on oxybenzone, a common sunscreen ingredient. For example, research in the US Virgin Islands found no substantial settlement of coral larvae, survival of juvenile corals, or regeneration of adult tissue in induced lesions over a 5-year period in Trunk Bay, where high levels of recreational swimming resulted in up to 1.4 mg of oxybenzone per liter of seawater (4). Meanwhile, a thriving coral community was found at neighboring Caneel Bay, with lower recreational use but presumably the same impacts from global stressors. Exacerbation of coral declines by sunscreens washed off tourists would be ironic and particularly pernicious, given the promotion of ecotourism in the interest of protecting coral reefs.

Although previous research has indicated that oxybenzone is an endocrine disruptor (5, 6), studies using corals and zebrafish have suggested that it might also be phototoxic (4, 7). However, the mechanism(s) of phototoxicity have not been explained. Here, we demonstrate that oxybenzone is indeed a sunscreen in vitro but is metabolized into phototoxic glucoside conjugates in the sea anemone *Aiptasia* and the mushroom coral *Discosoma* sp. (see materials and methods and fig. S1). We also show that *Aiptasia* without algal symbionts accumulate more glucoside metabolites in their tissues and die faster than animals with algae,

suggesting that the algae protect the hosts by sequestering the phototoxins. Thus, oxybenzone may be particularly phototoxic to bleached corals, exacerbating the damage due to global stressors. With recent moves by regulatory authorities in Hawaii and elsewhere to ban oxybenzone, understanding the mechanism(s) of its phototoxicity is important to ensure that

the sunscreen components that are selected as alternatives are truly safer for corals.

For most experiments, we used *Aiptasia*, a well-established model system for the study of symbiotic anthozoan cnidarians such as corals (see materials and methods and table S1). We exposed symbiotic anemones to 8.8 μ M (2 mg/liter) oxybenzone in artificial seawater (ASW) at 27°C in a solar simulator that approximates the 24-hour diurnal sunlight cycle (see materials and methods and fig. S2), including ultraviolet (UV) wavelengths of 290 to 370 nm (Fig. 1A). Mortality was 100% within 17 days (Fig. 1B and table S2). By contrast, negligible mortality was observed over 21 days when the animals were exposed to simulated sunlight without oxybenzone or to 8.8 μ M oxybenzone without UV light (Fig. 1B).

The importance of 290- to 370-nm light for oxybenzone-induced phototoxicity was surprising, given that oxybenzone's strong absorption within this waveband (Fig. 2, A and B) is precisely why it is expected to protect sunscreen users from UV damage. Phototoxicity frequently involves the photoexcitation of sensitizing molecules to excited triplet states that then degrade biomolecules either directly or indirectly via reactive oxygen species (ROS) and/or reactive halogen species (RHS) (8). To test this possibility, we conducted in vitro

