Advanced harmonization techniques result in accurate establishment of in vitro–in vivo correlations for oxybenzone from four complex dermal formulations with reapplication

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Abstract
Due to high variability during clinical pharmacokinetic (PK) evaluation, the prediction of in vivo exposure from in vitro absorption testing of topical semisolid and liquid dermal products has historically proven difficult. Since absorption from unoccluded formulations can be influenced by environmental factors such as temperature and humidity, maximal effort must be placed on the harmonization of experimental parameters between in vitro and in vivo testing conditions to establish accurate in vitro/in vivo correlations (IVIVC). Using four different sunscreen formulations as a model, we performed in vitro permeation testing (IVPT) studies with excised human skin and maintained strict harmonization techniques to control application time, occlusion, temperature, and humidity during in vivo human serum PK evaluation. The goal was to investigate if increased control over experimental parameters would result in decreased inter-subject variability of common topical formulations leading to acceptable IVIVC establishment. Using a deconvolution-based approach, excellent point-to-point (Level A correlation) IVIVC for the entire 12-h study duration was achieved for all four sunscreen formulations with <10% prediction error of both area under the curve (AUC) and peak concentration (Cmax) estimation. The low variability of in vivo absorption data presents a proof-of-concept protocol design for testing of complex semisolid and liquid topical formulations applied over a large surface area with reapplication in a reliable manner. This work also presents the opportunity for expanded development of testing for the impact of altered temperature and humidity conditions on product absorption in vivo with a high degree of precision.

Keywords In vitro/in vivo correlation (IVIVC) · Sunscreen · Oxybenzone · Pharmacokinetics (PK) · In vitro permeation test (IVPT)

Introduction
In vitro/in vivo correlation (IVIVC) modeling is a currently approved surrogate for biowaiver approval in oral extended-release formulations with wide acceptance by the pharmaceutical industry. Its use allows for bypassing lengthy clinical trials saving time and money when post approval changes, scale-up, or bioequivalence need to be assessed [1]. The application of IVIVC to dermal products is currently being investigated and shows promising results for transdermal delivery systems under both baseline and elevated skin temperature conditions with in vitro permeation testing (IVPT) proving to be predictive of in vivo pharmacokinetic (PK) profiles [2–12]. Unfortunately, their use for predicting drug absorption from semisolid and liquid dermal formulations has proven challenging [13–16]. After the recent guidance released by the US Food and Drug Administration (FDA) on the administration of maximal usage trials (MUsT) prior to product approval, a successful IVIVC model may prove impossible with the abundance of variables requiring control for proper harmonization of semisolid and liquid dermal products [17, 18].

Predictive IVIVC models rely on a high degree of harmonization between the in vitro and in vivo testing conditions [19]. Since semisolid and liquid dermal products are unoccluded and open to the environment, harmonization becomes a challenge. Not only can application site
impact absorption, but so can temperature, humidity, and contact transfer (residual formulation removal), which are all typically left uncontrolled during in vivo clinical testing [20–23]. Baseline skin temperature is typically 32 ± 1°C according to the “Transdermal and Topical Delivery Systems – Product Development and Quality Considerations” Guidance released by the FDA while baseline relative humidity (RH) for in vivo testing and long-term stability testing of topicals is 50 ± 10% [24–27]. The maintenance of baseline skin temperature (~32 °C), ideal RH (~45%), and avoidance of residual formulation removal without occlusive coverings are essential variables that, without precise control during in vivo testing, can drastically impact the absorptive potential of each formulation and produce high variability in resultant serum concentration measurements [13–16, 28].

Since 1997, there has been a published guidance by FDA detailing the harmonization techniques needed to develop a successful IVIVC for extended-release oral dosage forms which includes recommendations for the following: number of subjects during in vivo testing, type of allowable reference formulations, fasted versus fed state considerations, preferred dissolution apparatuses, and buffer media for in vitro studies [1]. There is currently no guidance specifying the degree of harmonization needed to develop a successful IVIVC model for semisolid and liquid dermal products, especially when MUS-T studies are needed for products intended for full body application.

In the work presented, sunscreens were chosen as a model due to the wide variety of marketed formulations and similarity of active ultraviolet (UV) filter levels. Oxybenzone, a lipophilic broad-spectrum UV filter, is incorporated into a multitude of sunscreen products with a 6% inclusion limit [29]. There have been ample reports on its high absorptive potential [28, 30–39]. The objective of this work was to use four distinctly different marketed sunscreen products (cream emulsion, solid stick, lotion, continuous spray) that all contain 6% oxybenzone (Table 1), and develop an in vivo testing environment to control skin temperature and environmental relative humidity with sunscreen reaplication under unoccluded conditions. Under strict harmonization of experimental conditions, the variability from in vivo PK absorption for complex dermal formulations, previously proven difficult to predict from IVPT, can decrease and provide valuable maximal exposure predictions of full body application serum levels without the risk of full body exposure to healthy subjects. We hypothesize that when the experimental variables of temperature and relative humidity are highly controlled in vivo and harmonized to IVPT studies, predictive IVIVC models of multiple complex topical formulations can be achieved providing product innovators a way to generate reliable in vivo exposure estimates from in vitro studies.

Materials and methods

Materials

Four commercially available sunscreen products, Coppertone® ULTRA GUARD® SPF 70 (cream emulsion), Coppertone® Stick Kids SPF 50 (solid stick), Neutrogena® Age Shield® Face Oil-Free SPF 110 (lotion), and Banana Boat® Kids Clear UltraMix® SPF 100 (continuous spray), were purchased from Amazon.com, Inc. (Seattle, WA), and all contained 6% oxybenzone as one of the UV filters (Table 1). Oxybenzone-d5 internal standard (98 + %), acetonitrile (99.9 + %, high-performance liquid chromatography (HPLC) grade), and methanol (99.9 + %, HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO). Oxybenzone standard (98 + %) was a gift from Sigma-Aldrich (St. Louis, MO). Glacial acetic acid (99.7 + %) was purchased from AmericanBio, Inc. (Natick, MA). Acetonitrile (liquid chromatography–mass spectrometry (LC–MS) grade), methanol (LC–MS grade), water (LC–MS grade), methyl tert-butyl ether, formic acid (OPTIMA™ LC–MS grade), Brij® 98, potassium phosphate monobasic, and dibasic salts were purchased from Fisher Scientific, Inc. (Fair Lawn, NJ). All reagents were analytical grade. Nanopure water from an in-house Milli-Q system (EMD Millipore; Billerica, MA) was sourced for buffers.

