## 2.4 Nonclinical Overview

The section provides a comprehensive analysis of the nonclinical information contained in the electronic Common Technical Document (eCTD) for Bemotrizinol, submitted under IND 146892.

# Table of Contents

List of Abbreviations

| | |
|---|---|
| ADME | Absorption, distribution, metabolism, excretion |
| API | Active Pharmaceutical Ingredient |
| AUC | Area Under the Concentration-Time Curve |
| BEMT | Bemotrizinol |
| BLQ | below limit of quantification |
| b.wt. | body weight |
| $C_{max}$ | Maximum concentration |
| CYP | Cytochrome P450 enzymes |
| DART | Developmental & Reproductive Toxicity |
| DMSO | Dimethylsulfoxide |
| EC | European Commission |
| EU | European Union |
| FDA | Food and Drug Administration |
| GRASE | Generally Recognized as Safe and Effective |
| GLP | Good Laboratory Practice |
| HPLC | High-performance liquid chromatography |
| ICH | International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use |
| IND | Investigational New Drug |
| IVPT | *in vitro* permeation test |
| LOQ | Limit of Quantification |
| LLOQ | Lower Limit of Quantitation |
| MEDLINE | Medical Literature Analysis and Retrieval System Online |
| MGF | Monograph File |
| MUsT | Maximal Usage Trial |
| NOAEL | No Observed Adverse Effect Level |
| OECD | Organisation for Economic Cooperation and Development |
| OMOR | OTC Monograph Order Request |
| OTC | Over the Counter |
| PD | Pharmacodynamic |
| PK | Pharmacokinetic (may also occur as TK, toxicokinetic) |
| SD | Standard Deviation |
| SPCC | synthetic peptides containing cysteine |
| SPCL | synthetic peptides containing lysine |
| SULT | Sulfotransferase |
| $t_{1/2}$ | Half-life |
| UGT | Uridine 5'-diphospho-glucuronosyltransferases |
| US | United States of America |
| USP | United States Pharmacopoeia |
| UV | Ultraviolet |
| UVA | Ultraviolet A |
| UVB | Ultraviolet B |
| UVR | Ultraviolet radiation |
| vs. | Versus |

### 2.4.1 Overview of the nonclinical testing strategy

DSM is seeking an FDA determination that Bemotrizinol (BEMT) 6% is Generally Recognized as Safe and Effective (GRASE) as a broad-spectrum (UVA-B) sunscreen active ingredient under the FDA's Over-the-Counter Monograph M020: Sunscreen Drug Products for Over-the-Counter Human Use. This request is being made via FDA's OMOR Tier 1 process (monograph file MGF400105). This section provides a summary of the nonclinical testing strategy employed to support the GRASE determination for BEMT 6% and includes an integrated assessment of the pharmacologic, pharmacokinetic, and toxicologic evaluations conducted on BEMT.

The nonclinical safety of BEMT is supported by pharmacokinetic and toxicology test results submitted in 2005 and 2006 under a Time and Extent Application (TEA) for BEMT (FDA Docket No. 2005N-0446). These results have been supplemented by additional nonclinical tests sponsored and conducted by DSM following a meeting with the FDA on June 7, 2019. At this meeting, DSM received feedback on the necessary safety and efficacy data required to support the GRASE determination for BEMT under the OTC Sunscreen Monograph.

DSM's approach to supporting the nonclinical safety of BEMT adhered to the FDA's 2016 Guidance for Industry on Nonprescription Sunscreen Drug Products - Safety and Effectiveness Data, the FDA's 2019 Guidance on Maximal Usage Trials for Topically Applied Active Ingredients, and relevant ICH guidelines. This approach also incorporated advice and recommendations from DSM's proposed investigation plan and subsequent updates under IND 146892. All tests were planned and initiated following discussions with the FDA, and a chronology of these meetings and discussions is presented in Module 1 of this OMOR submission. Additionally, the FDA's 2001 Guidance for Industry: M4S - The CTD-Safety was used to organize the Module 2.4 nonclinical section of our OMOR (MGF 400105) submission.

The nonclinical studies recommended by the FDA to support a GRASE determination for BEMT 6% as an OTC sunscreen active ingredient include assessments of dermal carcinogenicity, systemic (oral) carcinogenicity, developmental and reproductive toxicity, toxicokinetic studies on oral and dermal absorption, distribution, metabolism, excretion (ADME), and hormonal effects. Additionally, pharmacokinetic (PK) in vitro permeation tests (IVPT) were indicated as being necessary to measure the percutaneous penetration of the BEMT in the market image sunscreen formulations proposed for use in the clinical pilot and pivotal pharmacokinetic Maximum Usage Studies (MUsT).

DSM's GLP-based nonclinical testing strategy, as outlined in our IND 146892 general investigational plan, investigational brochure and annual report updates, fully align with the FDA's nonclinical testing recommendations mentioned above. Additionally, the strategy incorporates the advice and recommendations received from the FDA under IND 146892. *Table 1* provides a list of the nonclinical studies conducted under IND 146892 to support a GRASE determination for BEMT 6%.

*Table 1* also provides a very short summary of the rationale for waiving the 26-week systemic carcinogenicity oral dosing study in transgenic (RasH2) mice. This request is justified based on ICH guideline S1B(R1) (https://database.ich.org/sites/default/files/S1B-R1_FinalGuideline_2022_0719.pdf), FDA's S1B(R1) Addendum to S1B Testing for Carcinogenicity of Pharmaceuticals Guidance for Industry (https://www.fda.gov/media/152777/download), and the FDA's 2016 Nonprescription

Sunscreen Drug Products – Safety and Effectiveness Data Guidance for Industry (https://www.fda.gov/media/94513/download). According to these guidelines, systemic carcinogenicity studies may be waived if both of the following conditions are met: (1) an adequately conducted human pharmacokinetic Maximum Usage Trial (MUsT) shows a steady-state blood level of less than 0.5 ng/mL, and (2) an adequately conducted toxicology program reveals no safety signals for the ingredient or any structurally similar compound that would suggest potential adverse effects at lower levels. The full version of the waiver justification is found in Module 1.6.2 (DSM's Dec 11, 2024 Type Y meeting background Part 1 and Part 2 submission package) of this OMOR.