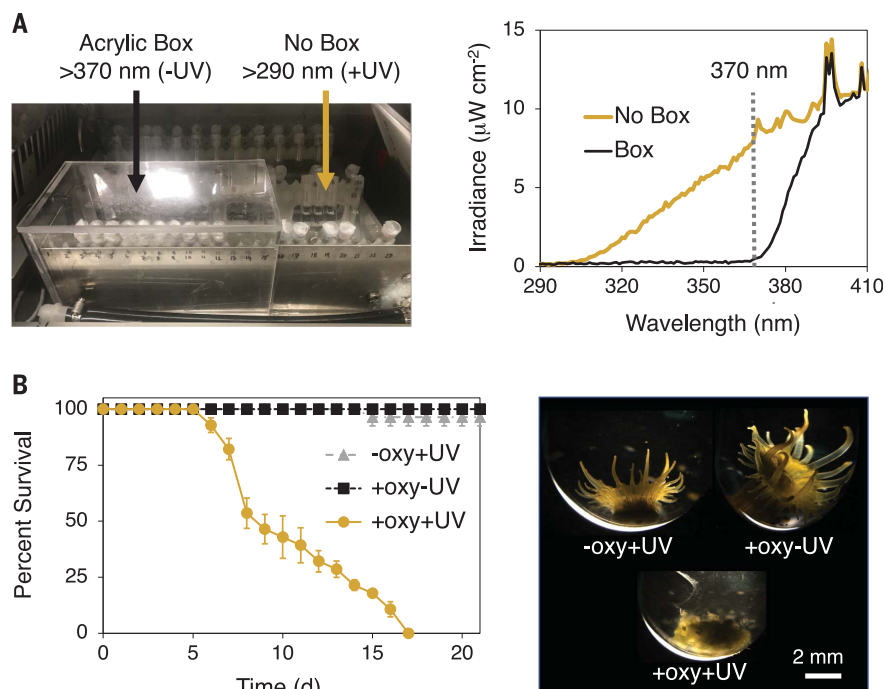


Fig. 1. Phototoxicity of oxybenzone to *Aiptasia* under simulated sunlight. (A) Solar simulator setup. An acrylic box (left) blocks UV light of wavelength <370 nm (right). (B) Survival of symbiotic *Aiptasia* in ASW exposed to full-spectrum light without oxybenzone (-oxy+UV; $n = 28$ animals in total) or to 8.8 μ M oxybenzone either with full-spectrum light (+oxy+UV; $n = 28$ animals) or with light >370 nm (+oxy-UV; $n = 16$ animals). Animals were exposed in three independent trials; means \pm SEM (weighted for the number of animals in each trial) are shown. Photographs show an example from each group after 9 days.

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experiments in a solution that contained seawater levels of halides using allyl-thiourea and sorbic alcohol as probes for photosensitization by oxybenzone (Fig. 2C), because their reduced sulfur and alkene groups are reactive with excited-state triplets, ROS, and RHS (8, 9). Consistent with oxybenzone's intended role as a sunscreen and with previous research indicating that it does not produce ROS upon sunlight illumination in vitro (10), oxybenzone decreased the first-order photodegradation rate of allyl-thiourea by ~40% (Fig. 2D, none versus **1**; $P = 0.006$; fig. S3; and table S3); similar results were observed with sorbic alcohol (fig. S4).

Because oxybenzone is similar in structure to the strong photosensitizer benzophenone (BP) (Fig. 2A, **1** and **6**) (11), we hypothesized that *Aiptasia* (and also corals) might metabolize oxybenzone to one or more photosensitizers. Previous studies of oxybenzone metabolites in corals only analyzed for 2,4-dihydroxybenzophenone and 2,2'-dihydroxy-4-methoxybenzophenone and either observed them at very low (~0.04 nmol/g wet weight) concentrations (12) or did not observe them at all (13). However, our analysis of *Aiptasia* tissue (see materials and methods) after exposure to 8.8 μM oxybenzone for 18 hours found that these compounds accounted for <2% of total metabolite mass. We instead recovered 1780 nmol/g dry

weight (~220 nmol/g wet weight) of oxybenzone-glucoside (Fig. 2A, **4**) and higher-order conjugates of oxybenzone (Fig. 3, A and B; figs. S5 to S7; and table S3).

Glucoside conjugates of oxybenzone were reported previously only in plants and suggested to be a pathway for oxybenzone detoxification (14). Here, however, whereas oxybenzone protected allyl-thiourea from photodegradation in vitro (Fig. 2D), its glucoside metabolite increased the photodegradation rate by ~5.6-fold (Fig. 2D, none versus **4**; $P < 0.001$). This photodegradation occurred only during illumination with the 290- to 370-nm waveband (fig. S3B), which corresponds to the glucoside's absorption spectrum (Fig. 2B). The glucoside's concentration did not change during the reaction (fig. S3C), indicating that it generates reactive species photocatalytically. We also observed enhanced allyl-thiourea photodegradation when the hydroxyl group that is adjacent to the carbonyl was deprotonated at pH 11 or replaced by a methoxy group [(MeO)₂BP] [**2** ($P < 0.0001$) and **3** ($P < 0.001$) in Fig. 2, A and D]; similar results were observed when sorbic alcohol was used as a probe (fig. S4). These results agree with previous ab initio calculations (15) and pump-probe spectroscopy (16) results indicating the importance of the hydroxyl group for sunscreen activity; trans-

fer of the proton from the hydroxyl group to the photoexcited triplet carbonyl promotes its relaxation to the ground state, which should prevent damage to biomolecules.