Skin preparation

Ex vivo surgical-waste abdominal skin sections from four consenting human donors were provided by the NCI funded Cooperative Human Tissue Network (CHTN) skin repository (Charlottesville, VA). All skin samples were deidentified prior to next day delivery on ice to the University of Maryland, Baltimore, where the stratum corneum (SC), viable epidermis, and a small portion of the dermis were removed by dermatome to a resulting skin thickness of 295 ± 35 µm. Table 2 contains a summary of the demographic features for donors used for IVPT. All skin samples were stored at ~20°C in aluminum foil and a sealed Ziploc® bag until the morning of each experiment when it was thawed to room temperature, cut into 4.84 cm² squares, and placed into an In-Line cell (PermeGear, Inc.; Hellertown, PA) between the donor and receiver chamber with the epidermal side facing up. Prior to dosing, each skin piece was allowed to equilibrate for 30 min as receiver solution flowed under the dermis side. A heated circulating water bath maintained skin temperature at a baseline of 32 ± 1°C. Skin integrity was confirmed by measuring transepidermal water loss (TEWL) with a cyberDERM RG-1 open chamber evaporator (cyberDERM, Inc.; Broomall, PA). Any TEWL reading above 15 g/m²/h is outside the
range reported for healthy skin and indicates a disruption of the skin barrier [40]. Any skin piece deemed unacceptable was removed, replaced, and allowed to equilibrate again prior to reevaluating TEWL and dosing.

**IVPT studies**

**IVPT method and experimental design**

A PermeGear flow-through In-Line diffusion system (Hellertown, PA) with cell membrane supports that offered a 0.95 cm² permeation area was used for all IVPT experiments. The receiver solution was phosphate-buffered saline at pH 7.4 with 0.1% Brij 98® as a surfactant. Surfactants are added at a low concentration to maintain the solubility of highly lipophilic compounds, like oxybenzone, in the receiver solution and preserve sink conditions throughout the 12 h study duration. Flow rate was set to 1 mL/h. After successful skin integrity verification, the semisolid sunscreens were dosed by gently rubbing them onto the skin surface using the flat bottom of a HPLC vial in a circular motion while the continuous spray sunscreen was dosed using a positive displacement pipette. Dosing occurred three times to mimic the harmonized in vivo protocol and simulate maximal dosing according to product labeling, once at 0 min and again at 80 min and 160 min. At each dosing time point, approximately 10 mg/cm² of sunscreen was applied. Samples were collected at 1:00, 2:00, 3:00, 3:30, 4:00, 4:30, 5:00, 5:30, 6:00, 6:30, 7:00, 7:30, 8:00, 8:30, 9:00, 9:30, 10:00, and 12:00 h post sunscreen application. The resulting samples were diluted 1:1 with HPLC mobile phase and analyzed using a validated HPLC method. Skin was maintained at a temperature of 32 ± 1°C.

Table 1 Characteristics of sunscreen products used in the study

| Cream Emulsion | Solid Stick | Lotion | Continuous Spray |
|----------------|-------------|--------|------------------|
| **Active Ingredients (UV Filters)** | Oxybenzone 6% Avobenzone 3% Homosalate 15% Octisalate 5% Octocrylene 10% | Oxybenzone 6% Avobenzone 3% Homosalate 15% Octisalate 5% Octocrylene 10% | Oxybenzone 6% Avobenzone 3% Homosalate 15% Octisalate 5% Octocrylene 10% | Oxybenzone 6% Avobenzone 3% Homosalate 10% Octisalate 5% Octocrylene 10% |
| **Inactive Ingredients** | Water, butylene glycol, microcrystalline cellulose, glyceryl stearate, behenyl alcohol, benzyl alcohol, diethyleneglycol syringylidene malonate, tocopherol (vitamin E), retinyl palmitate (vitamin A), sodium ascorbyl phosphate, stearic acid, palmitic acid, lauril alcohol, myristyl alcohol, cetyl alcohol, lecithin, caprylyl/caprylic triglyceride, chlorophenesin, cellulose gum, butylated PVP, disodium EDTA | Ozokerite, caprylyl/caprylic triglyceride, C12-15 alkyl benzate, lauryl laurate, behenyl alcohol, bis-PEG-12 dimethicone beeswax, isopropyl myristate, C20-40 alkyl stearate, synthetic beeswax, tocopherol (vitamin E), polymethylene, sorbitan oleate, VP/hexadecene copolymer, aloe barbadensis leaf extract, stea roxy dimethicone, helianthus annuus (sunflower) seed oil | Water, styrene/acylates copolymer, silica, beeswax, cyclopentasiloxane, ethylhexyglycerin, glyceryl stearate, PEG-100 stearate, acrylates/dimethicone copolymer, acrylates/c10-30 alkyl acrylate crosspolymer, chlorophenesin, disodium EDTA, triethanolamine, dipotassium glycyrrhizate, BHT, methylisothiazolinone, propylene glycol, fragrance | Alcohol denatured, isobutane, acrylates/octyl acrylate amide co-polymer, diethylhexyl syringylidene malonate, caprylyl/caprylic triglyceride, caprylyl glycol, tocopheryl acetate, mineral oil, aloe barbadensis leaf extract, fragrance |
for the 12 h experiment with temperature monitored prior to dosing and at 2:00, 4:00, and 6:00 h post-sunscreen application using a Traceable™ infrared thermometer (Fisherbrand™; Fair Lawn, NJ).

**Oxybenzone skin extraction**

At the end of the study, each skin piece was wiped clean with an alcohol swab, removed from the IVPT cells, and sliced into small pieces using a scalpel. The alcohol swab was placed in a 15 mL conical tube while the skin pieces and scalpel blade were placed in a separate 15 mL conical tube. Four milliliters of methanol were added to each conical tube to extract residual oxybenzone remaining on and within the skin. The alcohol swab, removed from the IVPT cells, and scalpel blade were placed in a separate 15 mL conical tube while the skin pieces were sliced into small pieces using a scalpel. An alcohol swab was placed in a 15 mL conical tube while the skin pieces and scalpel blade were placed in a separate 15 mL conical tube. Four milliliters of methanol were added to each conical tube to extract residual oxybenzone remaining on and within the skin at the end of the study. Conical tubes were capped, and centrifuged for 10 min. After centrifugation, all samples were sonicated for 10 min and then placed on a shaker at 200 rpm for 24 h before an aliquot was removed for HPLC analysis.

