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Preliminary clinical pharmacokinetic evaluation of bemotrizinol - A new sunscreen active ingredient being considered for inclusion under FDA's over-the-counter (OTC) sunscreen monograph

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ABSTRACT

Protection against sunburn, skin damage and the carcinogenic effects of ultraviolet light are the primary health benefits associated with UV filters used in topical sunscreen drug products. Countries such as Europe have 30+ UV filters approved for sunscreen products while the US has about 10, greatly reducing the options to provide diverse, effective sun protection products. Bemotrizinol (BEMT) is the first new sunscreen active ingredient to be evaluated for inclusion in the Over-The-Counter (OTC) sunscreen monograph using FDA's new Generally Recognized as Safe and Effective (GRASE) testing guidelines. An *in vitro* skin permeation test (IVPT) and clinical pilot pharmacokinetic Maximum Usage Trial (MUsT) were completed to support the GRASE determination for 6% BEMT. IVPT results indicated an oil +10% ethanol as the model sunscreen intervention for the pilot MUsT. The open-label trial revealed: BEMT concentrations rarely exceeded FDA's defined threshold (0.5 ng/mL) in plasma; no evidence for BEMT accumulation or steady-state concentrations above threshold; only one moderate and few mild treatment emergent adverse events (TEAEs). Therefore, maximal topical applications of 6% BEMT in a model sunscreen formulation did not contribute to meaningful systemic exposure. These results support the safety of BEMT 6% for human sunscreen use.

1. Introduction

Nonprescription sunscreen drugs are topically applied products regulated by the U.S. Food and Drug Administration and indicated to help prevent sunburn. Some are also indicated to decrease the risk of skin cancer and early skin aging, caused by exposure to the sun's ultraviolet radiation, as permitted under FDA's OTC sunscreen drug monograph (FDA, 2021a; 21 CFR 201.327, 2011). Ultraviolet light filters are active ingredients in sunscreen products that provide their protective effects by absorbing, reflecting, and/or scattering UV radiation.

Compared to other countries such as in Europe having 30 or more UV filters approved for use in sunscreen products (Mohiuddin, 2019; Sabzevari et al., 2021), the US has 16 UV filters currently listed on FDA's OTC sunscreen monograph (FDA, 2021a). Of these, only about 10 are currently viewed as technically suitable for formulating sunscreen products that provide adequate, effective, and broad-spectrum UV protection as prescribed by the OTC sunscreen drug monograph. This small number of approved UV filters greatly reduces the options to provide American consumers a variety of innovative, broad spectrum and effective sun protection products. This situation is further complicated by an absence of new UV filters added to the sunscreen monograph in over 20 years and by the FDA's 2021 deemed final order and proposed order for OTC sunscreens request that additional clinical and preclinical safety data be generated for 12 of the 16 sunscreen actives listed on the sunscreen monograph (FDA, 2021b). The data request is driven by the combination of increased consumer use of sunscreen products in the last decades and the results of recent clinical MUsT studies that revealed a measurable percutaneous absorption of those sunscreen actives (FDA, 2021a, b; Matta et al., 2019; Matta et al., 2020). In response to this

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request, the PCPC Sunscreen Consortium has been working with FDA to generate the requested data. This situation presents an urgency to develop and bring to the US market new GRASE UV filters eligible for the OTC sunscreen monograph.

Apart from these PCPC activities, DSM is seeking a GRASE determination for inclusion of bemotrizinol at a maximum concentration of 6 percent on FDA's OTC Sunscreen Monograph via FDA's SIA regulatory pathway. Eligibility for this pathway was based on global safe use of BEMT as a UVA and UVB light absorber since 2000, its regulatory approval outside the United States derived from an extensive nonclinical safety profile of completed tests substantiating 10% BEMT as safe for use in human topically applied sunscreen products. In FDA's 2014 PSO, BEMT was indicated eligible to be considered for inclusion on the OTC sunscreen monograph pending the submission of additional data needed to support a GRASE determination (US Government Accounting Office, 2017; FR 892, 2015). This eligibility decision and the requested additional data were based on FDA's review of the TEA data submitted for bemotrizinol per the requirements of 21 CFR 300.14 (CIBA Specialty Chemicals Corp.; April 11, 2005; 70 FR 72449, 2005).

Bemotrizinol is a high molecular weight, highly photostable, oil soluble broad-spectrum UV filter (Appendix A in Supplementary Material). Compared to older broad-spectrum chemical agents, bemotrizinol is more soluble in cosmetic oils to aid in preparing better, efficacious broad-spectrum sunscreens from oil-in-water emulsion formulations.

