Special Series

Development of a novel rinse-off method for improved sunscreen exposure assessment

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EDITOR'S NOTE:

This article is part of the special series "Consequences of Sunscreen Product Use on Aquatic Environments." This series documents the current state of knowledge concerning potential impacts of chemicals derived from sunscreen products on freshwater and marine ecosystems, including coral reefs. Specific topics discussed include use patterns, environmental loadings, potential exposures, toxicological effects, and future research needs.

Abstract

Recent legislative measures restricting the sale of sunscreens containing certain ultraviolet light filtering ingredients (UVFs) have been based on a perception of risk to aquatic life despite the lack of a robust data set to support these decisions. Concerns were focused on the potential for recreational swimmers' and divers' sunscreen use to result in exposures to coral already stressed due to climate change, disease, and other local conditions. In published environmental risk assessments for UVFs, exposure estimates were based on episodic environmental monitoring or estimates of typical sunscreen use, arbitrarily assuming the portion rinsed off from skin in seawater. To improve the accuracy of exposure estimates and thereby develop more robust risk assessments, we measured the amount of the UVFs, avobenzone, homosalate, octisalate, octocrylene, and oxybenzone released to seawater from four sunscreen products (two lotions, one spray, one stick) in a novel porcine skin model of typical human sunscreen use. Sunscreen was applied to porcine skin, allowed to briefly dry, then exposed to four rinse cycles in artificial seawater. The mass of each UVF in seawater, partitioned from seawater onto glassware, and extractable from skin after rinsing were determined. The proportion rinsed from skin varied by UVF, by formula type, and by application rate. Less than 1.4% of applied octisalate, homosalate, and octocrylene was detected in seawater samples (independent of formula) increasing to an average of 4% and 8% for avobenzone in stick and lotion forms, and to 24% for oxybenzone in lotions. The initial data show substantial differences in rinse-off among formulation types and sunscreen application rates, and stress the importance of using UVF-specific rinse-off values rather than a single value for all UVFs. This new method provides a tool for more robust exposure estimates, with initial data supporting lower rinse-off values than adopted in published risk assessments. *Integr Environ Assess Manag* 2021;17:961–966. © 2021 Johnson & Johnson Consumer Companies Inc. *Integrated Environmental Assessment and Management* published by Wiley Periodicals LLC on behalf of Society of Environmental Toxicology & Chemistry (SETAC).

KEYWORDS: Aquatic risk, Exposure assessment, Exposure modeling, Personal care products, Sunscreens

INTRODUCTION

Recent research has addressed the potential for adverse effects to aquatic species exposed to ultraviolet light filters (UVFs) from sun protection products rinsed from swimmers' or divers' skin (e.g., Carve et al., 2021; Danovaro et al., 2008; Downs et al., 2016; Schaap & Slijkerman, 2018; Sharifan et al., 2016). Some regional governments where coral reefs are important to the local ecology and economy—including Hawaii and the Virgin Islands in the United States, Aruba, and Palau—have chosen to ban the sale or restrict the use of specific UVFs in sunscreen based on these initial research findings. The substances most scrutinized are frequently

described as "chemical" UVFs because their chemical bonds absorb UV light, and include oxybenzone, octinoxate, and more recently homosalate, octisalate, and octocrylene (e.g., Palau). These legislative decisions were made on a precautionary basis, without the benefit of a robust environmental risk assessment (ERA) underpinning (Mitchelmore et al., 2020). There is a tension between the potential benefit to coral by using this approach—which has thus far relied on uncertain data—and the expected adverse human health outcome due to a potential reduction in consumer confidence in the use of sunscreens (Diffey, 2020). Hence, improved data and methods for ERA of UVFs are needed to support further decision making on the safe use of sunscreen products.