*Table 1. Nonclinical Studies Conducted under IND 146892*

| IND 146892 Study | Test Design and Objectives | Status |
|---|---|---|
| ***Non-Clinical Studies*** | | |
| *In Vitro Tests* | | |
| $^{14}$C-BEMT (6%) In Vitro Percutaneous Permeation Test (IVPT) with Human Skin Membranes Innovative Environmental Services (IES) Ltd Study No. 20190089 | This study was designed to provide information on the dermal penetration of BEMT formulated in different market image sunscreen formulations at a concentration of 6% (w/w) on human skin membranes. The IVPT was performed utilizing FDA's May 2019 guidance document: "Maximal Usage Trials for Topically Applied Active Ingredients Being Considered for Inclusion in an Over-The -Counter Monograph: Study Elements and Considerations. The tests were performed under GLP conditions, followed OECD Guideline No. 428 (2004) and considered the Guidance Notes on Dermal Absorption No. 156 from OECD (2011). Original study and summary was provided under Serial 0000, section 2.6.4.3. | Completed |
| $^{14}$C-BEMT (6%) *In Vitro* Percutaneous Permeation Test (IVPT) with Human Skin Membranes – Report/Project No.: 10B0377/21B110 | This IVPT was conducted to address the request from FDA to conduct a second IVPT to understand any effect of butyloctyl salicylate on the skin permeation of BEMT. Study summary and report submitted under Serial 0009, section 4.2.2.2. | Completed |
| *In vitro* investigations of the metabolic profile of BEMT by incubation of human hepatocytes and S9 fractions with $^{14}$C-Bemotrizinol. DSM Report RD-00068522 | $^{14}$C-BEMT incubated with human liver enzymes in hepatocyte suspensions and liver S9 fractions plus relevant co-factors to assess CYP, SULTs, UGTs, and GSH for Phase I & II metabolic conjugates formation. Study summary submitted under Serial 0010, section 1.13.8.1.1.2. | Completed |
| *Toxicokinetics* | | |
| Single and repeat dosing PK study by oral route, rat | A time course toxicokinetic study was conducted with single and repeat dosing by oral gavage to establish the rat plasma steady state level of BEMT to compare to human systemic levels for a margin of safety of human exposure. Dosing at 1000 mg BEMT/kg b.wt./day once or once daily for 21 days; not radiolabeled BEMT determined in plasma (LOQ 0.5ng/mL) sampled in time courses for each of 3 study groups. Study summary in OMOR Module 2.4.3.4.2. | Completed |
| Single and Repeat dosing TK study by dermal route, rat | This dermal route study had the same design as the above oral PK study. Study summary submitted under OMOR Module 2.4.3.4.1. | Completed |
| *Toxicology* | | |
| Dose range-finding by 5 and 28-days oral dosing, non- transgenic mice with the same genetic background as RasH2 mice | CByB6F1 Hybrid (non-transgenic) mice were given dosages up to 1500 mg BEMT/kg/day; plasma sampled for not radiolabeled BEMT determinations (LOQ 0.5 ng/mL) once at 14 and 28 days. Study summary submitted under Serial 0008 1.13.8.1.4. | Completed |
| Systemic Carcinogenicity, 26-weeks oral dosing study in transgenic (RasH2) mouse | A request for waiver of this study was submitted under Serial 0011, section 1.12.13 and in OMOR section 1.6.2.<br><br>Background: A systemic carcinogenicity study in a transgenic mouse model (Wild Type CByB6F1-Tg(HRAS)2Jic Mice) was previously proposed by DSM to provide both the required | Waiver requested. |

| IND 146892 Study | Test Design and Objectives | Status |
|---|---|---|
| | second species and a systemic carcinogenicity assessment in a shorter timeline.<br><br>However, based on a comprehensive review of the scientific results obtained from the nonclinical and clinical studies completed under IND 146892, a waiver request for this study is justified as Bemotrizinol (6%) does not meet any of the criteria for further carcinogenicity testing as set out in the ICH Harmonized Guideline on Testing for Carcinogenicity of Pharmaceuticals S1B(R1) (August 2022 Final). | |
| Developmental & Reproductive Toxicity (DART) Segment I Fertility and Reproduction and III Reproduction Studies | DSM provided the complete final reports for the DART Segments I and III studies missing from the OTC Docket under Serial 0011 (sections 4.2.3.5.1 and 4.2.3.5.3). | Completed |

An overview of the approach taken and findings supportive of the nonclinical safety of BEMT 6% are presented below.

### 2.4.2 Pharmacology

No specific studies have been conducted to assess the nonclinical pharmacological effects of Bemotrizinol, as it is intended for topical use as a UV light absorber under the FDA's OTC Sunscreen Drug monograph. Pharmacological effects are not a primary endpoint since the main indication is sunburn prevention. Additionally, completed nonclinical toxicity studies did not indicate any pharmacological effects.

### 2.4.3 Pharmacokinetics

#### 2.4.3.1 Bioanalytical methods

This section includes bioanalytical method validation reports for determining BEMT in mouse (CRL 00825013) and rat (CRL 00825015) plasma, with a lower limit of quantitation (LLOQ) of 0.5 ng/mL. These methods were developed and validated based on the FDA Guidance for Industry, Bioanalytical Method Validation (FDA 2018) and were GLP-compliant. The validated methods were applied to samples collected in a 5-day and then 28-day oral gavage study with mice (CRL 2029876) and to rat blood samples taken during the parallel designed oral gavage (CRL 00825016) and dermal (CRL 00825024) pharmacokinetic single and repeat dose studies with rats.

Development of these methods established sample extraction conditions and chromatographic and mass spectrometer parameters to reliably validate the quantitation of BEMT in mouse or rat $K_2$EDTA plasma samples. Precision and accuracy, matrix stability, stock stability, recovery, matrix effects, selectivity, linearity, and reproducibility were assessed prior to initiation of the validation. Plasma volumes were 0.025 mL for mouse and 0.050 mL for rat samples analyzed by UHPLC-MS/MS (API 6500) with Electrospray ionization (positive-ion mode) detection. The precision and accuracy confirmed a robust and reproducibly reliable quantitative instrumental determination at a LLOQ of 0.5 ng BEMT per mL plasma from mice and rats. Plasma concentrations were shown to be fully representative of whole blood BEMT concentrations.