The high concentration of oxybenzone-glucoside and other presumably photosensitizing metabolites in *Aiptasia* co-occurred with oxybenzone itself (Fig. 3B, whole animal, oxy). It was not possible to determine the phototoxicities of the metabolites directly by introducing them into *Aiptasia* through the water column because of their low lipophilicity (octanol-water partition coefficient $\log K_{ow} = 1.1$ for oxybenzone-glucoside versus 3.6 for oxybenzone). Thus, we treated *Aiptasia* with (MeO)₂BP, which provides photosensitization comparable to that of oxybenzone-glucoside (Fig. 2D, **3** versus **4**; $P = 0.35$) but readily partitions into *Aiptasia* from the water column ($\log K_{ow} = 3.3$). Exposure to 8.3 μM (MeO)₂BP caused little to no mortality in the absence of 290- to 370-nm illumination [Fig. 3C; compare with the absorption spectrum of (MeO)₂BP, Fig. 2B]. However, under full-spectrum light, 8.3 μM (MeO)₂BP caused rapid mortality, and even 0.83 μM (MeO)₂BP caused mortality at a rate threefold faster than 8.8 μM oxybenzone (Fig. 3C; $P = 1 \times 10^{-10}$).

Despite the greater lethality of (MeO)₂BP, *Aiptasia* exposed to this molecule at 0.83 μM