As topical sunscreen drugs are intended for long-term use in products with chronic or repeated intermittent use lasting longer than 6 months, the clinical and nonclinical safety requirements are consistent with those data needed for the approval of a new chronic-use topical drug product. These FDA requirements for the studies used in their GRASE determination for new or existing sunscreen active ingredients are set forth in their November 2016 Guidance for Industry (FDA, 2016). Among the requirements are topical safety studies, bioavailability and pharmacokinetic assessments, the evaluation of TEAE observed in clinical studies, and post marketing safety information. Nonclinical safety testing is focused on potential long-term adverse effects or effects not otherwise readily detected from human use like carcinogenicity and reproductive toxicity (FDA, 2021a). Additionally, in May 2019, FDA finalized guidance for industry for conducting Maximal Usage Trials (MUsT) for UV filters to be included on the OTC sunscreen monograph (FDA, 2019; Bashaw et al., 2015). Bemotrizinol represents the first new sunscreen active ingredient to be evaluated under these FDA revised GRASE and MUsT testing guidelines for OTC substances.

1.1. MUsT as GRAS concept

A demonstrated adequate safety margin for an OTC sunscreen active ingredient used at its maximum concentration in finished sunscreen products is a key element of FDA's risk assessment for a GRAS designation, wherein a standard safety margin calculation takes the highest animal NOAEL and compares it to the expected systemic exposure of humans (FDA, 2021a, b). As such, MUsT pharmacokinetic results are important predictors for human systemic exposure and clinical outcomes arising from a topically applied active ingredient because the protocols maximize the potential for drug absorption in a manner that is consistent with anticipated clinical use of the UV filter at amounts allowed on the drug product label (Bashaw et al., 2015).

Variations between individual sunscreen product formulations—in particular, characteristics of the vehicle or form (e.g., cream, lotion, or oil) in which active ingredients are delivered—can affect the percutaneous absorption of sunscreens and thereby have an impact on their safety and effectiveness (FDA, 2021a). Therefore, as the first step in supporting design of the MUsT, FDA requires that a market survey be conducted to identify those formulations and formulation attributes having the highest potential for market use, which are evaluated by an IVPT to determine highest dermal absorption of the UV filter. From these evaluations, at least four unitary target formulations that are representative of the types of dosage forms that could potentially be marketed with the active ingredient should be selected for evaluation in the MUsT pilot and pivotal study phases. At least one of these formulations should contain a known permeation enhancer at a relevant concentration. The formulations selected are not expected to be marketed formulations and are only representative vehicles or product dosage forms that deliver the active ingredient at the highest concentration proposed for use. The MUsT designs call for open label applications of maximal use amounts consistent with FDA OTC sunscreen monograph labeling requirements (21 CFR 352.52, 2003). The formulation selected for evaluation in the pilot study must be the IVPT formulation that shows the highest permeation among the those tested.

1.2. IVPT integral to MUsT design

The IVPT is an evaluation tool that has been used in dermal formulation development for over 30 years and is widely used to measure relative skin permeability and characterize the potential systemic exposure of topically applied drugs (Santos et al., 2020; Oh, 2020; FDA, 2021a). Historically, the lack of a standardized approach to conducting IVPTs has contributed to high variability and low reproducibility observed in published IVPT studies. The regulatory utility of the IVPT is as a tool to characterize systemic absorption of potential sunscreen active ingredients from various product formulations and reduce the number of non-clinical studies needed to support selecting candidate formulations or reformulating existing products. FDA recognized this utility and has improved the accuracy and reproducibility of IVPTs (Oh, 2020; Yang, 2020) with several recommendations and requirements as formalized into a guidance for industry (FDA, 2019). Additional IVPT guidelines have also been published by the EU under OECD Guideline no. 428 and EC test method No. 440/2008, Method B.45 (OECD, 2004; EC, 2008). These guidelines and recommendations were followed to conduct the IVPT for BEMT. Consequently, as part of the testing requirements to support an FDA GRASE determination for BEMT, the IVPT preceded a clinical pilot MUsT. Below we present the approach and results for these tests.

2. Material and methods

2.1. In vitro percutaneous permeation testing

The IVPT (IES 2019) used human skin membranes, obtained surgically from 8 different donors and stored frozen until use, fitted to flow-through chambers in 5 groups dosed for 24 h with one of the following 6% BEMT-containing market image formulations: Sunscreen Oil, Sunscreen Oil + 10% ethanol as skin permeation enhancer, Oil-in-Water emulsion without or with 5% propylene glycol permeation enhancer, and Water-in-Oil cream emulsion. The 14C-BEMT in a formulation was applied onto the skin membranes at a nominal dose level of 2 mg formulation/cm2 corresponding to 0.12 mg BEMT/cm2 for a 24h exposure by nonoccluded conditions. Flow-through receptor fluid was phosphate buffered physiological saline with the addition of 5% (w/v) bovine serum albumin (BSA) collected in hourly intervals between 0 and 8 h and thereafter in 2 h intervals until the end of the experiment at 24h after dosing. The 14C-BEMT radioactivity was determined in skin wash, tape-stripped stratum corneum, remaining skin membrane, receptor chamber and dosing chamber rinses.