The published ERAs for UVFs in seawater referenced above have relied on modeling and environmental monitoring data to characterize exposure. The studies using models to estimate exposure assume that 25%–100% of the UVFs applied are rinsed off during swimming (Danovaro et al., 2008; Schaap & Slijkerman, 2018; Sharifan et al., 2016). The study cited as the basis for the assumption that high rinse-off (50%–100%) of UVFs is expected in seawater (Wright et al., 2001) does not actually support that conclusion. In a survey of beachgoers in Texas, Wright et al. (2001) report that reapplying sunscreen every 1–2 h prevented sunburn ($n = 8$) while no reapplication ($n = 22$) resulted in sunburn among all the beachgoers who were using sunscreen and were not already sunburned. The authors erroneously made statistical comparisons between swimming and non-swimming groups, pooling people who reapplied sunscreen with those who did not. The authors did not consider that fewer swimmers than non-swimmers reapplied sunscreen, hence drawing the unsupported conclusion that swimming predicted sunburn occurrence due to high rinse-off rates, when reapplication alone completely predicted sunburn occurrence, regardless of swimming. We identified no published study that quantitatively addressed the expected rinse-off proportion of UVFs from sunscreen products as typically used by consumers.

To address this data gap, we developed a new method for simulating UVF rinse-off from skin in sequential seawater rinses following a typical application of commercial sunscreen products. The specific scenario and test condition were chosen to reproducibly simulate potential exposure to sea life, given recent regulatory attention on the safety of sunscreen use near coral reefs. We hypothesized that UV rinse-off would vary due to three factors: (1) among UVFs because of their range of water solubility and hydrophobicity; (2) among sunscreen formulations (e.g., lotion, stick, or spray) because of interactions with inactive ingredients and formulation chemistry; and (3) between typical and recommended sunscreen application rates, proportional to the application rate. Here, we describe the method and initial results with four sunscreen formulas: one spray, one stick, and two lotions.

METHODS

Reference material for avobenzone (AVO), homosalate (HS), octisalate (OS), octocrylene (OC), and oxybenzone (OXY) were obtained from Fisher Scientific (Pittsburg, PA) and were 97.8% or greater purity. See Supporting Information (SI) for more information on reagents. Test samples were provided by the commercial manufacturer
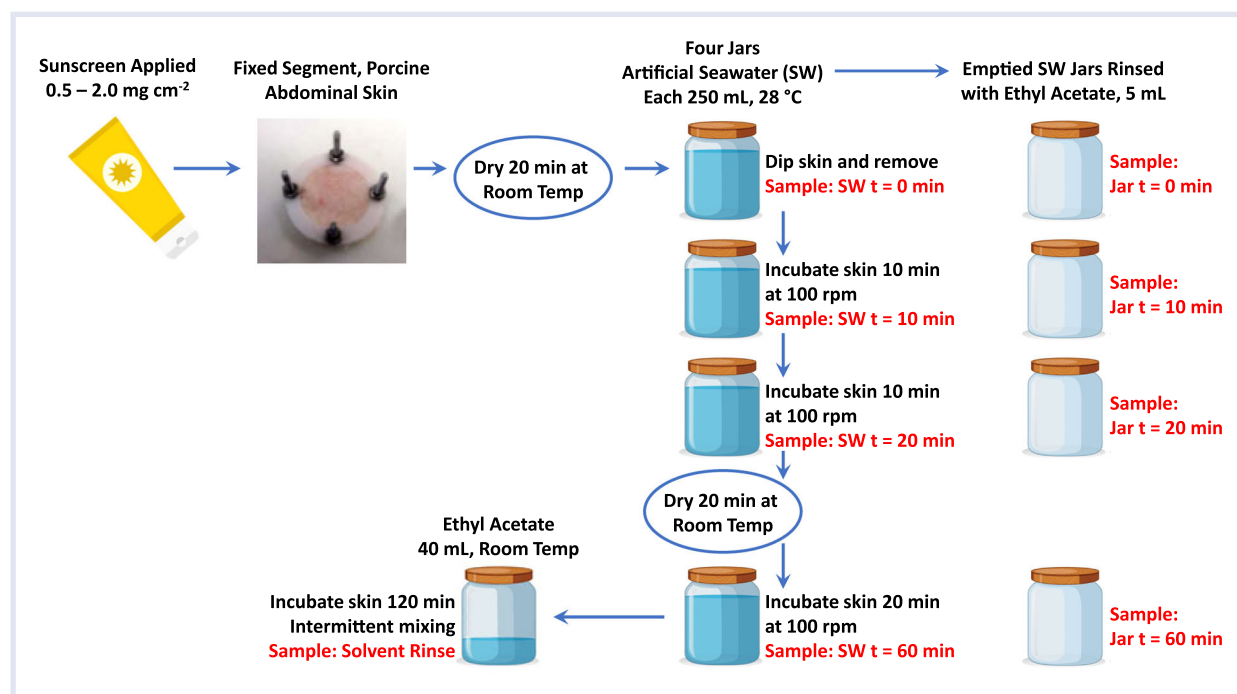