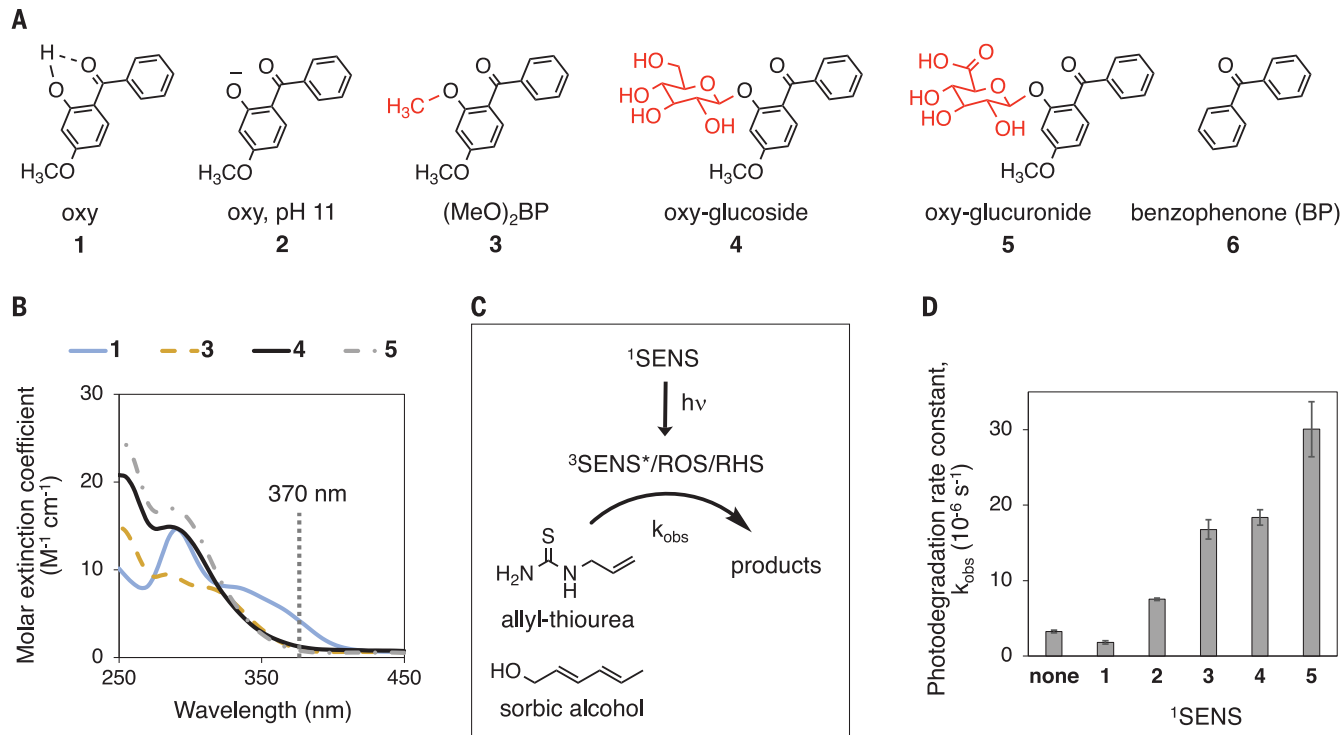


Fig. 2. Photosensitization by oxybenzone and related molecules.

(A) Chemical structures of oxybenzone (oxy) and related molecules (red: structural additions to oxybenzone). (B) Light-absorption spectra of compounds **1** and **3** to **5**. (C) Allyl-thiourea and sorbic alcohol as probes for reactive species. ¹SENS, photosensitizing molecule in its ground state;

³SENS*, photosensitizing molecule in its excited, triplet state. (D) First-order photodegradation rate constants for 10 μM allyl-thiourea with 35 μM of compounds **1** to **5** at pH 8.1 in seawater-strength halide solution (see supplementary materials and methods). Error bars, SEM from three independent experiments.

contained ~22-fold less phototoxin than *Aiptasia* exposed to 8.8 μM oxybenzone [Fig. 3B, whole animal, (MeO)₂BP versus sum of oxybenzone-glucoside and higher-molecular weight (MW) conjugates ($P = 6 \times 10^{-9}$)]. The fractionation

of *Aiptasia* homogenates (see materials and methods) suggested two explanations for this apparent paradox. First, the glucoside metabolites of oxybenzone were sequestered either entirely (higher-molecular weight conjugates,

such as the oxy-malonyl-glucoside) or largely (the glucoside without further modification) within the algal symbionts (Fig. 3B, Algal versus Animal fraction), so that the animal tissue of *Aiptasia* exposed to oxybenzone contained

Fig. 3. Oxybenzone metabolism in *Aiptasia* and (MeO)₂BP effects.

(A) Hypothesized scheme of oxybenzone (oxy) metabolism. (B) Concentrations of oxybenzone, its glucoside metabolites, and (MeO)₂BP (Fig. 2A, 3) in the water column, whole *Aiptasia*, and its algal and animal fractions after an 18-hour exposure to 8.8 μM oxybenzone (O) or 0.83 μM (MeO)₂BP (M). (Inset) Concentrations of oxybenzone-glucoside and (MeO)₂BP in the animal fraction (note expanded scale). Error bars: SEM of six trials (two animals per trial). (C) Survival of *Aiptasia* exposed to the full solar spectrum (+UV) with 8.3 μM (MeO)₂BP ($n = 16$), 0.83 μM (MeO)₂BP ($n = 17$), or 8.8 μM oxybenzone (data from Fig. 1B) or to light >370 nm (-UV) with 8.3 μM (MeO)₂BP ($n = 19$). Error bars, SEM over three independent trials, weighted for the number of anemones in each trial.