**HPLC analysis of in vitro samples**

The HPLC system was comprised of a Waters® Alliance e2695 separations module connected to a Waters® 2489 dual-wavelength absorbance detector and Waters® Empower software (Milford, MA). A Waters® Symmetry C<sub>18</sub> column (5 µm, 4.6 × 150 mm) with Phenomenex® SecurityGuard™ C<sub>18</sub> cartridge guard column (5 µm, 4 × 3.0 mm) (Torrance, CA) was used for oxybenzone separation from other interferences in each sample. The mobile phase was a mixture of acetonitrile, methanol, and acidified water adjusted to pH 3.0 with glacial acetic acid at a ratio of 65:20:15 (v/v/v) under constant flow of 1 mL/min. All IVPT samples were diluted 1:1 with mobile phase prior to analysis and standards were created similarly in a 1:1 mixture of mobile phase and receiver solution prior to analysis. Oxybenzone elution occurred at 3.1 min with maximum UV detection at 287 nm. There were no interferences from any excipients or other UV filters from the commercial products or skin samples. The skin extraction and residual swab samples were diluted 4x and 50x, respectively, with a 1:1 mixture of methanol and mobile phase prior to HPLC analysis. Oxybenzone elution occurred at 4.5 min with maximum UV detection at 326 nm. The concentration of oxybenzone standards ranged from 0.05 to 10 µg/mL. All IVPT, extraction, and quality control (QC) standards (20 µL) were injected in duplicate. The developed method had a limit of detection (LOD) at 0.025 µg/mL with a linearity range of 0.05 to 25 µg/mL. All QC standards (20 µL) were injected in duplicate. The developed method had a limit of detection (LOD) at 0.025 µg/mL with a linearity range of 0.05 to 25 µg/mL. All QC, including the lower limit of quantitation (LLOQ), showed inter-day precision within 5% of the calculated nominal value and accuracy between 99 to 102%.

**In vivo PK study in healthy human subjects**

The in vivo PK study was approved by the University of Maryland, Baltimore Institutional Review Board and carried out in compliance with the ethical and scientific principles of the International Conference on Harmonization Good Clinical Practice E6 (ICH-GCP) at the General Clinical Research Center within the University of Maryland Medical Center.

**Clinical study subjects**

An open-label, randomized, four-way crossover study was completed by ten healthy adult subjects. Table 2 provides a summary of the demographic information. Study enrollment was open to both men and non-pregnant women from September 2020 to April 2021. Subject ethnicity was self-reported and collected by the clinical staff. Key eligibility criteria were ages 18 to 45 years with a body mass index (BMI) < 30 kg/m<sup>2</sup>, non-smoker/tobacco user, negative test results for drugs of abuse (cannabinoids, amphetamines, barbiturates, benzodiazepine, cocaine, methadone, opiates, PCP), normal screening laboratory values (white blood cells, complete blood count, hemoglobin, platelets, sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase, urine protein, and urine glucose), mid thighs of ≥ 42 cm in circumference, legs that measure ≥ 46 cm in length from the iliac crest to the top of the patella or large enough to accommodate the products to be tested at an area of 400 cm<sup>2</sup> per thigh, normal electrocardiogram readings as determined by the medically accountable investigator, and normal vital signs (temperature 35–37.9°C, systolic blood pressure (BP) 90–140 mmHg, diastolic BP 60–90 mmHg, heart rate (HR) 55–100 beats per minute, respiration rate 12–20

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**Table 2** Demographic information for in vitro study donors and in vivo PK study subjects

|                      | In Vitro Donors (n = 4) | In Vivo Subjects (n = 10) |
|----------------------|-------------------------|---------------------------|
| **Age in years**     |                         |                           |
| Mean (SD)            | 47.5 (23.9)             | 30.0 (5.5)                |
| Range                | 39–57                   | 24–42                     |
| **Sex, n (%)**       |                         |                           |
| Male                 | -                       | 3 (30)                    |
| Female               | 4 (100)                 | 7 (70)                    |
| **Ethnicity, n (%)** |                         |                           |
| Black                | -                       | 1 (10)                    |
| Caucasian            | 4 (100)                 | 5 (50)                    |
| Asian                | -                       | 3 (30)                    |
| Hispanic             | -                       | 1 (10)                    |
| **BMI (kg/m<sup>2</sup>)** |                   |                           |
| Mean (SD)            | Unknown                 | 25.2 (2.5)                |
| Range                | 21.2–29.5               |                           |
upper thighs were examined for any signs of skin irritation, infections. Once continuance in eligibility was confirmed, both restrictions regarding oxybenzone as well as medication restrictions to changes in medical history and adherence to instructions were recorded, and the subject was asked questions pertaining to a clinical staff member prior to dosing. Vital signs were asked to provide a negative urine pregnancy test confirmation. At the beginning of each procedure day, female subjects were examined for any signs of skin irritation, infections, excessive hair, sunburn, raised moles, scars, open sores, scar tissue, tattoos, or discoloration that would interfere with product placement or skin assessment of both upper thighs. Additionally, subjects were instructed to refrain from using any products containing oxybenzone (makeup, lip balm, face creams, sunscreen, etc.) 0–7 days prior to the start of each procedure day as well as refrain from using any chronic prescription medications 0–30 days and over-the-counter (OTC) medications/short-term prescription medications 0–3 days prior to the start of each procedure day with the exception of vitamins, herbal supplements, and birth control.

Randomization

There were 24 combinations of procedure day orders that could be assigned to subjects. After screening and confirmation of eligibility, each subject was randomly assigned a different procedure day order to prevent any treatment bias. Once a procedure day order was used, it was not reassigned to any other subject.

Clinical PK study design

The IVPT and clinical PK studies were harmonized to the maximum possible extent, replicating sunscreen products, temperature, humidity, dosing times, sampling time points, and study length. The duration of each clinical PK procedure day was 12 h with sunscreen applied at 0, 80, and 160 min. A minimum of 1-week washout period between procedure days was required to remove residual oxybenzone from systemic circulation.

Clinical PK study procedures

At the beginning of each procedure day, female subjects were asked to provide a negative urine pregnancy test confirmed by a clinical staff member prior to dosing. Vital signs were recorded, and the subject was asked questions pertaining to changes in medical history and adherence to instructions regarding oxybenzone as well as medication restrictions. Once continuance in eligibility was confirmed, both upper thighs were examined for any signs of skin irritation, abrasions, or sunburn and a template measuring 400 cm\(^2\) was marked as the dosing area on each thigh. An intravenous (IV) catheter was placed under sterile technique and approximately 4 mL of blood was drawn as a pre-dose blood sample. The application area on each thigh was cleaned with water, carefully dried, and a temperature sensor (Novatemp\(^{\circledR}\) skin temperature sensors 400 series; NOVAMED USA; Elmsford, NY) was placed near the knee adjacent to the application area on one leg to measure skin temperature throughout the entire 12 h procedure day.