FIGURE 1 Experimental design for seawater rinse-off simulation method. Each experiment was performed in triplicate for four sunscreen formulas applied to skin at approximately $0.5\,\mathrm{mg\,cm^{-2}}$ (typical application rate) and for one formula (Lotion 1) applied to skin at approximately $2\,\mathrm{mg\,cm^{-2}}$ (recommended application rate)

and included a total of eight samples consisting of four formulations (two lotions, one stick, and one spray) with and without UVFs. UVF concentrations in each formula were determined in triplicate (see Supporting Information) using gas chromatography—tandem mass spectrometry (GC-MS/MS) as described below and in Supporting Information.

Figure 1 depicts the method for simulating sunscreen rinse-off in seawater. The rinsing procedure follows part (a) of US FDA test method 352.76, *Determination if a product is water resistant or very water resistant* (https://www.ecfr.gov/cgi-bin/retrieveECFR?gp=1&SID=4bc79838759baa6124e57801dd484a11&ty=HTML&h=L&mc=true&r=SECTION&n=se21.5.352_176) with modification to obtain data for more time intervals. The procedure was performed in triplicate for each experimental condition. Porcine abdominal skin was selected as a model for human skin, stored overnight in a saturated salt box to maintain hydration between 20% and 50% until testing. For testing, a 20-cm^2 skin patch was affixed to a custom holder fabricated from Delrin washers with foil backing where it remained throughout the entire experiment as shown in Figure 1, leaving a 10-cm^2 area of exposed skin for testing. A pre-weighed portion of sunscreen product was applied to the skin and allowed to dry at room temperature for 20 min. After drying, the skin patch was dipped into and immediately removed from a glass jar containing 250 mL artificial seawater at 28 °C to determine if a large pulse UVFs would rinse off upon initial exposure to water (sample SW $t = 0$). The water temperature selected is representative of areas where coral thrives and swimming is comfortable. The skin patch was then placed into a second, identical seawater jar, agitated at 100 rpm for 10 min, and removed (sample SW $t = 10$). This was repeated once more in a third jar (sample SW $t = 20$). The skin patch was then allowed to dry at room temperature for 20 min followed by agitation in a fourth and final, identical jar for 20 min (sample SW $t = 60$). Seawater rinsates from each jar were processed and analyzed separately as described below and in Supporting Information. After the fourth seawater rinse, the skin was placed in a glass jar containing 40 mL ethyl acetate and allowed to sit at room temperature for 120 min with intermittent manual agitation, after which the solvent was processed and analyzed as described below to detect UVFs extractable from skin. Samples were shaded from ambient light using foil during shaking, during "rest" stages, and in storage after collection. Each empty jar was also rinsed with 5 mL ethyl acetate and analyzed to quantify UVFs adhering to the glass (jar samples: $t = 0$, $t = 10$, $t = 20$, and $t = 60$).

Each of the four sunscreen formulas was applied to skin at a rate of 0.5 mg cm^{-2}, reflecting typical sunscreen application practices (e.g., Autier et al., 2001; Diaz et al., 2012; Heerfordt et al., 2017; Lademann et al., 2004). One additional condition was tested, with one lotion applied to skin at a rate of 2.0 mg cm^{-2}, reflecting the US FDA-mandated application dose for SPF determination, which the agency recommends for consumer use.