Bemotrizinol permeating into and through the skin was at a maximum of $1.35 \pm 0.70\%$ (mean $\pm$ SD), normalized to 100% recovery, that was associated with the sunscreen oil formulation without 10% ethanol; all other formulas used in the IVPT showed lower permeation amounts, indicating that the sunscreen oil formulations provided one of the best options for BEMT dosing. A statistical comparison of the results obtained for the two sunscreen oil formulations showed that while the permeation value obtained for the formulation with 10% ethanol was lower than the value obtained for the second sunscreen oil formulation

(0.85 ± 0.77% vs.1.35 ± 0.70%, respectively), this difference was not statistically significant (*t-test*, $p = 0.26$). Therefore, based on FDA's MUsT Guidance (FDA, 2019) that without meaningful differences in permeation being observed a formulation with a skin permeation enhancer is preferred to be tested, the sunscreen oil containing 10% ethanol as skin permeation enhancer was selected as the dosage form for the MUsT pilot phase. (APPENDIX B, Supplementary Material).

2.2. Clinical testing: MUsT

Ethics and Consent to Participate. All pertinent study documents received ethical clearance for research involving human participants according to the Declaration of Helsinki, from DSM Nutritional Products AG and by the clinical research unit's institutional review board (Integreview, Austin, Texas). Study participants gave written informed consent before initiation of any study-specific procedures. The clinical trial is registered at Clinical Trials (http://ClinicalTrials.gov) identifier NCT04355286.

2.2.1. Study design

As noted above, the IVPT results and FDA Guidance determined the intervention selected for the pilot phase clinical trial. The MUsT protocol design is a single center clinical trial conducted in 2 parts: Part 1 as a phase 1 pilot MUsT; and Part 2 as a phase 3 pivotal MUsT. The pilot MUsT was an open-label, 1-arm trial to determine if BEMT reaches quantifiable plasma concentrations after multiple-doses of a market image sunscreen formulation containing 6% BEMT applied to healthy adult participants. The drug product intervention was an oil formulation of recognized safe for use excipients with 10% ethanol added as a skin permeation enhancer, plus 6% (w/w) BEMT. The trial was conducted in November 2020 at Spaulding Clinical Research LLC, West Bend, Wisconsin, USA.

Each trial participant provided their written informed consent. Fourteen participants, 7 male and 7 female, were admitted to the clinical research unit on Day 1 and remained in-clinic until discharged on Day 5, with outpatient return visits once on Days 8 and 12. On the morning of Days 1 through 4, participants received a topical application of study drug between 07:00 and 10:00 h followed by 3 more applications each day at 2, 4, and 6 h after the first application. Study drug application after first dose were at: 0, 2, 4, 6 h; on Day 2 at 24, 26, 28, 30h; on Day 3 at 48, 50, 52, 54h; and on Day 4 at 72, 74, 76, 78h (last dose). Participants showered only once daily after the days' first blood sample but before the first daily dose and wore loose-fitting garments (clinical "scrubs") during the day. As a maximum usage pharmacokinetic study for topical sunscreen drug ingredients, the dosing frequency is considered representative of consumer sunscreen usage (Bashaw et al., 2015; FDA, 2019**)**.

A research unit staff member weighed the study drug container in advance and then after each dose that was applied at 2 mg formulation per 1 cm^2 of skin over 75% of total body surface area (DuBois and DuBois, 1916) typically exposed to sun, excluding the covered antecubital blood sampling area; participants wore typical swim wear during dosing. Blood sample collection was by stick phlebotomy into K_2EDTA treated vials, centrifuged, and the collected plasma stored frozen until analysis. Specific sample times after first daily dose were: Day 1, -0.5, 2, 4, 8, 12 h; Day 2, 23.5, 28, 32 h; Day 3, 47.5, 52, 56 h; Day 4, 71.5, 74, 76, 78, 80, 82, 84, 86 h; Day 5, 96 h; Day 8, 168 h; and Day 12, 264 h. Bemotrizinol plasma concentrations, determined after protein precipitation extraction, were analyzed by Ultra-high Pressure Liquid Chromatography with tandem Mass Spectrometry and electrospray ionization detection, according to a validated method with a lower limit of quantitation (LLOQ) of 0.5 ng BEMT/mL plasma (**Appendix C; Supplementary material)**.

2.3. Intervention

The identified market image formulation containing 6% BEMT (PARSOL® Shield, DSM Nutritional Products AG, Switzerland) was a sunscreen oil + 10% ethanol as skin permeation enhancer prepared for this open-label trial by the combination of commonly used sunscreen topical excipients compatible to ensure BEMT was stable in the applied mixture. The drug ingredient was of standard quality of at least 97.5% purity. The model (not to be marketed) formulation was considered appropriate, based on FDA's MUsT design guidance (FDA, 2016; FDA, 2019), for the pilot study to give controlled clinical indication for BEMT to reach systemic circulation and evaluate trial design and analytical instrumentation performance.

2.4. Participant cohort

The number of participants was based on recommendations in the sunscreen guidance for MUsT pilot investigations (FDA, 2019; Bashaw et al., 2015) and direction from the FDA reviewers of our study protocol. Healthy participants were recruited from the clinical research laboratory database of regular study participants and via open advertisements by email, telephone, and online during August and September 2020. A CONSORT flow diagram for the enrollment of subjects, their allocation to treatment, disposition status and if included for analysis in the trial is shown in Fig. 1.