For each semisolid sunscreen product (cream emulsion, lotion, and solid stick (Table 1)), a teardrop silicone applicator (Qosmedix; Ronkonkoma, NY) covered by a XL nitrile finger cot was used to apply 800 mg of sunscreen onto the marked 400 cm\(^2\) location on each thigh simultaneously by two clinical staff until evenly distributed (~1 min). For the continuous spray product (Table 1), approximately 3 mL was sprayed into a glass scintillation vial and left to sit for 15 min so the propellant could evaporate. After 15 min, two 1 mL topical syringes were used to draw up 0.95 mL of liquid sunscreen. Similar to the semisolid products, dosing occurred by releasing the liquid from the syringe onto the marked 400 cm\(^2\) application area on each thigh and rubbed in using a teardrop silicone applicator covered by a XL nitrile finger cot. Approximately 800 mg (2 mg/cm\(^2\)) of sunscreen product was applied simultaneously to each thigh repeatedly at two additional times (80 and 160 min) during each procedure day, representing maximal usage and reapplication recommendation as stated on the respective sunscreen labels.

Before the scheduled dosing time of the first application, the environmental control chamber was set up and allowed to equilibrate for approximately 30 min so that the RH within the chamber measured between 35 and 55%. The individual components as well as the setup procedure of the environmental chamber are depicted in Fig. 1. The chamber consisted of a 3D printed dome (ARTLAB Virtual Studio; Lexington, KY) made of polyethylene terephthalate glycol filament with the dimensions 749.3 mm × 330.2 mm × 539.8 mm, designed to provide ample space underneath for the subjects’ upper thighs without touching the application area. Velcro\(^{\circledR}\) was used to attach a far infrared tourmaline heating pad (UTK\(^{\circledR}\) Technology Store; China) to the underside of the dome. The hose and humidity monitor of an ultrasonic humidifier (Nebula\(^{\circledR}\), River Systems; Campodarsego, Italy) were attached at the base of the dome and angled inward towards the subjects’ thighs. The humidifier was set to 45% RH and self-regulated to reach the set target based on the connected mini-USB humidity sensor. RH within the environmental chamber was also measured with a wireless humidity monitor (TP-60S Digital Hygrometer, ThermoPro; Duluth, GA) attached under the dome near the subjects’ feet. The entire 3D dome was covered by a polyester disposable aluminized
rescue blanket (Dynarex Corporation; Grainger; Lake Forest, IL) and a hospital provided bed sheet.

After the initial sunscreen dose at time 0 min, the subjects’ upper thighs were covered with the environmental control chamber. The environmental control chamber was only removed for subsequent dosing at 80 and 160 min or if the subject needed to use the restroom. During either event, skin temperature and RH were recorded prior to chamber removal, after chamber replacement, 5 min post replacement and 10 min post replacement to verify that skin temperature and RH returned to $32 \pm 2^\circ$C and $45 \pm 10\%$, respectively. The infrared heating pad was only turned on if subjects’ skin temperature fell below $30^\circ$C after the 10 min replacement window; otherwise, it remained off.

At 12 h post initial application, the sunscreen was removed by clinical personnel. Each application site was wiped with a dry cotton gauze, followed by four alcohol swabs. Left and right application sites were processed separately for residual oxybenzone concentration. Alcohol swabs and gauze for each site were placed into 500 mL glass bottles containing 200 mL of methanol for extraction.

Safety

Adverse events (AE) were recorded during each procedure day with vital sign monitoring (temperature, BP, HR, respiratory rate) prior to initial sunscreen application and periodically throughout the 12 h visit. Sunscreen application and temperature sensor sites were assessed subjectively on a scale of 0–4 (0: no evidence of irritation; 1: faint but definite erythema; 2: moderate erythema; 3: severe erythema; 4: generalized vesicles or eschar formations) by clinical staff for signs of erythema, itching, hyperpigmentation, and edema prior to initial sunscreen application and at the end of the 12h study after sunscreen and temperature sensor removal. Subjects were asked about oxybenzone allergies prior to enrollment and to confirm that the heating pad within the environmental chamber was not in contact with the skin; therefore, sunscreen application was not anticipated to cause any health concerns. Any subject with ongoing AEs at the end of a procedure day was contacted by phone until resolution was reported.

Serum sample collection

Collection of approximately 4 mL of whole blood with a vacutainer (BD Vacutainer® Plus plastic serum tube; BD; Franklin, NJ) from an IV catheter occurred prior to initial application of sunscreen, and at 1:00, 2:00, 3:00, 3:30, 4:00, 4:30, 5:00, 5:30, 6:00, 6:30, 7:00, 7:30, 8:00, 8:30, 9:00, 9:30, 10:00, and 12:00 h post initial sunscreen application. The sample was allowed to clot for 30–60 min and centrifuged at $1,300 \times g$ for 20 min at $4^\circ$C. Resultant supernatant serum was aliquoted into two identical cryovials. Cryovials were immediately placed in an ultralow (−80°C) freezer until analysis with a validated ultra-performance liquid chromatography-tandem mass spectrometry (LC–MS/MS) method.

LC–MS/MS assay for serum oxybenzone concentrations

For oxybenzone serum extraction, 200 µL of each sample was placed in a glass screw-top culture tube along with 50 µL of oxybenzone-d5 (50 ng/mL) as an internal standard (IS). Two milliliters of methyl tert-butyl ether was added to each culture tube with a glass pipette as an extraction solvent. Samples were vortexed for 5 sec, placed on a high-speed shaker (Fisher Scientific, Inc.; Fair Lawn, NJ) at 2,500 rpm for 20 min, and centrifuged for 5 min. The upper organic layer (1.5 mL) was transferred to a clean glass culture tube.
while a second extraction was performed repeating the above procedure. After centrifuging the second extraction, 1.5 mL of the upper organic layer was combined with the previous extraction and the sample was evaporated to dryness under nitrogen gas (N-EVAP; Organamation Associates, Inc.; Berlin, MA) at ambient temperature. Residue was reconstituted in 100 µL of mobile phase consisting of methanol and water with 0.1% formic acid (73:27 v/v) and centrifuged for 5 min. Five microliters were injected into the LC–MS/MS for analysis.