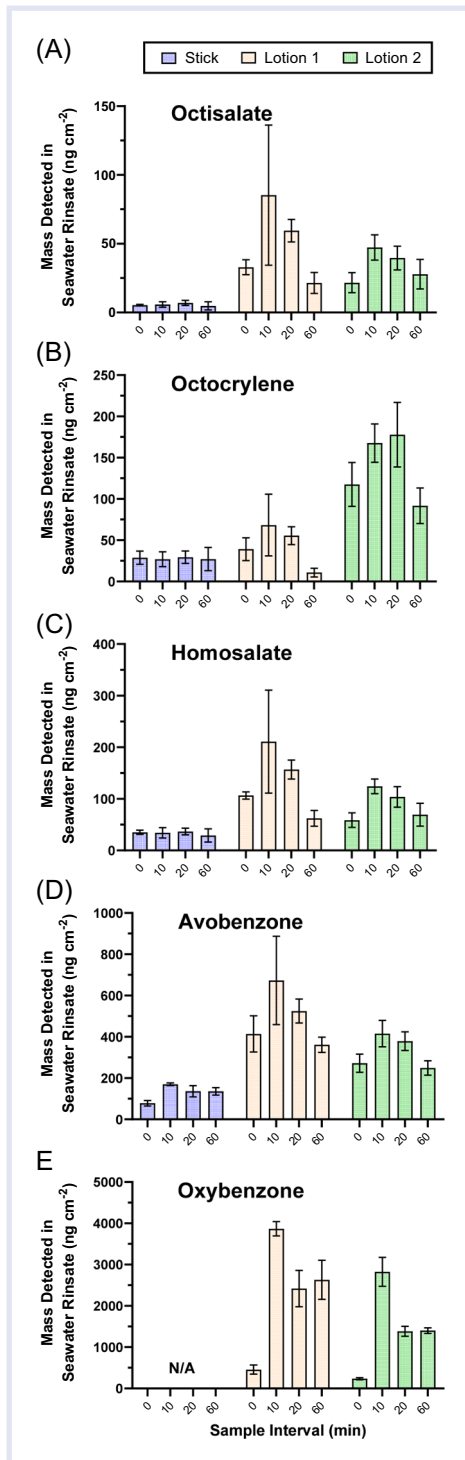Target compounds were extracted from seawater samples by SPE. See procedure details in Supporting Information. Initially, liquid chromatographic separation was performed on a Waters Acquity I-Class UPLC using a Waters BEH XbridgeTM C18 column (1.0 × 100 mm, 1.7 μm particle size) maintained at 40 °C. See Supporting Information for further details. Liquid chromatography (LC) was ultimately abandoned due to significant carryover of OXY, with detection continuing even after 24 h of solvent rinse between samples. To remedy this issue, we utilized gas chromatographic separation performed on an Agilent 7890 using an Agilent HP-1MS capillary column (30 m × 0.25 mm i.d. × 1 μm film thickness consisting of 100% polydimethylsiloxane). Triple quadrupole mass spectrometry (Waters Xevo TQ-S) was used for the identification and quantification of analytes. See Supporting Information for further details. Statistical analyses were performed using GraphPad Prism version 8.4.3 software.

RESULTS

Quantitative data are presented for three of the four sunscreen formulations. During the SPE processing step, the spray formula recoveries were all less than 20%, resulting in unacceptably high uncertainty. The spray formula is anhydrous and tended to stick to the walls of the experimental apparatus more than the other formulas. While refinement of analytical procedures was undertaken here (e.g., LC vs. GC), further refinement of the method to optimize the selection of SPE cartridge, organic solvent, test vessel material (e.g., polytetrafluoroethylene (PTFE) vs. glass), and rinsate volume would be necessary to obtain reliable quantitative data for the spray formula. Among formulas, mass balance was highest for the Stick (97%–138%) and lowest for Lotion 2 (51%–67%). See Supporting Information for all results. Refinement of the skin extraction procedure to improve recovery for lotion formulas is expected to improve the mass balance.