In support for two legacy toxicology studies, a 39-week dermal dosing of minipig and a life-time dermal dosing of rats, the BEMT plasma concentrations were determined with validated

methods using instrumental reverse phase chromatography and mass spectrum detection; the limit of quantitation was 2 ng/mL.

### 2.4.3.2 In vitro percutaneous penetration

Highlighted below are three percutaneous penetration testing reports that are most relevant to this OMOR for BEMT 6%. From an historical perspective, a fourth study (AnEx 1998) completed in 1998 was the first IVPT with BEMT 10% and used as support for the regulatory submission in Europe. However, the study results will not be presented as they are no longer in scope for current BEMT 6% uses.

The primary focus is on 2 GLP-compliant IVPT protocols (IES 20190089; BASF 10B0377/21B110) with human skin membranes used in flow-through chambers and dosed with $^{14}$C-BEMT applied in several variations of model sunscreen complete formulations to estimate skin penetration and permeation of BEMT 6%. These tests aimed to evaluate formulation components (excipients), including skin penetration-enhancing ingredients ethanol and propylene glycol, and their influence on variations of the rate of BEMT skin absorption, ultimately identifying the highest penetrating market image sunscreen formulations. The IVPT results served as the basis for selecting the market image formulations to be used in the human pharmacokinetic maximum usage trial (MUsT) protocol, reflecting sunscreen formulations marketed commercially in the United States.

Five market image formulations containing BEMT 6% were selected based on their safety, compatibility with BEMT stability, and consistency with sunscreens representing a majority of those sold globally, particularly in the US marketplace. The selected formulations included two oil-based sprays, one without and one with a 10% ethanol penetration enhancer, two oil-in-water emulsions, one without and one with propylene glycol as penetration enhancer, and one water-in-oil cream emulsion.

A second IVPT was required to evaluate FDA-recommended changes to one of the included excipients, ensuring better alignment with those acceptable for the US market and confirming that BEMT was the only UV absorbing ingredient present in the formulations. In both tests, similar formulation types were used, and BEMT 6% did not exhibit measurable skin penetration into the receptor chamber, regardless of the formulation used. The absorbed dose was found to be below 0.6% of the dose applied to human skin membranes in the formulations that were used later in the MUsT clinical protocol; the oil spray formulation without a penetration enhancer was used in each of the clinical safety study protocols.

In one IVPT (IES 2019090) rat skin membranes were used and dosed with $^{14}$C-BEMT from the oil-based spray containing BEMT 6%, following the same study design as with human skin as cited above. The penetration results with rat skin membranes demonstrated an approximately 6-10 times higher BEMT permeation compared to human skin and provided insight into the nonclinical differences in systemic BEMT concentrations when compared across mice, rat, minipig and human plasma data profiles. These comparisons are pivotal to our conclusions for justifying waiving the mice systemic carcinogenicity study.

### 2.4.3.3 In vivo pharmaco*kinetic testing: single doses*

The nonclinical characterization of BEMT systemic availability and elimination was determined with a dermal single dose of $^{14}$C-BEMT in cosmetic-type emollients (Tegosoft TN

and Surfadone LP100) as the dosing vehicle (CTL UR0722; 2002) and in a separate study with PEG 400 for oral gavage dosing (CTL UR0698; 2002). Both studies followed standard OECD study guidelines and were GLP compliant. The BEMT used had an assay purity of 98% and a radiochemical purity of 99%.

A dermal dosing formulation of 4% BEMT was investigated for 2 different doses in the male rat (Alderley Park Wistar-derived strain; Alpk:AP$_f$SD). Each of 4 rats in each of 8 study groups received measured amounts (54 µl or 22 µl) of each formulated dose applied to 10 cm$^2$ of shaved skin per rat, corresponding to doses of 2 mg and 0.8 mg BEMT per rat, respectively. The non-occluded doses were intended to simulate human topical exposures for a 6-hour exposure period wherein rats were held in individual metabolism cages for urine and feces collection. Groups were terminated 6, 24, 72 and 120 hours (about 5 days) after dosing. Results indicated 90 to 96% of applied dose was in the dermal site rinses, about 2% of dose remained at the application site. Tissue and organ samples' (brain, spleen, kidneys, pituitary, thymus, adrenal glands, testes and epididymis) radioactivity amounts indicated a very low dermal absorption of about 0.2 to 0.3% of applied dose after 6 and 24h from both doses. The systemic absorbed dose was not associated with any examined specific tissue/organ.

As a comparison to BEMT dermal absorption, a single oral gavage dose to rats at 50 mg BEMT/kg b.wt. (4mL/kg b.wt.) included $^{14}$C-BEMT (5.11 MBq/kg b.wt.) in a PEG 400 suspension; administration was to 4 males and 4 females in an oral balance determination with metabolism cages for 96h after dosing. The kinetics phase used 9 males and 9 female dosed rats of the Alderley Park Wistar-derived strain Alpk:AP$_f$SD for timed blood samples taken at 1, 2, 4, 6, 8 and 10h and at terminations 12, 18, and 24h after dosing. Urine and feces samples were collected 24, 48, 72 and 96h after dosing.

Results showed BEMT by single oral gavage was rapidly excreted mainly in feces with 94 to 97% of dose to males and females found as parent material within 96h, whereas blood and plasma contained <0.01% of the dose in total for male and female samples. In a 24h time course, all samples were below $^{14}$C-BEMT mean detection limits of <0.038 µg/g and <0.019 µg/g in blood and plasma respectively. Tissue/organs examined (brain, spleen, kidneys, pituitary, thymus, adrenal glands, testes, epididymis, mammary tissue, ovaries and uterus) were <0.1% of applied dose and residual carcass with 0.3% in males and 0.1% in females. Radioactivity total recovery was 95% for males and 97% for females.

Comparing these two single dose studies does not reveal meaningful differences between dermal and oral dosing routes and both show only very low systemic exposure to BEMT not exceeding 1% of the applied doses. While these single doses were low, when compared to the more recent studies in rat with 1000 mg/kg b.wt. per dose, discussed in the next section, this 200 times higher single oral dose did not show any quantitative systemic exposure ($AUC_{tlast}$), even with a 40 times lower LLOQ (19 ng/g as radiolabel vs. 0.5 ng/mL plasma). The single dermal dose of 1000 mg/kg b.wt. (ca 200 mg BEMT) resulted in a 12 ng/mL $C_{max}$, equivalent to about 96 ng BEMT in rat plasma (ca. 8 mL plasma in 200 g b.wt.[1]), compared to the 20 ng BEMT (0.01% of 2 mg BEMT dose) reported in the single dose dermal study.