The eligible 14 selected participants were admitted to the clinic after showing a negative test result for COVID-19 (polymerase chain reaction for SARS-CoV-2). Inclusion criteria were met for those male and females 18 up to 75 (inclusive) years of age with body mass index of 18.5–29.9 kg/m^2, and at screening had a normal medical history, including negative test results for alcohol and drugs of abuse. The main exclusion criteria included skin irritation, broken or unhealed skin, active sunburn, tanning bed use in previous month, blood abnormalities (e.g., anemia) or other underlying chronic or medical conditions, or within 2 weeks before clinic check-in had used products containing BEMT.

2.5. Technical information

The primary objective was to determine whether BEMT at 6% in a high-penetrating sunscreen-like formulation is absorbed into the systemic circulation when applied under maximal-use conditions. A secondary objective was to obtain information to support a successful pivotal MUsT study.

Bemotrizinol plasma concentration analytical results from 22 blood samples per subject, collected from before the first topical study drug application to 264 h (12 days) after the first application, were used for primary descriptive pharmacokinetic parameters, when sufficient data permitted.

TEAEs were recorded when observed at the daily dosing times, categorized and summarized by seriousness, severity, relationship to treatment, and by treatment. Additional safety assessments were based on vital sign measurements (blood pressure, heart rate, respiratory rate, oral body temperature) conducted at physical examination of the participants. A summary of the TEAEs recorded for each subject by MedDRA System Organ Class Preferred Term is presented in APPENDIX E (Supplementary **Material**).

2.6. Statistics

Plasma concentrations and PK parameters of BEMT were listed and summarized using descriptive statistics (frequency, arithmetic mean, geometric mean, standard deviation, minimum, median, maximum, percent coefficient of variation [CV%], and geometric CV%) by nominal sampling time and parameter. By post hoc analysis any plasma BEMT concentration not quantifiable (BLQ) was treated as zero for descriptive statistics and set as zero for calculations.