Analysis was conducted on a Waters® LC–MS/MS system consisting of a Waters® Acquity H class UPLC connected to a Waters® Xevo TQ-XS mass spectrometer (Milford, MA). All samples were injected by an autosampler onto a Waters® BEH C18 column 130 Å (1.7 µm, 2.1 x 100 mm) with a Waters® VanGuard™ BEH C18 guard column (1.7 µm, 2.1 x 5 mm). Mobile phase was a mixture of methanol and water with 0.1% formic acid (73:27, v/v) held constant for 3 min at a flow rate of 0.3 mL/min. Each sample was proceeded by a gradient column wash. From 3–8 min, mobile phase was primarily organic (99:1, v/v). From 8–12.5 min, initial conditions (73:27, v/v) were reestablished allowing the column to return to equilibrium. Multiple reaction monitoring was carried out with the parent to daughter ion transition of 229.06→150.62 at a cone voltage of 2 V and a collision energy of 20 eV for oxybenzone. The IS had similar monitoring with a parent to daughter ion transition of 234.06→150.75 at a cone voltage of 2 V and a collision energy of 18 eV. Calibration standards had a linearity range from 0.1 to 100 ng/mL. Method validation was conducted according to the Bioanalytical Method Validation Guidance for Industry [41]. All QCs, including the LLOQ, showed inter-day precision within 11% of the calculated nominal value and accuracy between 96 and 101%. Freeze–thaw, bench top, processed sample, stock solution, and long-term stability (6 months) of both the low QC (0.3 ng/mL) and high QC (50 ng/mL) had an accuracy between 90 and 103%. Recovery at all QC levels was high (91–94%) with minimal matrix effect. During clinical sample analysis, the deviation from nominal values did not exceed 15% for any standards or QCs except for the LLOQ with a maximum allowable error of 20%.

**IVIVC approaches**

A point-to-point (Level A) IVIVC was performed comparing the entire 0–12 h observed serum PK profile in vivo to the harmonized predicted in vitro oxybenzone absorption data from the IVPT study using a unit impulse response (UIR)–based numerical deconvolution method [7, 11]. Briefly, relative clinical PK parameters for a two-compartment oxybenzone base model were generated and absolute PK parameter estimates (supplemental material) were derived using bioavailability ($F_{IVPT}$) calculated from IVPT studies. $F_{IVPT}$ is the ratio of total oxybenzone remaining on/in the skin (cum.amt.skin) plus the total oxybenzone absorbed through the skin (cum.amt.absorbed) to the total oxybenzone dose applied (Eq. 1).

$$F_{IVPT} = \frac{(\text{cum.amt.absorbed} + \text{cum.amt.skin}) \times 100}{\text{total dose}}$$ (1)

To validate that the base model absolute PK parameter estimates were correct, allometric scaling of central clearance using subjects’ weights from the clinical study was performed. According to Huh et al., allometric scaling presents an optimal approach for interspecies scaling of clearance at a fixed exponent of 0.65 with a high degree of prediction accuracy and an average-fold error (AFE) of 2.31 when scaling from rats to humans [42]. The input of each individual subjects’ weight into an allometric scaling calculation of clearance from rats resulted in an arithmetic mean human systemic clearance estimate of 16.1 L/h (13.34–18.08 L/h) and an AFE of 0.67 confirming an adequate model prediction of the absolute PK parameters of interest after relative IVPT bioavailability adjustment [42, 43]. Because numerical UIR-based deconvolution requires input from an IV administration and oxybenzone is not approved for IV dosing, a simulation was conducted to generate a hypothetical IV PK profile based on model predicted PK parameter estimates. From the simulation profile, UIR estimates were obtained with the following bi-exponential equation where $C_T$ represents the concentration at time $T$, $A$ and $B$ are the initial concentration of oxybenzone following IV administration in the central and peripheral compartments respectively, and $\alpha$ and $\beta$ are the elimination rate constants from the central and peripheral compartments respectively:

$$C_T = (A \times e^{-\alpha T}) + (B \times e^{-\beta T})$$ (2)

Parameter estimates for Eq. (2) are as follows:

- $A = 5.92 \text{ (units: ng/mL)}$
- $B = 0.38 \text{ (units: ng/mL)}$
- $\alpha = 0.29 \text{ (units: h}^{-1}\text{)}$
- $\beta = 0.02 \text{ (units: h}^{-1}\text{)}$

Based on the above parameter estimates, UIR values were calculated using a stripping dose of 1703.75 mg, equivalent to the IV bolus dose utilized for oxybenzone IV simulation:

- $A1 = A \text{ (stripping dose: mL}^{-1}\text{)}$
- $A2 = B \text{ (stripping dose: mL}^{-1}\text{)}$
- $\alpha1 = \alpha \text{ (units: h}^{-1}\text{)}$
- $\alpha2 = \beta \text{ (units: h}^{-1}\text{)}$

The in vitro fraction permeated ($F_p$) for oxybenzone was obtained from IVPT studies carefully harmonized to the in vivo study design by dividing the cumulative amount
of oxybenzone permeated at time \( t \) by the total amount of oxybenzone hypothetically on the skin surface at time \( t \). Due to sunscreen reapplication to the skin, there is an additive nature in total oxybenzone concentration on the skin surface for the first 160 min of the study, after which further additions of oxybenzone were ceased. The in vivo fraction absorbed \( (F_a) \) for oxybenzone was obtained from the numerical deconvolution of the clinical PK samples for the entire 12 h time course using the UIR values mentioned above on Phoenix® WinNonlin® software (Pharsight Corporation; San Diego, CA). Both linear as well as polynomial models were tested to relate \( F_a \) and \( F_p \) for IVIVC model construction using GraphPad Prism® software (version 6.01, La Jolla, CA). The highest regression coefficient \( (R^2) \) value was achieved with a polynomial equation correlation for all four formulations (Fig. 2).