[1] Bijsterbosch et al. 1981. Experientia 37: 381-382

The key observations from the two single dose studies with $^{14}$C-BEMT are that nearly all of oral gavage BEMT was cleared principally into feces as unchanged parent material, and oral and dermal dosing did not show labelled BEMT in any of the observed organs at termination.

### 2.4.3.4 In vivo single and repeat dose pharmacokinetic tests

The two most recent tests in rats, involving single dose oral (CRL 00825016) or dermal (CRL 00825024) applications of BEMT, utilized a parallel design with three dose groups in each study, wherein 1000 mg/kg b.wt./d of BEMT in PEG 400 was administered. Both studies used Wistar Han rats, followed standardized design guidelines (OECD Method 417 and ICH Tripartite guideline S3A), and were GLP compliant. The lower limit of quantitation for BEMT was 0.5 ng/mL in rat plasma, as established by the validated bioanalytical method. The objective of these studies was to determine the systemic pharmacokinetic parameters of BEMT after one and 21 days of oral or dermal dosing at 1000 mg/kg b.wt./day, which represents the NOAEL for the pivotal repeat-dose studies with BEMT.

In both studies the PK parameters were generated with a non-compartmental approach by Phoenix® software using the composite concentrations in plasma from Day 0 and Day 20 for Groups 1 and 2, respectively. For Group 3 the parameters were generated from individual concentration in plasma from Day 0 through Day 31.

The BEMT test item used in these two studies had a minimum purity of 98% and was sourced from the same single lot number (PARSOL® Shield, 0818120254) that was also used in each of the recent nonclinical and clinical studies supporting this OMOR. It was manufactured according to DSM and the currently proposed and updated USP specifications for BEMT and is fully representative of the drug ingredient provided for incorporation into current and future globally marketed sunscreen products (see section 2.3 Quality overall summary).

#### 2.4.3.4.1 Dermal dose PK study

The dermal study design was like the oral study (section 2.4.3.4.2). The once, or once daily dermal applications were at 5 mL/kg body weight to approximately 10% of the skin area within a 20-25% of total body surface area clipped of fur. For the dermal PK study, three groups of male rats were assigned oral gavage treatments as follows:

- Group 1: 8 rats given a single oral dose on Day 0; blood sampled over 48h
- Group 2: 8 rats, dosed once daily for 21 consecutive days; blood sampled after Day 20 during a 48h period
- Group 3: 4 rats, dosed once daily for 21 consecutive days; blood sampled 24h after doses on days 1, 2, 4, 7, 10, 14, 18, and 20 then samples on Days 24, 27 and 31.

*Results.* Following a single dermal application of BEMT in PEG 400 at 1000 mg/kg to 8 rats (Group 1), plasma BEMT mean concentrations were quantifiable from 1 through 48 hours post-dose (the last collection time point) in animals on Day 1. Observed were an $AUC_{tlast}$ 203 h*ng/mL, a $C_{max}$ =12.2 ng/mL, and $t_{max}$ at 10 h, after the single dermal application.

An additional 8 rats (Group 2) and another 4 rats (Group 3) received a daily dermal application of BEMT at 1000 mg/kg/day for 21 days. After 21 daily doses, the Group 2 rats had mean plasma BEMT concentrations on Day 21 that were quantifiable from before dosing through 48 hours post-dose (the last collection time point). Observed were an $AUC_{tlast}$ of 339 h*ng/mL, a $C_{max}$ =18.3 ng/mL, and $t_{max}$ at 24h, over the 48h after day 21 dosing. The repeated

dosing showed only a limited accumulation of BEMT as 1.67 times higher after 21 daily doses ($AUC_{tlast}$ 339 h*ng/mL) when compared to the Day 1 AUC result ($AUC_{tlast}$ 203 h*ng/mL).

In all Group 3 animals, plasma BEMT concentrations were also quantifiable before dosing on Days 2, 3, 5, 8, 11, 15, and 19, and on Day 21 from pre-dose through 168 hours (7 d) after dose application. Plasma concentrations were below the lower limit of quantitation (0.5 ng/mL) on the last collection at 264 hours (11d) after dosing on Day 21. Observed were a mean $AUC_{tlast}$ 1460 h*ng/mL (676 SD), a $C_{max}$ =28.6 (24.7) ng/mL, and $t_{max}$ at 12.0 (13.9) h, over the 48h after day 21 dosing. Maximum BEMT concentrations were observed at 10 hours post-dose on Day 1, at 24 hours post-dose on Day 21 and at pre-dose or 24 hours post-dose for Group 3 animals on Day 21. Systemic exposure was generally similar on Day 21 compared with Day 1. High variability in plasma concentrations between groups and the individuals within groups rendered the PK parameters of limited reliability; a steady state plasma concentration could not be determined.

Dermal doses of BEMT were absorbed into systemic circulation with an exposure profile after one dose like that after 21 consecutive daily doses. Plasma accumulation with repeated daily dosing occurred but was low ($Ratio_{AUC}$ 1.67; Day 21: Day 1) and a clear concentration-time profile was not apparent from the plasma mean concentrations.

2.4.3.4.2 Oral dose PK study

As for the design and protocol of the dermal PK study, three groups of male rats were assigned oral gavage treatments as follows:

- Group 1: 8 rats given a single oral dose on Day 0; blood sampled over 48h
- Group 2: 8 rats, dosed once daily for 21 consecutive days; blood sampled after Day 20 during a 48h period
- Group 3: 4 rats, dosed once daily for 21 consecutive days; blood sampled 24h after doses on days 1, 2, 4, 7, 10, 14, 18, and 20 then samples on Days 24, 27 and 31.

*Results for Group 1*. The single oral gavage dose with 1000 mg/kg b.wt. did not result in quantifiable BEMT plasma concentrations at any of the collection times, all were BLQ, except one sample at the 30h (0.506 ng/ml) and two samples at the 48h sample time (0.579, 0.572 ng/ml) that were only slightly above 0.5 ng/mL LLOQ. An insufficient number of measurable concentrations were available to estimate the PK parameters for this dose group.