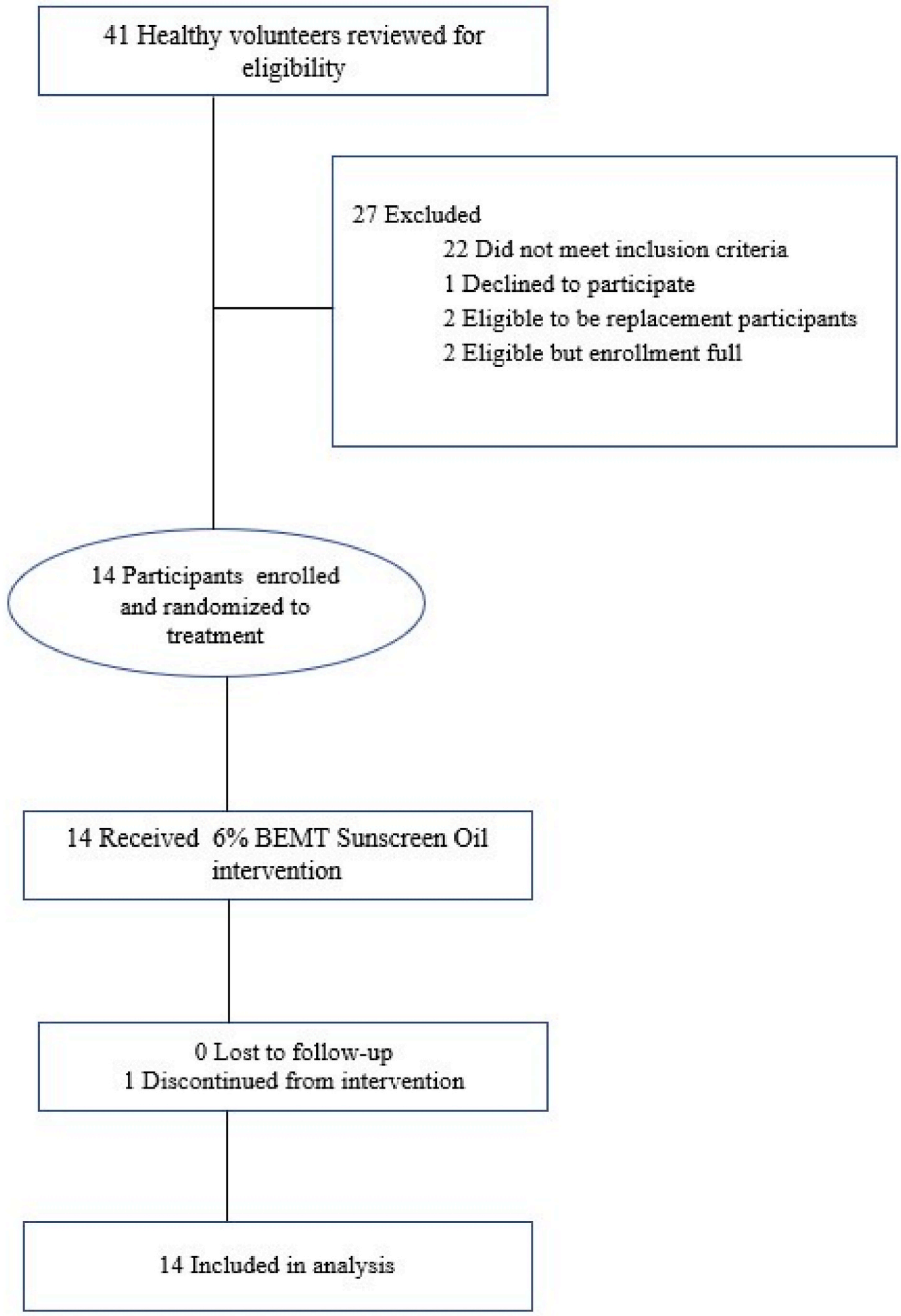

Fig. 1. Flow of participants in pilot study of bemotrizinol in sunscreen oil.

Plasma pharmacokinetic parameters were calculated, data permitting, by noncompartmental methods using Phoenix® WinNonlin® Version 8.3 and observed drug concentration time-course data for each dosing day as follows: C_{max}, maximum bemotrizinol concentration; T_{max}, time of C_{max}; concentrations 2 h after each of the 4th daily doses (C_{2h}), and at the end of each of the 4 study days (C_{trough}); area under the plasma concentration time curve from before first daily dose to the last measurable concentration over 24 h for Overall, Day 1 and Day 4 ($AUC_{overall;}$ $AUC_{0–24h}$). Additionally, overall C_{max} and overall T_{max} were calculated for the 12-day (0–264 h) trial period. Elimination parameters included terminal half-life ($t_{1/2}$) as computed from the apparent elimination rate constant (λ_z).

The PK population included all participants receiving at least one application of study drug and had at least one PK parameter after study drug application; this population was used for descriptive statistics of BEMT concentrations and PK parameters. Values for λ_z or $t_{1/2}$ were not reported for cases not exhibiting a terminal log-linear phase in the concentration-time profile and PK parameters were not calculated for participants with fewer than 3 quantifiable concentrations following BLQ imputation. If all concentrations for a participant were BLQ, then only the C_{2h} and C_{trough} parameters were reported for that individual.

The safety population included all participants who received at least one application of the study drug. The incidence of AEs, organized by system organ class and preferred term, were summarized with a focus on TEAEs. Clinical laboratory test results and change from baseline, summarized using descriptive statistics, were evaluated for clinically significant results. Vital sign measurements and change from baseline were summarized using descriptive statistics by treatment and visit. Fitzpatrick skin type assessments, determined during the check-in process by the admitting Clinician, were listed for each participant.

3. Results

3.1. Disposition

A total of 14 participants were enrolled and 13 (92.9%) completed the study. One participant was discontinued from the study on Day 4 due to TEAEs of erythema and urticaria and therefore did not receive any of the Day 4 applications (Fig. 1).

3.2. Demographic and baseline characteristics

Of the 14 participants enrolled, 9 (64.3%) were White, and 5 (35.7%) were Black or African American, and most participants were not Hispanic or Latino (85.7%). The Fitzpatrick skin type assessments on Day 1 were IV (8 participants, 57%), III (5, 36%), and V (1, 7%). Other demographic information is included in Appendix D (**Supplementary Material**). Demographic and baseline characteristics were the same for the safety and PK populations. All inclusion criteria and no exclusion criteria were met; none of the participants had on-going medical history findings at screening to preclude entry into the study.

3.3. Compliance

All 14 participants received each application of the 6% BEMT in oil except one (no. 1010) who discontinued before Day 4 dosing. The completing 13 participants (92.9%) received 16 applications of study drug and 1 (7.1%) received 12 applications. The mean weight of study drug applied per subject and application was 27.75 g, which contained 1.66 g BEMT for 6.64 g BEMT per day. Four protocol deviations included: follow-up visit conducted outside of scheduled window (1 subject on Days 8 and 12) and PK blood sample collected outside of scheduled window (2 subjects on Day 4); they were deemed not to affect the data analysis or interpretations.

3.4. Pharmacokinetics

Analytical comparative results showed that BEMT was stable in stored-frozen plasma samples for at least 6 months and that whole blood and plasma represented BEMT equally in systemic circulation.

Thirteen of the 14 participants completed the PK sample collection schedule; one (7.1%) discontinued before Day 4 sampling and their data were excluded from any PK parameter for Day 4 and afterwards. A total of 299 plasma samples collected and analyzed showed 13 samples (4.3%) with quantifiable BEMT, 9 (3.0%) as not reportable (NR) per the laboratory's standard operating procedure for lack of internal standard reproducibility after multiple assays, and 277 (92.6%) were BLQ (<0.5 ng/mL). In 7 (50%) participants BEMT was BLQ; in the remaining participants, 13 of 154 samples were quantifiable and the maximum number of quantifiable concentrations per participant was 1, 2, and 5 on Day 1, Day 4, and overall, respectively. All samples after 82 h had BLQ BEMT concentrations as shown in Table 1.