To validate if the established correlation was predictive of the reported in vivo results, the respective correlations for each formulation were used to calculate a predicted \( F_a \) time course from the IVPT \( F_p \) data. The predicted \( F_a \) values were convoluted in Phoenix® WinNonlin® software using the Wagner-Nelson method and the same UIR input as the deconvolution step to obtain a predicted in vivo systemic oxybenzone concentration verses time PK profile for each formulation. The predictability of the developed IVIVC models was assessed by calculating the prediction error \( \%PE \) for both oxybenzone AUC and \( C_{\text{max}} \). \%PE represents the absolute difference in the reported and the model predicted oxybenzone exposure estimates (Eq. 3)\[1\].

\[
\%PE = \left| \frac{\text{Observed value} - \text{Predicted value}}{\text{Observed value}} \right| \times 100
\]

Data and statistical analysis

Statistical analysis was conducted with GraphPad Prism® software. All in vitro data is presented as arithmetic mean ± SD of the three replicates for each product. An ANOVA followed by Tukey’s post hoc analysis was used to find significance among the four sunscreen formulations. Differences were considered statistically significant when \( p \leq 0.05 \) and are denoted as follows: \(* p \leq 0.05\), \(** p \leq 0.01\), or \(*** p \leq 0.001\). In vivo oxybenzone exposure estimates (AUC and \( C_{\text{max}} \)) for 0–12 h were calculated using NCA linear up, log down methodology on Phoenix® WinNonlin® software (Pharsight Corporation; San Diego, CA). Phoenix® WinNonlin® software was also used for numerical deconvolution and convolution procedures.
Results

Human skin IVPT study

Dermatomed ex vivo human abdominal skin was used to conduct pivotal studies for four sunscreen formulations under baseline skin temperature conditions (32 ± 1 °C) with application occurring at 0, 80, and 160 min, simulating maximal product usage according to product labeling. Fig. 3 shows the mean flux versus time profile as well as the mean total cumulative amount of oxybenzone permeated for all four formulations. The cream emulsion formulation consistently demonstrated a significant (ANOVA with Tukey’s post hoc; \( p \leq 0.001 \)) increase in both maximum flux (\( J_{\text{max}} \)) and cumulative amount of oxybenzone permeated compared to all other formulations. Not significantly different from each other, the solid stick and the lotion formulations had similar cumulative amount of oxybenzone permeation and \( J_{\text{max}} \) values. These two formulations differed in the time to reach \( J_{\text{max}} \) with maximum flux occurring at 5 h for the lotion and 12 h for the solid stick. The continuous spray consistently resulted in the lowest \( J_{\text{max}} \) and cumulative amount of oxybenzone permeation, although the difference between the continuous spray and lotion or solid stick formulation was not statistically significant. The rank order of the formulations from highest to lowest in regard to total oxybenzone permeation is cream emulsion > solid stick ≈ lotion > continuous spray.

In vivo PK study

Serum PK concentration versus time profiles for all study procedure days are shown in Fig. 4 separated by formulation. The upper plots represent the arithmetic mean profile of each formulation with associated standard deviation at each sampling time point (\( n = 10 \) subjects) while the lower plots represent the individual concentration versus time profiles for each subject separated by formulation. There was not a significant difference in the mean \( C_{\text{max}} \) values for any of the formulations. However, the rank order was similar to the in vitro study results with the highest oxybenzone absorption from the cream emulsion > solid stick > lotion ≈ continuous spray. Since the recommended application area of sunscreens is full body coverage, maximal full body exposure estimates were made under the assumption that an average adult has a body surface area of 2 m² (Table 3) [44]. The calculated full body maximum exposure estimates were similar to previously reported \( C_{\text{max}} \) values from full body reapplication studies conducted by the FDA in 2019 [28, 38]. Differences are expected due to contrasting product formulation design, product oxybenzone concentration levels, and time/number of reapplications performed. However, since the mean \( C_{\text{max}} \) values for each of the four formulations have a relatively low coefficient of variability (%CV), the full body estimates are assumed to have a high reliability. There were two subjects (OBENZ-08 and OBENZ-09) with outlier serum concentration measurements at 12 h for the continuous spray formulation. It was concluded that because...
these were the first two subjects receiving the continuous spray treatment, possible contamination at that time point occurred during sample processing and steps were taken to ensure contamination risk was mitigated for future subjects. No other outlier measurements were recorded.

**Safety**

The ten subjects determined eligible were enrolled and completed all four procedure days without any serious or unexpected AEs. Nine out of ten subjects experienced at least one AE throughout the study. All AEs were reported as mild (grade 1) with the most common being decreased HR (31%), erythema due to sunscreen or temperature sensor removal (26%), and decreased BP (21%). Typically, erythema was attributed to sensitivity to alcohol wipes at the end of the study or flushed vasodilation of the legs after prolonged exposure to 45% RH, while decreases in BP and HR were attributed to spending 12 h sitting in a hospital bed. Any AE, other than erythema, was resolved prior to the subject leaving at the end of the day with resolution of erythema by the end of the following day as self-reported by the subjects. Other AEs (<10%) included headache, increased BP, dizziness, somnolence, and lightheadedness.

**Room temperature and humidity monitoring**

Room temperature and humidity monitoring during each procedure day was conducted with a wireless temperature/humidity monitor (ThermoPro; Duluth, GA) set near the center of the room. Fig. 5 plots both arithmetic mean (black lines) and individual (gray lines) temperature or humidity versus time profiles for all procedure days separated by formulation. Readings were recorded every hour from 0 to 12 h. The mean room temperature for each formulation was consistently around 22°C with a low %CV because the room temperature could be manually controlled within each room of the clinical testing facility (Table 4). The mean RH of

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**Table 3** Arithmetic mean oxybenzone exposure \( (C_{\text{max}}) \) in vivo for all four sunscreen formulations with associated %CV and estimation of full body \( C_{\text{max}} \) \((n=10 \text{ subjects})\)

| Formulation       | Mean \( C_{\text{max}} \), ng/mL (%CV) | Mean \( C_{\text{max}} \), ng/mL | Full Body Estimate |
|-------------------|----------------------------------------|---------------------------------|--------------------|
| Cream Emulsion    | 12.2 (41.3)                            | 304.6                           |                    |
| Solid Stick       | 11.5 (27.0)                            | 287.1                           |                    |
| Lotion            | 8.7 (33.7)                             | 217.6                           |                    |
| Continuous Spray  | 9.0 (22.4)                             | 225.4                           |                    |

![Fig. 4](image) a Arithmetic mean (± SD). b Individual serum concentration versus time profiles for four sunscreen formulations over 12 h at baseline skin temperature \((32 ± 2°C)\) with doses applied at 0, 80, and 160 min \((n = 10 \text{ subjects})\).
the room had a high degree of variability for all formulations ranging from 10 to 52% RH and mean room RH values all fell below our target range of 35–55% (Table 4). The humidity was controlled by the building and not for each room; therefore, day to day humidity readings could fluctuate depending on weather.