*Results for Group 2*. After 1000 mg/kg b.wt. was administered by oral gavage once daily to 8 rats on each of 21 days, the measurable mean plasma concentrations showed variability between sample collection cohorts, which was not unexpected as the mean concentrations covered a narrow range of 1.72 ng/mL to 2.81 ng/mL over 48 hours following the last administration of BEMT. A clear relationship between plasma concentration and time of collection was not found; the PK parameters could not be reliably calculated.

*Results for Group 3*. Sample collection occurred 24 hours after doses on days 1, 2, 4, 7, 10, 14, 18, and 21. The plasma concentration of BEMT appeared to increase to a maximum concentration of approximately 1.45 ng/mL 24 hours after the last dose and then returned to BLQ by 264h after the final dose. The calculated PK mean (SD) parameters were as follows: a mean $AUC_{tlast}$ 748 (271) h*ng/mL, a $C_{max}$ =2.17 (1.26) ng/mL, and $t_{max}$ at 444 (79.6) h, over the 48h after day 20 dosing.

The overall results for this oral dosing study indicate BEMT has a very limited systemic exposure not exceeding 3 ng/mL. ($AUC_{0\text{-}inf}$ 1080 ng*h/ml) after 21days of a NOAEL dosage.

2.4.3.4.3 Comparisons of Rat PK 21d Oral and 21d Dermal Results.

We used parallel study designs to allow more direct comparison of the BEMT systemic exposures from oral and dermal dosing and derive general indications and differences. As noted above, the single doses of 1000 mg/kg b.wt. induced measurable systemic concentrations that by dermal application resulted in a $C_{max}$ of 12 ng/mL ($AUC_{tlast}$ 203 h*ng/mL) compared to 0.288 ng/mL by oral gavage (AUC not calculable: insufficient number of measured concentrations). The repeat dose study groups indicated that both oral and dermal dosing resulted in higher and sustained plasma BEMT concentrations that after 21 doses were differentiated by the about 2 times higher AUC concentrations after dermal dosing and 9 to 14 times higher $C_{max}$ than by oral gavage. A consistent time-concentration plasma profile did not emerge in either study. Measurable BEMT concentrations decreased to BLQ in both studies at 264h after a last dose (Day 21).

Direct comparisons of oral to dermal dosing indicate, in relative (qualitative) terms, that by dermal dosing BEMT appeared sooner in systemic circulation, showed about a 2-fold accumulation in plasma (Day 1 vs. Day 21) and at a high daily dose of 1000 mg/kg/day (ca. 7 mg BEMT/$cm^2$; 20% BEMT formulation) did not show a consistent time-concentration plasma profile, decreasing after dosing stopped. See tabular comparison in 2.6.5.4.1 Table 9.

The pivotal conclusion from these PK data is that compared to nonclinical oral dosing, dermal dosing induces the highest systemic BEMT concentrations, a conclusion relevant to the conduct of a systemic oral carcinogenicity study and to supporting the human exposure risk assessment through a clinical pharmacokinetic (MUsT) result.

2.4.3.5 Metabolism

In general terms, the stability of a molecule or a substance is crucial to its sustained reliability in meeting performance expectations and objectives. This is particularly true for the UV filter and the complete sunscreen formulation in which it is incorporated. Equally important is stability required to prevent the release of harmful degradation products or reactive intermediate molecular structures. Sunscreen formulations and their individual components should remain stable throughout their intended shelf-life before use, as well as after topical application and exposure to UV irradiation and other skin-resident situations. For the UV filter molecule, stability under UV radiation is essential to ensure targeted efficacy and human safety, and, after systemic absorption, its metabolic stability in response to human enzyme systems and related cellular processes is also critical.

The nonclinical and clinical investigations with BEMT have demonstrated nearly complete stability to UV irradiation and with photoirradiation an absence of local (phototoxicity, genotoxicity, sensitization) and systemic adverse effects after topical dosing in single and chronic frequencies. From nonclinical oral and dermal in vivo tests without UV irradiation, BEMT at maximum achievable doses did not demonstrate remarkable toxicity, lethality, immune system effects, or chronic toxicities to reproduction systems; neoplastic changes or adverse effects were not evident in testing. Systemic exposures, higher by dermal than oral doses, were quantifiable but without dose, time, or sex-related correlations or adverse effects.

Drug substance purity and stability and low bioavailability were key contributors to the good outcomes of all the nonclinical testing.

The FDA considered the legacy safety data as insufficient evidence for BEMT stability to human metabolic systems, leading to a request for such data from clinical samples analyzed for metabolites, or from other approaches to address human-specific BEMT metabolites. Our approach was to conduct a study with robust and validated in vitro test systems (RD-00068522, 2022). The study used well-established in vitro models of human liver enzymes in hepatocytes suspensions and liver S9 fractions co-incubated with radiolabelled $^{14}$C- BEMT. These incubations did not result in any phase I or phase II metabolites of BEMT, indicating an absence of disproportionate drug metabolites specific to humans. These findings indicate that additional clinical testing or plasma analytical measurements for such metabolites are not required for samples collected during the MUsT clinical phase. These results also support the conclusion that the safety of any BEMT metabolites present in the nonclinical test species was adequately evaluated based on those studies' findings and conclusions.

The pivotal conclusions from these findings are that BEMT is stable within biological systems, is not readily absorbed after high oral or dermal doses and is safe for repeated and prolonged, long-term, topical applications in humans using BEMT 6% as UV drug ingredient in OTC drug product sunscreens.

### 2.4.4 Toxicology

#### 2.4.4.1 Single dose Studies

Toxicity testing by single oral gavage and dermal applications of BEMT up to 2000 mg/kg b.wt. did not show adverse effects or lethality in rat test systems assessed in guideline consistent GLP compliant study protocols (RCC No. 651407, 1997; RCC No. 651420, 1997). Study groups were 5 male and 5 female HanIbm: WIST (SPF) rats given a single dose of BEMT (CGF-C1607) suspended in PEG 400: oral gavage of 0.2 g/mL with 10 mL/kg b.wt.; dermal 0.5 g/mL with 4 mL/kg b.wt. under semi-occlusive dressing for 24h. Observations for 14 days after dosing did not find any signs of toxicity, adverse effects or deaths in any of the animals before study terminations.