The small number (13/299; 4.3%) of quantifiable plasma samples precluded calculation of meaningful PK parameters for C_{max} and T_{max} on Days 1 and 4, and of C_{2h} and C_{trough}. In post hoc data analysis, all BLQ concentrations were set to zero, NR concentrations were set to missing, and a minimum number of concentrations was not required to report any PK parameter. Applying these rules provided a data set with some estimated PK parameters (Table 2).

The limited amount of concentration data and estimated PK parameters did not indicate BEMT accumulation or steady state above 0.5 ng/mL (LLOQ), the FDA threshold of relevance. The $AUC_{0\text{-}24}$ was based on only 1 or 2 quantifiable concentration results, rendering them as not reliable indicators of systemic exposure. All the plasma concentrations above 0.5 ng/mL in the 5 of 7 participants with quantifiable BEMT concentrations were BLQ at the next measurement time point, while 2 participants each showed quantifiable but decreasing BEMT concentrations at the next time point, 2 h later . The 13 quantifiable BEMT values were 0.56–2.29 ng/mL; an overall C_{max} mean of 0.55 ng/mL (SD 0.72) was derived after post hoc analysis (Fig. 2).

3.5. Adverse events

In the five participants with skin irritation, laceration, or ecchymosis their BEMT plasma concentrations did not indicate a concomitant or enhanced BEMT absorption profile, including that for the discontinued subject (1010). The TEAEs were reported in 8 of 14 participants (57.1%), consisting primarily of mild skin irritation for 7 subjects; the discontinued participant showed moderate severity erythema and urticaria. Results for completing participants were not remarkable for the clinical laboratory tests, vital sign measurements or the physical, other than skin, examinations (**Appendix E, Supplementary Material**).

4. Discussion

The concepts behind the MUsT have been part of FDA drug regulatory requirements to show bioavailability of dermally applied products since the mid-1970s. Study design refinements, increased analytical detection sensitivity, and standardization of FDA requirements in the 1990s coalesced into the current MUsT design (Bashaw et al., 2015). Unlike the NDA process, which is for a single drug formulation, an OTC

Table 1
Plasma Bemotrizinol Concentration Data by Subject: each cell represents one plasma sample. (Color printing requested).

	Day 1								Day 3													
						Day 2						Day 4									Day 8	Day 12
	Nominal Time (h)																					
	0	2	4	8	12	23.5	28	32	47.5	52	56	71.5	74	76	78	80	82	84	86	96	168	264
SUBJID	Reported Concentration (ng/mL)																					
1001														2.29	0.74							
1002																						
1003																						
1004				0.74				1.63			0.91			1.08	0.82							
1005			0.67																			
1006																						
1007													0.56				0.58					
1008																						
1009																						
1010							0.61															
1011								1.23														
1012																						
1013															0.74							
1014																						

Note: Blank cell = BLQ (0.5 ng/mL); orange shading = NR; grey shading = no sample; red font = C2h.

Table 2
Post hoc analysis of plasma BEMT pharmacokinetic parameters.

	C_{max}(ng/mL)			T_{max}[a](h)			$AUC_{0\text{-}24}$[b](ng*h/mL)	
	Day 1	Day 4	Overall	Day 1	Day 4	Overall	Day 1	Day 4
n	14	13	14	14	13	14	14	13
Mean	0.1004	0.3613	0.5537	–	–	–	0.3581	1.032
SD	0.25548	0.68355	0.72481	–	–	–	0.92929	1.8696
Min	0.000	0.000	0.000	0.00	0.00	0.00	0.000	0.000
Median	0.0000	0.0000	0.2915	0.000	0.000	1.962	0.0000	0.0000
Max	0.738	2.29	2.29	8.01	10.00	82.00	2.98	5.81

	C_{2h}(ng/mL)				C_{trough}(ng/mL)			
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
n	14	14	14	13	14	14	14	13
Mean	0.0527	0.2043	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SD	0.19724	0.52518	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
Min	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Median	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Max	0.738	1.63	0.000	0.000	0.000	0.000	0.000	0.000

Abbreviations: BEMT, bemotrizinol; Min, minimum; Max, maximum.
[a] For T_{max}, only n, min, median, and max are shown.
[b] The maximum number of quantifiable concentration data points used to compute a subject's $AUC_{0\text{-}24h}$ was 1 for Day 1 and 2 for Day 4.