**Skin temperature and environmental chamber humidity monitoring**

Skin temperature and RH readings under the environmental chamber were recorded every 30 min as well as 5 min and 10 min after each sunscreen application to confirm the target range for each measurement was re-achieved after chamber removal and replacement. Fig. 6 plots the individual skin temperature or RH versus time profiles for all procedure days separated by formulation. The thick dashed lines represent the upper and lower target range while the thin dashed lines for the temperature profiles are the clinical range specified for each procedure day. A tighter window for temperature was utilized to maintain maximum harmonization between in vitro and in vivo study protocols. Only one subject had a single RH reading out of the target range (34%) due to clinical staff error removing the thermal blanket and sheet during the third sunscreen administration which released the equilibrated humidity environment within the chamber. Once dosing was completed and the coverings were replaced, RH quickly returned to the target range. All subjects began each procedure day with skin temperatures within the target range (28–36°C). Any subject with skin temperature measurements below the clinically designated range (30–34°C) quickly achieved the target of 32 ± 2°C within 30 min of initial sunscreen application. No subject had skin temperature readings outside the target range and very few had measurements outside the clinically designated range for extended periods of time. Skin temperatures above 34°C were lowered by venting the cover of the environmental chamber, allowing the skin to slowly cool while maintaining humidity within the target range. Skin temperatures below 30°C were allowed to rise naturally by confirming that the covers were secure over the environmental chamber. This scenario typically only occurred when a measurement was recorded directly after a subject

| Formulation    | Mean Room Temp, °C (%CV) | Mean Room RH, % (%CV) |
|----------------|---------------------------|-----------------------|
| Cream Emulsion | 22.6 (4.5)                | 21.5 (42.0)           |
| Solid Stick    | 22.7 (3.2)                | 23.9 (44.3)           |
| Lotion         | 22.4 (4.7)                | 23.8 (41.4)           |
| Continuous Spray | 22.8 (3.2)            | 23.7 (52.8)           |
had returned from the restroom. Fig. 7 shows the mean skin temperature and RH measurements versus time for all 10 subjects separated by formulation. All formulations maintained mean skin temperature within the clinically designated range (32 ± 2°C) and mean RH under the environmental chamber within the target range (35–55%) for the 12h study duration. Mean skin temperature measurements were also highly correlated to in vitro testing conditions of 32°C with low %CV for all formulations (Table 5).

**Fig. 6** Individual a skin temperature versus time b RH under the environmental chamber versus time recorded for four sunscreen formulations over 12 h (n = 10 subjects). Thin dashed lines represent the target range while thick dashed lines represent the clinically acceptable range.

**Fig. 7** Arithmetic mean a skin temperature versus time b RH under the environmental chamber versus time recorded for four sunscreen formulations over 12 h (n = 10 subjects). Thin dashed lines represent the target range while thick dashed lines represent the clinically acceptable range. Arrows designate when dosing occurred at 0, 80, and 160 min.
IVIVC

IVIVC by deconvolution was able to be achieved by a point-to-point prediction of serum concentration for all four formulations tested (Fig. 8). There was a slight underprediction in \( C_{\text{max}} \) and AUC estimates compared to the observed data. Based on predictability criteria set by the FDA for IVIVC model evaluation of extended-release oral dosage forms, this model successfully predicted oxybenzone exposure (\( C_{\text{max}} \) and AUC) from all four formulations with %PE less than 10% (Table 6) [1]. The cream emulsion had the highest %PE for oxybenzone \( C_{\text{max}} \) and AUC, 8.71 and 5.27%, respectively (Table 6). However, the cream emulsion also had the highest variability in the observed absorption data out of all the formulations likely attributing to the slight underprediction of both model-predicted exposure metrics (Table 3).

Discussion

Clinical studies to assess maximal exposure levels of dermal penetrants, especially those designed for full body application or reapplication, can be both expensive and time-consuming while putting healthy subjects at risk of increased drug or other chemical exposure. Therefore, it is necessary to develop surrogate methods for accurate prediction of maximum exposure levels after dermal complex formulation application. The use of IVIVC to predict in vivo exposure estimates of oral extended-release products from in vitro dissolution testing is accepted by the FDA to assess bioequivalence, scale-up, and post approval changes [1]. The use of IVIVC models for dermal formulations has proven to be challenging with no formal guidance currently published by any regulatory agency for biowaiver claims. However, there have been successful demonstrations of IVIVC models using carefully harmonized IVPT and clinical PK studies [2, 3, 5–11].

Point-to-point (Level A) correlations represent the most robust level of prediction over the entire serum concentration versus time profile [1, 9, 45]. It is the most predictive but also the most difficult to successfully develop without complete harmonization between in vitro and in vivo testing conditions. Level A IVIVC models for prediction of penetrants from patches at both baseline as well as elevated skin temperature conditions have been successfully developed previously with the first published model reported by Yang et al., who adapted a deconvolution approach typically used for oral formulations for prediction of estradiol exposure [7]. Although successful point-to-point prediction models for other patch products have also been established since, there have been very few successful Level A IVIVC models for semisolid or liquid dermal formulations.

Unlike transdermal delivery systems, semisolid and liquid dermal formulations remain unoccluded and absorption profiles can be greatly influenced by the surrounding environment, reapplication time, and accidental removal [20–23, 46]. Although these variables are easily controlled in vitro, precise harmonization during clinical PK testing can pose challenging especially for MUsT studies where the recommendation from the current guidance is to apply the product to 75% of the total body surface area when full body application is recommended [17]. Without precise harmonization between in vitro and in vivo protocol designs, attaining a successful and predictive IVIVC model from IVPT data is nearly impossible.

IVPT testing of dermal formulations have previously been used as a reliable tool for the establishment of predictive

| Formulation        | Mean Skin Temp, °C (%CV) | Mean RH Under Environmental Chamber, % (%CV) |
|--------------------|--------------------------|---------------------------------------------|
| Cream Emulsion     | 32.2 (2.0)               | 45.8 (8.0)                                  |
| Solid Stick        | 32.3 (2.9)               | 44.9 (6.4)                                  |
| Lotion             | 32.2 (2.9)               | 45.5 (5.4)                                  |
| Continuous Spray   | 32.2 (3.2)               | 44.8 (5.8)                                  |

Table 5 Arithmetic mean skin temperature and RH under the environmental chamber for all four sunscreen formulations with associated %CV (n = 10 subjects)

Fig. 8 Predicted (arithmetic mean ± SD) and observed (arithmetic mean ± SD) oxybenzone serum concentrations
Table 6  Observed versus predicted oxybenzone exposure estimates (AUC_{0-12 h} and C_{max}) as well as the %PE for four sunscreen formulations

|                | Cream Emulsion | Solid Stick | Lotion | Continuous Spray | Cream Emulsion | Solid Stick | Lotion | Continuous Spray |
|----------------|----------------|-------------|--------|------------------|----------------|-------------|--------|------------------|
| **Observed**   | 75.79          | 71.07       | 49.71  | 53.20            | 10.82          | 10.01       | 7.98   | 8.74             |
| **Predicted**  | 69.19          | 66.69       | 48.18  | 50.19            | 10.25          | 9.76        | 7.75   | 9.01             |
| **%PE**        | 8.71           | 6.16        | 3.08   | 5.66             | 5.27           | 2.50        | 2.88   | 3.09             |