The simulation included four successive seawater rinses totaling 40 min of submergence under continuous agitation. Figure 2 shows the pattern of UVF detection in each rinse. Sample SW $t = 0$ min was a rapid dip into seawater, yielding detectable UVF in the water phase for all formulas and filters. As expected, the sample SW $t = 10$ min generally yielded the maximum amount of UVFs in seawater, with few exceptions. Four filters—OS, HS, OC, and AVO—generally yielded a lower mass in water with each successive rinse after $t = 0$ for the two lotions. Only OXY was detected to a similar extent in water in the $t = 20$ and $t = 60$ samples, which included a 20-min drying cycle between rinse cycles. Oxybenzone also yielded the highest mass in water (4 μg cm^{-2} for Lotion 1, $t = 10$), consistent with the fact that OXY has the highest water solubility among the UVFs (3.7 mg L^{-1} at 25 °C). The stick formula resulted in the smallest releases with less than 185 ng of UVF detected in seawater per cm^2 of treated skin per rinse cycle. These data could be used to support a modeling parameter that relates the mass of UVFs released to water to the area of sunscreen-treated skin for various UVF-formulation combinations.

Exposure models in the literature, cited above, used a highly conservative percentage of sunscreen expected to be
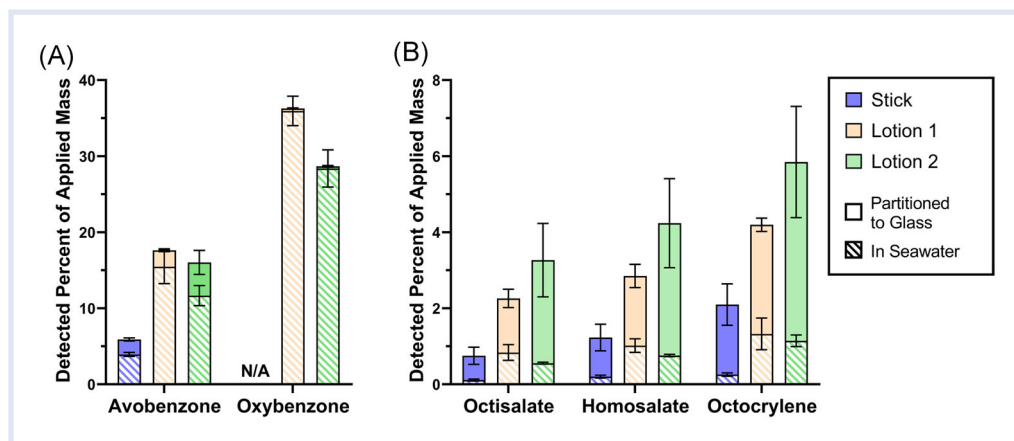
FIGURE 2 Mass of UVFs detected in successive seawater rinsates from stick (violet), Lotion 1 (tan), and Lotion 2 (green) formulas trending in order of increasing mass found in water arising from typical application rate (0.5 mg cm^{-2}) on skin: (A) OS < (B) OC < (C) HS < (D) AVO < (E) OXY. Error bars show standard error of the mean. AVO, avobenzone; HS, homosalate; OC, octocrylene; OS, octisalate; OXY, oxybenzone

rinsed from skin to seawater during a swimming event. The data in Figure 3 could be used to support a more realistic value for this model parameter. The total proportion of all UVFs rinsed from skin (i.e., detected in SW plus that

redeposited from water to jar) was in the order, OXY > AVO > OC > HS > OS with maxima occurring for lotions, averaging 33%, 17%, 5%, 4%, and 3%, respectively and maxima for the stick: (OXY not a constituent), 6%, 2%, 1%, and 1%, respectively.

The UVFs' behavior in SW fell into two categories: (A) AVO and OXY, which were primarily detectable in the water phase, with a smaller proportion partitioning to the glass jars, and (B) OS, HS, and OC, which primarily partitioned to the glass jars, with a smaller proportion detectable in the water phase. This behavior suggests partitioning from seawater to sand, which is chemically similar to glass, might be expected for OS, HS, and OC in nature, though these substances are expected to preferentially partition to natural organic matter (either biological or as other particulate matter) when it is present. This points to sediment sampling as a potentially sensitive monitoring tool for these UVFs relative to AVO and OXY. Less than 1.5% of applied OS, HS, and OC, total, over the four rinse cycles, was detectable in the water phase, regardless of the formulation applied. For AVO, 4% of the applied mass was detectable in water for the stick formulation, while the result was 16% and 12% for Lotions 1 and 2 (for the 0.5 mg cm^{-2} application rate), respectively. These proportions for AVO were unexpected, given its relatively high octanol–water partition coefficient (log K_{ow} = 6.1) and low water solubility (27 µg L^{-1}), which are more similar to those of OS, HS, and OC than to those of OXY. Consistent with its higher water solubility, the total OXY detected in seawater from the two formulas containing this ingredient were 36% and 28% in Lotions 1 and 2 (for the 0.5 mg cm^{-2} application rate) respectively, noting that rinse-off differs by application rate (Figure 4).