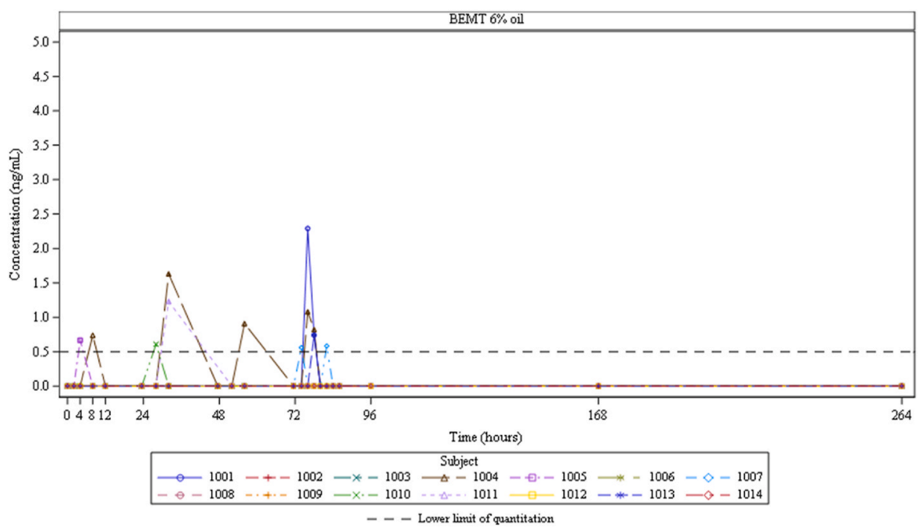

Fig. 2. Individual Plasma Bemotrizinol Concentration-Time Profiles Relative to First Dose. (Color printing requested).

GRASE drug substance is anticipated to be prepared into any number of inactive formulating ingredients (excipients) to make a commercial drug product, which is the reason the MUsT uses several representative market image formulations (FDA, 2019; 2021a).

The key application of the *in vivo* PK results is as the human systemic exposure for comparison to the highest no-observed-adverse-effect level from non-clinical tests. This safety margin calculation represents the quantified level of human risk for substances with demonstrated nonclinical adverse effects (FDA, 2019; 2021a). Thus, the revised FDA Guidance for GRASE determinations sets forth nonclinical and clinical testing requirements (FDA, 2016; FDA, 2021a; FDA, 2021b) as the regulatory pathway to listing an UV filter on the OTC sunscreen monograph. Any existing or new UV filter like BEMT is expected to follow this path.

The pilot MUsT study demonstrated that maximal topical applications of 6% BEMT in a high penetrating model sunscreen formulation showed limited above threshold systemic exposure. These results are in stark contrast to MUsT results obtained by FDA for 7 common UV filters in US-market sunscreens that showed plasma concentrations notably above 0.5 ng/mL after four Day 1 doses and after the subsequent 3 dosing-days, remaining quantifiable in over 50% of participants up to 7–21 days for six of the UV filters evaluated. Using two sprays, a lotion and cream formulations, plasma geometric mean concentrations (% coefficient of variation) for Avobenzone were 4.0 (60.9), 3.4 (77.3), 4.3 (46.1), and 1.8 (32.1) ng/mL, respectively; for Oxybenzone 209.6 (66.8), 194.9 (52.4), 169.3 (44.5), and not applicable; for Octocrylene 2.9 (102), 7.8 (113.3), 5.7 (66.3), and 5.7 (47.1) and for Ecamsule 1.5 (166.1) ng/mL, cream was the only applicable product (Matta et al., 2019, 2020). These MUsT designs followed the general principles for PK studies but were intending to show systemic concentrations of the UV filter active ingredients currently on the sunscreen monograph and therefore used commercially acquired sunscreen products not market image formulations.

While the low systemic availability of BEMT in this small sample population may be related to formulation differences from those used in the seven commercial sunscreens cited above, it is likely most accountable to the physical-chemical characteristics of the BEMT molecule, regardless of formulation excipients. This was apparent in the very low (<2% of applied dose) percutaneous permeation of BEMT from the 5 different market image sunscreen formulations used in the IVPT (IES, 2019). Excipients and molecular structure, among other factors, influence behavior of substances in formulations and on their percutaneous permeation (Benson 2000; FDA, 2021a; Surber et al., 2021). Indeed, the large variances around the plasma mean concentrations for the different UV filters reported by Matta et al. (2019, 2020) indicate that other factors, such as excipients used and the molecular characteristics of the formulation, can influence percutaneous absorption.

This multidimensional construct of factors influencing skin absorption, systemic exposure, and the anticipated human risk estimation for a GRASE candidate is addressed in the FDA determination pathway for all existing and new sunscreen active ingredients as announced on September 24, 2021, in its PO to amend the OTC monograph for sunscreen drug products (FDA, 2021a). This encodes particularly the IVPT linkage to the MUsT program as primary relevance for estimating human safety of a UV filter on the sunscreen monograph and extending that assessment to other proposed drug product formulations by requiring manufacturers to perform IVPT evaluations before marketing each new formulation. Under a final sunscreen monograph FDA anticipates each UV filter will establish a standard “control formulation” based on the primary IVPT and MUsT program formulations for comparison to IVPTs of any new market formulations containing that active ingredient. Other risk-relevant considerations like nonclinical safety outcomes being unremarkable, the IVPT results may be used to assess a new formulation as equivalent to or less safe than the primary formulation. From this new regulatory perspective for GRASE determinations these first results with BEMT illustrate the FDA approach that will set the IVPT results and our completed MUsT program into the perpetuity of the regulatory record.