#### 2.4.4.2 Repeat dose Dermal Studies

Dermal dosing was selected as a principal route of exposure considering the drug use of BEMT as a UV filter in sunscreens with foreseeably long-term dermal application in humans. Each of the pivotal repeat dose studies in rats and minipigs was preceded by a 14d dose range finding study by dermal or oral once-daily doses of BEMT in PEG 400 vehicle. In mice a 5d and then 28d oral gavage study was also conducted but a follow on pivotal systemic carcinogenicity study is not completed pending FDA decision on our request to waive conduct of that study.

Rats. A life-time dermal dosing study with rats was preceded by two dose range finding dermal studies, the first for 14d dosed only with 1000 mg BEMT/kg b.wt./d, or vehicle (PEG 400) to groups each with 5 male and 5 female Wistar Han rats. The principal terminal finding included dose site dermal scabs on 1 dosed male and slight desquamation on 1 control female and 4 of 5 dosed females. These first observations after 14d were initially considered not to be test item related; however, this topical effect occurred in the subsequent 13-week and 104-

week studies, and in retrospect, dermal scabs may have been the first indication of a test item related effect predictive of a topical maximum tolerated dose. Other effects or adverse changes did not occur in the rats during this study (CIT No. 4-2-25455 TSR, 2003).

A subsequent pivotal dermal 13-weeks' study (CIT No. 4-2-25378 TCR, 2006) followed OECD testing guidelines and met GLP compliance standards. Groups of 13 male and 13 female Wistar rats were assigned to one of five groups given un-occluded treatments; 10 male and 10 females were in an untreated control group. Dosages were in 2.5 mL/kg b.wt. at 1.5, 5.0 or 6 mg BEMT/$cm^2$ from dosing formulations in PEG 400 vehicle applied at 250, 500 or 1000 mg/kg b.wt./d, each without protective collar or dose site rinsing; a second high dose group at 1000 mg/kg b.wt./d each wore a protective collar for 6h per day followed by dose site rinsing. Each of the untreated control, PEG 400 vehicle control, and dosed groups contained an additional 3 males and 3 females as satellite animals used only for plasma sampling after 8 days and 6- and 13-weeks' dosing.

The in-life observations, samples' analyses results, and post-sacrifice examinations recorded from this study did not indicate any adverse effects or test item related changes. The early dermal desquamation observations were determined early in this study to be attributable to residual dosing formulation, whereas reversible dermal scabs occurred mainly in males of the low (1 of 13), mid (6/13), and high (5/13; without restrictive collar) dose groups while only 1/13 females were affected in each of their dose groups. Skin from the dosing site was given particular attention by macroscopic and microscopic examinations and effects related to BEMT dosing were not revealed. Plasma analytical mean results for the two high-dose groups were about 10 ng/ml or less, with most individual results below the 2 ng/mL LLOQ. The results indicated a NOAEL of 1000 mg/kg b.wt./day, equivalent to about 6 mg BEMT/$cm^2$/day. These results also supported dose selection for the life-time dermal carcinogenicity study in the same test system.

Minipigs. Dermal 14-days' dosing study outcomes with Goettingen minipigs demonstrated the topical safety of BEMT in a non-rodent test system in standard guideline consistent (European Agency for Evaluation of Medicinal Products, 2000) and GLP compliant study protocol. A preliminary dose range finding study used 1 male and 1 female animal given topical applications of BEMT suspended in PEG 400 vehicle at 2.5 mL/kg b.wt. dosing volume to the shaved dorsum area considered 90% as test site and 10% for vehicle control. Animals were dosed at 1000 mg/kg b.wt./d for 2 days without adverse dermal changes and the subsequent 12 days at 1250 mg/kg b.wt./d; treatment sites were rinsed after 6h each day. Mortality did not occur, body weights and food consumption were unaffected, clinical signs and skin treatment sites were unaffected by BEMT, as confirmed by microscopic examination of skin biopsies. The 2 minipigs were returned to the animal stock. The experimental results indicated the Maximum Tolerated Dose was higher than 1250 mg/kg/d but dosing of BEMT higher than the 50% w/w concentrations in vehicle could not be technically achieved for a spreadable, reproducible application at a higher dosage. The subsequent 9-months' study used the daily dosages of 250, 500, and 1250 mg/kg b.wt./d (4-2-25383 TCN, 2004).

The pivotal 9-months dermal dosing study, like the 14d study, was GLP-compliant and guideline consistent, used 3 groups each of four male and four female Göttingen minipigs given a once daily cutaneous application of the test article suspended in the vehicle (PEG 400) dorsally at constant dose volume of 2.5 mL/kg b.wt./day. Daily applications of BEMT (a.i.; 97.6% assay, 2004) were applied unoccluded for 6h per day then rinsed from the dorsal sites; equivalent dose metrics are summarized in the next table. The vehicle control group included

4 males and 4 females. Plasma samples were collected from each animal at single time points in study weeks 12, 26, and 38; BEMT LC-MS analytical method had an LLOQ of 2.0 ng/mL plasma.

| **mg a.i./kg b.wt./d** | **% a.i.** | **mg a.i./cm$^2$** | **mg a.i./ m$^2$ (Human equivalent dose)** |
|---|---|---|---|
| 250 | 10 | 0.875 | 8,750 |
| 500 | 20 | 1.75 | 17,500 |
| 1250 | 50 | 4.38 | 43,750 |
| **Human dose BEMT (a.i.) at 6% in sunscreen** | | | |
| 37 | 6.0 | 0.13 | 1,295 |

1. Human equivalent dose is calculated as mg/kg/d dose x $k_m$ (Table 1 in FDA 2005)
2. $k_m$ is a scaling factor to convert mg/kg (/d) doses to the human body surface area (mg/m$^2$) equivalents. For HED as mg/kg the $k_m$ is 1.1 for minipig. (FDA 2005)

The gross macroscopic and microscopic observations revealed some changes considered unrelated to the test item. Microscopic findings for treated skin included hyperkeratosis, parakeratosis and acanthosis reported in control and treated group animals and was attributed to the skin sites' preparation for dosing. Local topical adverse effects or internal changes indicating a target organ-effect were not related to the test item. Oral ingestion of the test item could not be excluded by the open topical applications and may have contributed to the quantifiable systemic concentrations of BEMT observed, which did not exceed a plasma mean of 15 ng/mL in any group; 5 ng/mL could be estimated as an approximate median systemic exposure across the groups during the dosing period. However, consistent and clear dose-, time- and sex-related profiles were not established. The NOEL was 1250 mg/kg b.wt./day based on the study design (25384 TCN, 2006). *These study results are a fundamental part of the nonclinical safety conclusions supporting the acceptability of long-term human topical application of BEMT.*