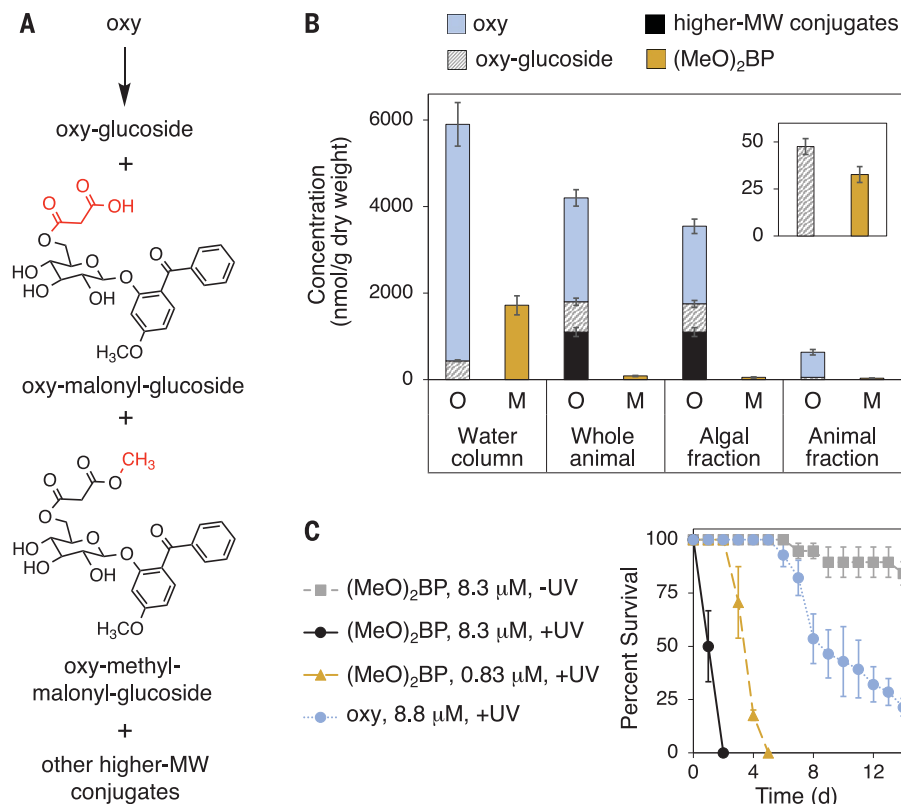
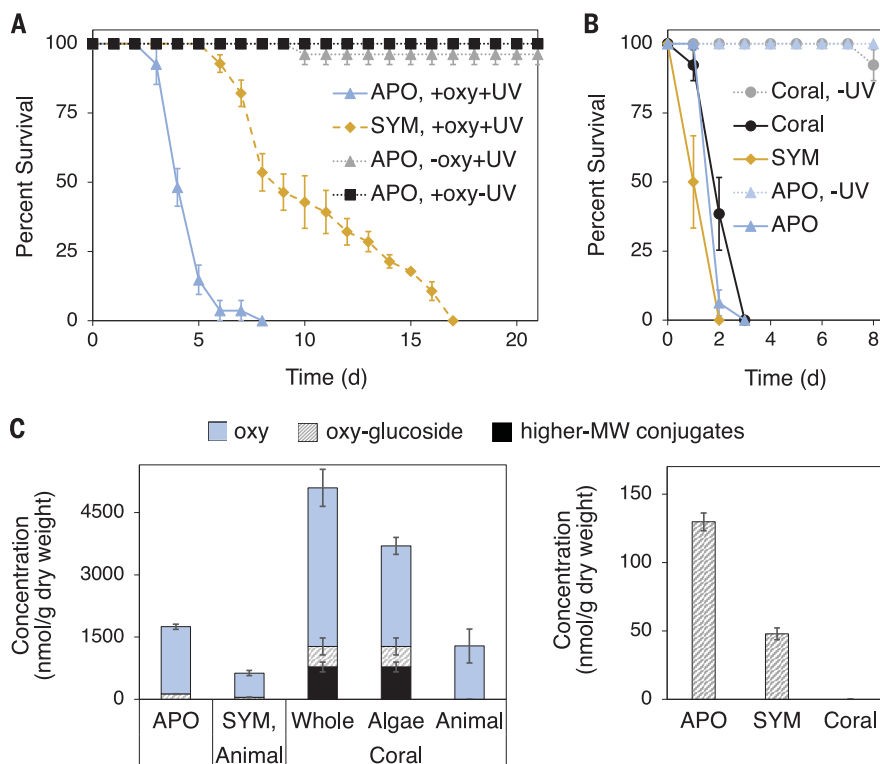