4.1. Limitations

Several limitations of the pilot trial design were unavoidable to meet the objectives of this controlled clinical trial. Participants were confined to the clinic's conditions that excluded those usually associated with sunscreen usage, such as outdoor full sun exposures, temperatures, and other personal activities. The number of trial participants were an abbreviated cross-section of consumer skin types, ages, behaviors, and concomitant topical product uses compared to an approved UV filter in widely marketed commercial consumer products. Trial duration was necessarily short to accommodate participation while allowing compliance with regulatory test design guidance and showing pharmacokinetic indicators of systemic exposures. The planned pivotal MUsT with more subjects exposed to one of three 6% BEMT formulations will represent a more complete market image constellation as recommended by FDA and effectively measure the percutaneous pharmacokinetics of BEMT as a single active ingredient across wider ages and population sample set.

5. Conclusions

This first clinical trial evaluated whether 6% bemotrizinol, the sunscreen active ingredient in a market image formulation also containing 10% ethanol as a skin permeation enhancer, reached systemic plasma at concentrations exceeding a threshold of 0.5 ng/mL under the FDA MUsT design. Results showed BEMT rarely exceeded the defined plasma threshold without evidence of accumulation or steady-state concentrations above 0.5 ng/mL. While the study was designed to attain steady state levels, the 13 of 299 plasma samples with quantifiable BEMT did not support a reliable plasma AUC estimation, although a steady state concentration below the 0.5 ng/mL LLOQ could not be excluded. TEAEs for the model not to be marketed sunscreen formulation were few and not severe. As such, results of the pilot MUsT support the safety of BEMT 6% for human use.

Based on results and findings of this pilot trial, the study design of the planned pivotal MUsT has been modified as follows: the implemented analytical method has been validated to a lower limit of quantitation of 0.1 ng BEMT/mL; the upper age limit for participants has been removed; a defined statistical factorial model of participant's age, gender and ethnicity per study arm will be implemented for recruitment guidance; plasma samples will be collected additionally at 75, 77, and 79 h, but not collected after study day 5; all BLQ concentrations will be set to zero, and no minimum number of quantifiable samples will be required to report any PK parameter other than λ_Z or $t_{1/2}$.

From a regulatory perspective, the results of the BEMT full MUsT will determine the potential systemic exposure to BEMT from topically applied sunscreens in a risk assessment to inform FDA's GRASE determination for sunscreen use. Equally important, the approach taken to date in conducting these studies demonstrated for the first time an implementation of FDA's recent sunscreen safety guidance and illustrate the early steps on the pathway to obtain an FDA GRASE determination for both new and existing sunscreen active ingredients under the OTC sunscreen monograph.

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DSM Contributors for study support and execution

Szabolcs Péter: supervised the study as the medical monitor. **Stefan Kaiser**: consulted on the study design and development phases. **Elodie Chenal**: coordinated and managed analytical methods development and clinical trial plasma samples analysis at the contract laboratory. **Karina Radomsky and Anne Janssen:** provided technical and logistics support in preparation and shipment of the DSM test formulations.

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CRediT authorship contribution statement

Carl D. D'Ruiz: Conceptualization, design, monitoring, Writing – original draft, Writing – review & editing. **James R. Plautz:** Design, Writing – review & editing. **Rolf Schuetz:** Design, clinical study director, writing, and manuscript review. **Carlos Sanabria:** Investigation. **Jody Hammonds:** Methodology, writing, Formal analysis. **Cassandra Erato:** Design, Formal analysis, writing. **Jochen Klock:** Formulations, Writing – review & editing. **Juergen Vollhardt:** Design, Formal analysis, Writing – review & editing. **Szilvia Mesaros:** Project administration, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Carl D. D'Ruiz reports a relationship with DSM Nutritional Products Inc that includes: employment. James R. Plautz reports a relationship with DSM Nutritional Products AG that includes: consulting or advisory. Rolf Schuetz reports a relationship with DSM Nutritional Products AG that includes: employment. Carlos Sanabria reports a relationship with Spaulding Clinical Research that includes: employment. Jody Hammonds reports a relationship with Spaulding Clinical Research that includes: employment. Cassandra Erato reports a relationship with

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Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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Glossary

ASEAN: Association of Southeast Asian Nations
BEMT: bemotrizinol
BLQ: below limit of quantitation
CONSORT: Consolidated Standards of Reporting Trials
EC: Council Regulation
EU: European Union
FDA: U.S. Food and Drug Administration
GRASE: generally recognized as safe and effective
IVPT: *in vitro* percutaneous permeation test
LLOQ: lower limit of quantitation
MED: Minimal Erythemal Dose
MERCOSUR: Mercado Común del Sur
MedDRA: Medical Dictionary for Regulatory Activities
MUsT: Maximum Usage Test
NDA: new drug application
OECD: Organization for Economic Cooperation and Development
OTC: over-the-counter
PCPC: Personal Care Products Council
PK: pharmacokinetic
PSO: Proposed Sunscreen Order
SPF: Sun Protection Factor
SIA: Sunscreen Innovation Act
TEA: Time and Extent Application
(TEAE): Treatment Emergent Adverse Events
US: United States of America
UV: ultraviolet
UVA: Ultraviolet light (radiation) in wavelengths 315–400 nm
UVB: Ultraviolet light in wavelengths 280–315 nm