### 2.4.4.3 Oral dose Studies

Mice. A 5-day then 28-day dose range finding study according to OECD guidelines and GLP compliant, was conducted with BEMT based on the FDA request for a second species systemic carcinogenicity study. We anticipated using oral gavage of transgenic RasH2 mice in the main study that in these studies were represented by the CByB6F1 Hybrid non-transgenic mice with the same genetic background as RasH2. The 5d study had groups of 5 male and 5 female mice dosed once daily at 10 mL/kg b.wt. with dosages of 0 (PEG 400), 500, 750, 1000, 1500 mg/kg/d; the high dose was the maximum achievable dosage. These study outcomes indicated a 28d study with gavage doses 0 (PEG 400 vehicle), 500, 1000, or 1500 mg/kg b.wt./day; each group was assigned 10 males and 10 females for the main study and an added 6 mice per sex for plasma sampling at study days 14 and 28. Adverse effects related to the test item did not occur in any dose group during the study. Plasma sample analysis group mean BEMT results were all below 10 ng/mL and without clear trends or meaningful differences within or across study groups, and duration of dosing. The high dose was a maximum dose deliverable to the mice based on characteristics of the dosing formulation, the gastric capacity, gavage needle dimensions, and, as indicated by the experience of the study director, animal health and welfare concerns for the test mice.

Rats. Bemotrizinol in PEG 400 was administered once daily, 7 days per week, over 14 days in a dose range finding study to support a 90-days' study. Doses of 50, 200, 800 or 2000 mg/kg

b.wt./day to separate groups of 5 male and 5 female Wistar rats did not reveal test item related effects and demonstrated a NOAEL of 2000 mg/kg/day. This study was not audited by the test laboratory's Quality Assurance Unit but did follow all of the applicable Standard Operating Procedures. (RCC No. 667530, 1998)

The 90 days' pivotal study used BEMT in PEG 400 administered by oral gavage once daily, 7 days per week, in a GLP-compliant OECD TG 408 study with 20 male and 20 female Wistar rats per group given vehicle, 100, 500 or 1000 mg/kg b.wt./day. Seven deaths occurred during the study, each attributed to technical errors during dose administration or blood sampling: Necropsy findings indicated dose intubation of the respiratory tract of 3 male rats (1 control; 2 low-dose group) each on separate days and 2 female rats (1 control;1 high dose group) on the same day of dosing; 1 mid-dose male died after blood sampling on dose-day 29 and 1 mid-dose female died during anesthesia on dose-day 86. The animals were not replaced during the study as the remaining numbers of rats were considered sufficient to meet the study objectives.

No adverse effects occurred in clinical appearance; functional observational battery testing and grip strength; survival; food consumption; body weights; ophthalmoscopy findings; hematology, clinical chemistry, and urinalysis values; organ weights; and macroscopic or microscopic findings. The gamma globulin fraction decreased 21-22% in high dose males and 30% in high dose females. Urinalysis showed increased output and decreased specific gravity in high dose males and in mid and high dose females. A NOAEL of 1000 mg/kg/day was established. (RCC No. 667541, 1998)

#### 2.4.4.4 Mutagenicity and Genotoxicity Studies

Three separate bacterial reverse mutation studies, the first two using BEMT from a legacy test item (Tinosorb® S) batch (CCR-RCC No. 582800, 1997; 618300, 1998) and the third with the DSM (PARSOL® Shield) test item (WIL Research 512974, 2016). They each followed OECD test guideline specifications and were GLP compliant; bacterial strains of S. typhimurium were TA98, TA100, TA 1535 & TA1537; E. coli WP2uvrA were also used. The studies from 1997 and 1998 used BEMT in DMSO by plate incorporation and pre-incubation and the 2016 study used top agar admix for BEMT; cultures were tested without and with metabolic activation by rat liver S9 exposures. An increase in numbers of revertant colonies did not occur in any of the studies' strains without or with metabolic activation indicating BEMT is not mutagenic by standard testing criteria.

Genotoxicity testing used Chinese hamster V79 cells in vitro according to a GLP compliant OECD protocol with exposure to Bemotrizinol in acetone up to 210 µg/ml, without metabolic S9 activation, exposed for 4, 18, and 28 hours then harvested at 18 or 28 hours for metaphase analysis. Cultures with S9 activation were dosed up to 210 µg/mL for 4 hours then harvested at 18 or 28 hours. With and without S9 activation, clastogenic responses did not occur. (CCR-RCC 597700, 1998)

Genotoxicity in vivo was assessed in mouse bone marrow erythrocytes according to the OECD guideline under GLP compliance. Bemotrizinol in corn oil was given as a single intraperitoneal injection to each of five male and five female Swiss mice (strain Ico: OF1 (IOPS Caw)) per group at 500, 1000, and 2000 mg/kg b.wt.; vehicle and positive control groups of mice were also used in the study. Signs of toxicity did not occur in any animals

during the study. Cytogenetic adverse effects seen as micronuclei in erythrocytes did not occur; the test substance was not genotoxic in this assay. (CTL 25608 MAS, 2003)

Genotoxicity expressed as unscheduled DNA synthesis was assessed according to the OECD guideline and GLP compliance using rat hepatocytes from animals administered Bemotrizinol by oral gavage with one dose of 2000 mg/kg b.wt., and a second group given 1000 mg/kg b.wt. Three male Fischer rats were used in both the 12- and 16-hour and the 2 to 4-hour expression times after each of the gavage doses; BEMT was suspended in 5% carboxymethylcellulose in distilled water as vehicle. The vehicle and positive control groups each showed their expected results. Compared to vehicle control results, the Bemotrizinol exposed hepatocytes did not have significantly increased mean net nuclear grain counts for cells in repair, and the number of cells in repair and S-phase were similar or below the vehicle control group responses. Bemotrizinol did not induce a proliferative response or genotoxic effects. (FSR-IPL 040012, 2004)

The overall indications are that BEMT is not mutagenic or genotoxic in guideline compliant in vitro and in vivo test systems.