Fig. 4. Oxybenzone sensitivity and metabolite concentrations in aposymbiotic *Aiptasia* and in the mushroom coral *Discosoma*.

(A) Survival of aposymbiotic *Aiptasia* (APO) in the presence of 8.8 μM oxybenzone and full-spectrum light (+oxy+UV; $n = 27$), full-spectrum light without oxybenzone (-oxy+UV; $n = 26$), or 8.8 μM oxybenzone without light <370 nm (+oxy-UV; $n = 16$). Survival data for symbiotic *Aiptasia* (SYM) from Fig. 1B are included for comparison. Error bars, SEM over three trials (weighted by anemones per trial). (B) Survival of *Discosoma* coral ($n = 13$), SYM *Aiptasia* ($n = 16$), data from Fig. 3C), and APO *Aiptasia* ($n = 16$) exposed to 8.3 μM (MeO)₂BP under full-spectrum light and of APO *Aiptasia* ($n = 8$) and *Discosoma* ($n = 13$) exposed to 8.3 μM (MeO)₂BP without light <370 nm (-UV). (C) Left: oxybenzone and metabolite concentrations in the animal fractions of SYM (from Fig. 3B) and APO *Aiptasia* and in the whole organism and algal and animal fractions of *Discosoma* after exposure to 8.8 μM oxybenzone for 18 hours. Right: concentrations of oxybenzone-glucoside in the animal fractions replotted on an expanded scale. Error bars, SEM over six trials for SYM and APO and three trials for *Discosoma* (two animals per trial).



only ~1.5-fold more phototoxin than the animal tissue of *Aiptasia* exposed to (MeO)₂BP [Fig. 3B, inset: oxy-glucoside versus (MeO)₂BP; $P = 0.03$]. Second, the animal tissue of oxybenzone-treated *Aiptasia* also contained a substantial amount of oxybenzone itself (Fig. 3B, Animal fraction), which probably provided a UV-screening effect that was absent from *Aiptasia* treated with (MeO)₂BP.

These results suggested that the algae protect the animals by sequestering phototoxic oxybenzone metabolites and that the overall phototoxicity depends on the balance in the animal tissue between screening of UV light by oxybenzone and photosensitization by its glucoside metabolites. Support for this model was obtained from experiments with aposymbiotic (lacking algae) *Aiptasia* (see materials and methods) and with the mushroom coral *Discosoma*. First, aposymbiotic *Aiptasia* died much faster in the presence of 8.8 μ M oxybenzone than symbiotic *Aiptasia* (Fig. 4A; $P = 6 \times 10^{-12}$), and correspondingly, the aposymbiotic tissue contained ~2.7-fold more oxybenzone-glucoside than the animal fraction of symbiotic anemones (Fig. 4C; $P = 1 \times 10^{-6}$). Second, *Discosoma* showed no death during 8 days under full-spectrum light with 8.8 μ M oxybenzone (fig. S8), even though they were as susceptible as *Aiptasia* to the phototoxin (MeO)₂BP (Fig. 4B; $P = 0.2$). Correspondingly, tissue analyses indicated that the algae in *Discosoma* were even more effective than those in *Aiptasia* at sequestering the oxybenzone metabolites, which resulted in undetectable concentrations of the phototoxins within the surrounding animal tissue (Fig. 4C). Relative to *Aiptasia*, the *Discosoma* samples contained lower numbers of algal cells per unit host protein (fig. S1C; $P = 6 \times 10^{-6}$). Thus, the difference in phototoxin sequestration probably reflects distinct intrinsic properties of the different algal species in the two hosts (fig. S1), so that the exchange of algal symbionts that may

occur during stress and subsequent recovery (17) could alter susceptibility to oxybenzone-induced phototoxicity.