Partitioning of individual filters into the water phase can be influenced by formulation matrix effects. Lotions are a mixture of two phases (oil and water) containing emulsifying ingredients to maintain a homogeneous formulation. The emulsifiers can increase solubility of hydrophobic UVFs in the water phase. Sunscreen stick and spray formulations, on the other hand, are anhydrous, and do not require emulsifiers to maintain homogeneity. In addition, OXY and AVO are solid at room temperature, and could potentially precipitate out of solution to form a suspension rather than fully dissolving in the water phase. No examination of these potential mechanisms for observed solubility differences was made in this study, but these initial findings suggest that developing a deeper understanding of intra-formulation factors influencing rinse-off could be a route to designing formulas that mitigate future environmental exposure to UVFs from sunscreen.

We tested Lotion 1 at two application rates with a hypothesis that UVF rinse-off would be proportional to application rate. We used 0.5 mg cm^{-2}, which is representative of typical use levels for sunscreen products (e.g., Autier et al., 2001; Diaz et al., 2012; Heerfordt et al., 2017; Lademann et al., 2004), and 2.0 mg cm^{-2}, which is the rate mandated for SPF-testing by US FDA, and underlies their recommendation that consumers should use at least one

FIGURE 3 Total rinse-off in seawater as a percent of the mass applied from Stick (violet), Lotion 1 (tan), and Lotion 2 (green) formulas. Cross-hatched segments represent the portion detected in seawater rinsates (cumulative for the four successive seawater rinses). Solid segments represent the portion detected in Jar Samples (removed from skin in seawater rinsate but partitioned to the glass jar rather than remaining in seawater, cumulative for the four solvent extracts of seawater rinse jars). (A) Proportion remaining in water exceeds that partitioning to glass for avobenzone (AVO) and oxybenzone (OXY). (B) Proportion partitioning to glass exceeds that remaining in water for octisalate (OS), homosalate (HS), and octocrylene (OC). Error bars show standard error of the mean

ounce of sunscreen per application. These data are shown in Figure 4. There was no significant difference between the results for typical and recommended application rates for OS, HS, and OC in seawater, that is, the amount detected in seawater was proportional to the amount applied. For AVO and OXY, a significantly lower proportion was detected in seawater at the higher application rate: 15% versus 3% for AVO and 36% versus 16% for OXY. The percent detected in extract jar rinsates from the typical and recommended treatments were proportional to their application rates (no significant difference) for all UVFs. So, while the absolute mass of AVO and OXY rinsed from skin increased at the higher application rate, the proportion of the total amount on skin decreased. These initial data suggest that the use of a flat percentage rinse-off rate may not be appropriate, but instead, should be coupled with its corresponding application rate.

DISCUSSION

Skin cancer is one of the most common forms of cancer in the United States (Silva et al., 2018). According to the World Health Organization, four out of five cases can be prevented by following safe sun practices, including use of sunscreen (World Health Organization [WHO], 2003). In the United States, where sunscreen is regulated as an OTC drug, there are only nine FDA-approved sun filters in common use. Policy development in this area should therefore be grounded in reliable and validated risk assessments. This new method was developed to fill a gap in knowledge about rates of UVF rinse-off when considering recreational sunscreen use at the beach.

This method is expected to be useful for testing any sunscreen formula to estimate differences in UVF rinse-off during seawater bathing, which can support proactive formulation choices that mitigate the potential for aquatic exposure. Additionally, the method and experiments described herein provide a more rigorous basis for selecting

UVF rinse-off values and estimating exposure concentrations for risk assessment.