IVIVC models for patch products and therefore was also used for semisolid and liquid dermal formulation testing in these studies. IVPT studies offer precise control over skin selection, dosage time, sampling time, and skin temperature while maintaining an unoccluded nature after product application. They also utilize a flow-through system for maintenance of sink conditions of oxybenzone and continuous sampling at predetermined time points to match in vivo PK time points. Dermatomed ex vivo human skin was selected as the skin source. Using dermatomed abdominal human skin provides the highest degree of harmonization to the absorption potential of the application site (thighs) during in vivo testing. The rate limiting barrier to absorption, SC, is maintained with similar follicular density to the thighs as well as TEWL readings in vitro [47–50]. Along with harmonizing the absorptive potential of the skin site, skin temperature was also highly controlled at a representative in vivo baseline skin temperature with a cell warmer around each cell placed on the diffusion apparatus. Increases in temperature have shown to amplify skin permeability through fluidization of the lipids within the skin, and prolonged solubility of the permeant within the formulation [21, 22, 51–57]. High temperatures have also slowed permeability of penetrants through the skin for unoccluded products by accelerating vehicle drying time leading to faster thin film formation and permeant precipitation out of the supersaturated residual layer on the skin surface. Although maintenance of precise control over environmental temperature, humidity, and removal is easy with an IVPT apparatus, in vivo control is more challenging.

The environmental chamber design used during our in vivo PK studies offers the ability of testing semisolid and liquid dermal formulations with the highest known degree of harmonization to IVIVC studies. Previously in vivo semisolid formulation absorption testing required the use of occlusive coverings to avoid product removal. However, occlusion has proven to impact dermal absorption by increasing skin hydration, delaying formulation drying rates, and increasing humidity around the application site [58]. With products that require full body testing, occlusive coverings are not an option and if left unoccluded, the high degree of contact transfer can cause highly variable absorption profiles limiting the ability of IVIVC to be successful.

To maximize harmonization between our in vitro and in vivo studies, the use of a smaller application area instead of full body coverage along with the use of our novel environmental control chamber around the application site allowed for skin temperature to consistently remain within the clinical target range of 32 ± 2°C, RH within 45 ± 10%, and avoidance of occlusive coverings. This high degree of harmonization has not previously been achieved and resulted in low variability in absorption profiles in vivo as well as an excellent Level A IVIVC correlation for all four sunscreen formulations.

Despite the relatively small sample size of skin donors in vitro (n = 4) and subjects in vivo (n = 10), a point-to-point correlational model was successfully established with %PE < 10%. According to the FDA guidance for IVIVC development of oral extended-release dosage forms, a successful external prediction requires a %PE of < 10% for both C_{max} and AUC exposure metrics [1]. The IVPT studies were able to discriminate between the flux profiles of each formulation with significantly more oxybenzone absorption occurring from the cream emulsion compared to the other three sunscreens tested. The same significance in vivo for the cream emulsion attributing to the highest permeation was not obtained; however, the rank order of absorptive potential of each formulation was confirmed to be similar with the highest permeation of oxybenzone from the cream emulsion > solid stick > lotion ≈ continuous spray.

Although this study promotes promising results for future IVIVC modeling of complex semisolid and liquid dermal formulations, limitations need to be addressed. For example, the IVPT studies were conducted on excised abdominal skin while the clinical study was conducted on the thighs. Although the barrier function between the two sites via TEWL measurements has been shown to be comparable, the permeability of oxybenzone at the two sites may be different. The dosage of oxybenzone in vitro was higher than what is clinically used and could have an impact on the drying time of the product compared to the drying time in vivo. A larger product dose for IVPT is typically needed for even coverage of the application site within the IVPT cell. In preliminary studies testing 3, 5, and 10 mg/cm² dosing schemes, the 10 mg/cm² sunscreen dose offered the lowest variability in oxybenzone absorption across replicative cells for all of the products tested. However, because oxybenzone is primarily...
absorbed via passive diffusion and the bioavailability is low, the concentration gradient will remain substantially large when dosed at 10 or 2 mg/cm² and the change in application amount should not impact overall conclusions drawn from the IVIVC model. Finally, because this study was not conducted under full body application and skin permeability varies across anatomical sites, assumptions were made when calculating the estimations of maximum full body exposure ($C_{\text{max}}$), and these values should not be used to draw definitive toxicological maximum exposure assessments. However, the low variability associated with absorption of each formulation denotes promising full body exposure estimations that are likely more representative of maximal exposure than actual full body assessment where the variability can be substantially higher.

**Conclusions**

The presented work exhibited successful establishment of a strong IVIVC model for the prediction of maximal oxybenzone exposure in healthy human subjects under reapplication conditions from four commonly marketed semisolid and liquid dermal formulations. The mathematical model related IVPT studies using ex vivo human skin to observed in vivo PK profiles in healthy human subjects in a point-to-point manner for the entire 0–12h study duration. The environmental control chamber design during clinical testing allowed for a degree of harmonization between their vitro and in vivo experimental parameters of temperature, humidity, and contact transfer that were not previously possible. The results illustrated that with a high degree of harmonization, IVPT studies accurately predict maximal exposure levels from complex semisolid and liquid dermal formulations. Further expansion of this work can be performed to test the impact of altered temperature and humidity conditions on product absorption with increased precision. The low variability in the resultant absorption data in vivo present a proof-of-concept protocol design for testing of topical formulations in a more reliable strategy than historic guidelines. Although full body application was not performed in this work, the reliability of the IVIVC model allows for the estimation of expectant penetrant serum levels under full body application without the increased risk MUSt studies can place on the enrolled subjects.

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**Author contribution** Paige Zambrana: writing—original draft; data curation; formal analysis; investigation; methodology; validation; visualization. Dana C. Hammell: writing—review and editing; data curation; project administration; resources. Audra L. Stinchcomb: conceptualization; funding acquisition; methodology; supervision; writing—review and editing.

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**Data availability** N/A.

**Declarations**

**Ethics approval and consent to participate** The University of Maryland, Baltimore Investigational Review Board provided approval for protocol and informed consents for subjects in accordance with the Code of Federal Regulations and local requirements. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

**Consent for publication** The publisher has the authors’ permission to publish this contribution.

**Competing interests** The authors declare no competing interests.

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