If the symbiotic algae of corals and other anthozoans indeed protect them from the toxic effects of oxybenzone metabolites, then the widespread bleaching of corals in response to rising seawater temperatures (2) will make them more susceptible to oxybenzone-induced phototoxicity. This may be a practical problem at reefs where recreational swimming and sunscreen use are high, with the risk varying between species. The widespread occurrence of glucosides and related metabolites suggests that phototoxicity from oxybenzone might also affect species beyond anthozoans. Notably, oxybenzone-glucuronide, the dominant oxybenzone metabolite in humans (18), is as potent a photosensitizer as oxybenzone-glucoside (Fig. 2D and fig. S4). Thus, metabolic pathways that presumably evolved to detoxify contaminants by rendering them more water soluble apparently convert oxybenzone from a sunscreen to potent phototoxins. These same pathways may also produce phototoxins from other organic sunscreens [e.g., salicylates (19) and other benzophenones (20)], which have aromatic carbonyl structures similar to that in oxybenzone and also rely on an excited-state proton transfer as their main energy-dissipation mechanism. Such conversions will need to be taken into account in developing safer alternative sunscreens.

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ACKNOWLEDGMENTS

We thank M. Houlihan (survival experiments), A. Simpson, E. Sattely, and K. Smith (metabolite purification), M. Paul (protein analysis), G. Rosenfield (coral harvesting and advice), O. Barry (animal feeding), B. Sabala (test-tube rack), T. Xiang (algal cultures), J. Bolorinos (statistics advice), and A. Grossman and Mitch and Pringle laboratory members for thoughtful insights throughout the project. **Funding:** Woods Institute for the Environment at Stanford University (grant no. WTAWZ to J.R.P. and W.A.M.); National Science Foundation (NSF) Graduate Research Fellowship (to D.V.); and NSF (grant no. CBET-2114790 to W.A.M. and J.R.P.). **Author contributions:** Conceptualization: J.R.P., W.A.M., and D.V.; Methodology: D.V., A.I.T., L.L., C.R., J.R.P., and W.A.M.; Investigation: D.V., A.I.T., L.L., and C.R.; Funding acquisition: D.V., J.R.P., and W.A.M.; Supervision: J.R.P. and W.A.M.; Writing—original draft: D.V. and W.A.M.; Writing—review and editing: D.V., A.I.T., L.L., C.R., J.R.P., and W.A.M. **Competing interests:** The authors declare no competing interests. **Data and materials availability:** All data are available in the main text or the supplementary materials.

SUPPLEMENTARY MATERIALS

science.org/doi/10.1126/science.abn2600
Materials and Methods

Figs. S1 to S8
Tables S1 to S4
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MDAR Reproducibility Checklist
Data S1 to S10

Submitted 15 November 2021; accepted 16 March 2022
10.1126/science.abn2600

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Science, 376 (6593), • DOI: 10.1126/science.abn2600

Sunscreen turned toxic

Coral reefs face many serious threats from human activity. Sunscreens can cause reef damage, and although the precise mechanisms involved are still under study, some localities have already phased out common components such as oxybenzone. Using a sea anemone as a model system, Vuckovic *et al.* found that oxybenzone is modified within cells by attachment of glucose, turning it from a sunscreen into a potent photosensitizer (see the Perspective by Hansel). The glycoside conjugate is concentrated within the algal symbionts of anemones and corals, and bleached anemones are more susceptible to damage when exposed to ultraviolet light and oxybenzone, suggesting that the algae provide some protection to their hosts. These experiments add to our understanding of reef damage by sunscreens and may help to inform policy and new sunscreen development. —MAF

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