Even as larger numbers of monitoring studies are published, yielding a more robust exposure database for UVFs in seawater, modeling still has an important role in performing ERA. The use of models and monitoring together can be used for mutual cross-checking to detect errors, either in sampling or in model assumptions, helping risk assessors avoid the use of inaccurate exposure estimates in ERA. For example, Carve et al. (2021) used a comprehensive data set to support their ERA of UVFs in marine and fresh water, but selected a high, outlying marine exposure value of $1.395\,\mathrm{mg\,L^{-1}}$ for
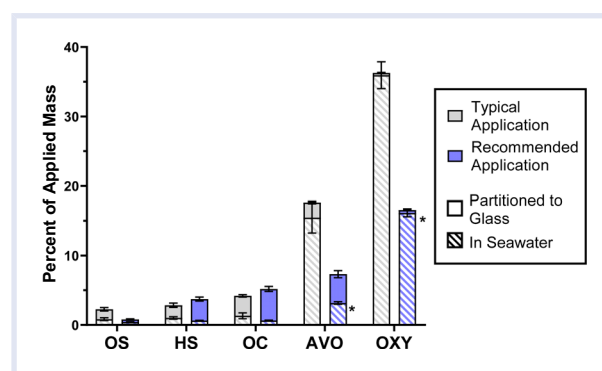

FIGURE 4 Mass of UVFs rinsed off in seawater for Lotion 1 applied to skin at the typical rate (gray, $0.5\,\mathrm{mg\,cm^{-2}}$) and at the FDA-recommended rate (violet, $2.0\,\mathrm{mg\,cm^{-2}}$). Cross-hatched segments represent the portion detected in seawater rinsates (cumulative for the four successive seawater rinses). Solid segments represent the portion detected in Jar Samples (removed from skin in seawater rinsate, but partitioned to the glass jar rather than remaining in seawater; cumulative for the four solvent extracts of seawater rinse jars). The proportion of applied UVF detected in seawater rinse-off is significantly smaller for the (higher) FDA-recommended application rate compared with the (lower) typical application rate for avobenzone (AVO) and oxybenzone (OXY). Statistical significance determined using the Holm–Sidak method, with $\alpha = 0.05$. Error bars show standard error of the mean

oxybenzone based on monitoring by Downs et al. (2016), although this value was shown to be erroneous in modeling (Maples-Reynolds et al., 2019; see Supporting Information). Also, Schaap and Slijkerman (2018) used redundant monitoring and modeling approaches to calculate hazard quotients for three UVFs, adopting scenarios of 25%, 50%, and 100% rinse-off and found substantial disagreement, noting that modeled values always exceeded measured values. The method and data presented here can be used to support more accurate, empirically generated rinse-off estimates, which will help close the gap between model estimates and monitoring data and will allow higher confidence in extrapolating exposure modeling to sites lacking monitoring data. Results for UVFs in seawater plus partitioned to glassware (Figures 3 and 4) demonstrate a significant reduction in exposure opportunity relative to scenarios adopted above (<20% vs. 25%, 50%, 100%), because these published scenarios assume a higher sunscreen application rate ($2.0\,mg\,cm^{-2}$), at which lower proportional rinse-off occurs for AVO (3.2%) and OXY (16.2%). Other rinse-off values could be justified for different application rates; for example, for a reasonable worst case scenario the rinse-off values for Lotions 1 and 2 at the low application rate can be averaged, representing UVF behavior at that sunscreen application rate, and that result can then be averaged with the rinse-off value for the higher application rate yielding 8% and 24% for AVO and OXY, respectively (see Supporting Information for details). The novel method described here is now being adapted for use with sunscreens containing mineral UVFs, and in the process, further optimized to improve its performance.

ACKNOWLEDGMENT

DATA AVAILABILITY STATEMENT

All laboratory data described in this manuscript were generated at Battelle Memorial Institute, and all results generated for this project are included in the Supporting Information.

SUPPORTING INFORMATION

FIGURE S1. Trunk Bay water volume estimate.
TABLE S1. Analysis details.
TABLE S2. Definition of quality control samples used.
TABLE S3. N-Evap recoveries.
TABLE S4. Amount of sunscreen formulation applied (rinse-off testing).
TABLE S5. Skin hydration level (rinse-off testing).
TABLE S6. UV filter content of four sunscreen formulas tested.

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