### 2.4.4.5 Carcinogenicity

Bemotrizinol in PEG 400 was administered once daily, 7 days per week, for 104-105 weeks in a GLP-compliant study following OECD TG 451 and ICH Guidance S1B. Each of five groups were assigned 50 male and 50 female Han-Wistar rats given 2 mL/kg b.wt. daily doses of 0 (PEG 400, vehicle), 100, 500, or 1000 mg/kg b.wt./day applied un-occluded and cleaned after 6 hours' exposure; the fifth group remained untreated during the study. Dosing equivalents are summarized in Table 2.

*Table 2. BEMT Carcinogenicity Study Dosing Equivalents*

| **Dermal dose mg a.i./kg/d** | **% a.i. applied** | **mg a.i./ $cm^2$ applied** | **HED[1] mg a.i./$m^2$** |
|---|---|---|---|
| 100 | 4 | 1 | 600 |
| 500 | 20 | 5 | 3000 |
| 1000 | 40 | 10 | 6000 |
| **Human Dose of BEMT (a.i.) from Usual Daily Sunscreen Use** | | | |
| 37 (human $k_m$) | 6 | 0.13 | 1295 |

1. Human equivalent dose calculated as mg/kg/d dose x $k_m$ (Table 1 in FDA, 2005).
2. The $k_m$ is a scaling factor to convert mg/kg (/d) doses to human body surface area (mg/$m^2$) equivalents. For rats, the estimated HED as mg/kg used the $k_m$ 6.2. (FDA 2005).

Survival was not adversely affected by the test item. Chronic moderate skin irritation expressed as topical scabs limited mainly to the mid- and high-dose Bemotrizinol-treated sites, and at higher incidence in males than females showed that a topical MTD was achieved.

The non-neoplastic correlates to these observations were microscopically observable increased incidence and severity of ulcer(s)/scab(s), multifocal/diffuse epidermal hyperplasia, multifocal/diffuse hyperkeratosis, subepithelial inflammatory cell infiltrate(s) and diffuse sebaceous gland hypertrophy/hyperplasia. These effects were primarily found in the mid- and

high dose groups and in some low-dose animals. The reading pathologists who reviewed these findings considered these effects as secondary to skin irritation caused by the test item, which was further exacerbated by the method of dose application. Notably, a related dermal tumor response was not observed.

Neoplastic changes did not occur at the dosing sites in any of the animals. For the study overall, statistically significant differences did not occur for tumor prevalence in the test item-treated groups compared to controls using the time-to-tumor method (Peto et al. 1980). The survival-adjusted Peto test (Peto et al. 1980) did not reveal statistically significant differences in the incidence of tumor at any site or overall, between the test item-treated groups and the combined control groups. Although most tumor types occurred at a low frequency, so that the statistical power to detect a possible effect is quite limited, the numbers of rats studied is typical of long-term carcinogenicity studies and the conclusion of no carcinogenic effect of the test item is appropriate. Comparing the rat dermal dosages of up to 10 mg BEMT/cm² and human topical application rates of BEMT 6% at 0.13 mg BEMT/cm² indicates a sufficient exposure margin to prevent adverse skin effects, as was confirmed by clinical dermal safety studies.

Plasma analysis (LOQ 2 ng/ml) for Bemotrizinol in each group during the study showed similarly low concentrations (2.2 to 81.2 ng/mL for individual rats) across the groups. The large standard deviations about a group's mean concentration obscured any clear dose-, sex- or time-related patterns or trends. It was concluded that BEMT was not a dermal or systemic carcinogen in rats; the overall study NOAEL was 1000 mg/kg b.wt./day. (CIT 25382 TCR, 2006).

### 2.4.4.6 Development and Reproductive Toxicity (DART)

Bemotrizinol has been tested for effects on all phases of the developmental and reproductive cycle in both male and female rats, as well as for in utero developmental toxicity in rabbits. These studies were conducted using legacy lot numbers in test item dose formulations, according to OECD guidelines and GLP compliance. No adverse effects related to the test item were observed in any of the oral gavage dosages used.

#### 2.4.4.6.1 Fertility and General Reproduction Toxicity (Segment I) Study in Rats

This GLP-compliant study evaluated stages A and B of the reproductive process, as outlined in the ICH Harmonised Tripartite Guideline, to detect effects on the estrous cycle, tubal transport, implantation, and preimplantation stages of embryo development in female rats. The study design also allows for the detection of functional effects, such as those on libido or epididymal sperm maturation, which may not be identified through histological examinations of male rat reproductive organs. Bemotrizinol, suspended in PEG 400 as the vehicle, was administered via oral gavage at a dose volume of 10 mL/kg body weight. The doses were 0 (vehicle control), 100, 300, or 1000 mg/kg body weight per day. Each study group consisted of 20 male and 20 female rats (Crj:®(SD)IGS SPF strain).

The results indicated that Bemotrizinol did not adversely affect body weight, food consumption, mating behavior, or fertility in either male or female rats. Additionally, male reproductive organs, sperm parameters, female estrous cycles, the number of corpora lutea, implantation sites, and the numbers of viable and non-viable fetuses were comparable to control group values. The No Observed Effect Level (NOEL) was determined to be 1000

mg/kg body weight per day for general toxicity in dams, reproductive function in mating animals, and early embryonic development. (Hashima Nihon Laboratory No. 100721, 2002)

#### 2.4.4.6.2 Prenatal Developmental Toxicity Study (Segment II) in Rats

Bemotrizinol in PEG 400 was tested for its effects on rat developmental toxicity under GLP compliance and according to OECD TG 414 and ICH S5-R2. Each group of 22 timed pregnant Wistar rats received a single daily oral gavage of vehicle (PEG 400) or test substance at 100, 300 or 1000 mg/kg b.wt./day on each of gestation days (GD) 6-17, the accepted dosing period at the time of the study; terminal sacrifice was on GD 20.

The only statistically significant differences in reproductive parameters were an increase in post-implantation loss and a reduction in the number of fetuses in the 100 and 1000 mg/kg groups. However, these effects were not considered related to the test article, as no dose-response relationship was evident, and the parameters remained within the ranges of historical control data. Test article-related effects did not occur in other reproductive parameters, including the mean number of corpora lutea and implantation sites and the percentage of pre-implantation loss. Similarly, no effects occured in fetal outcomes, including external, visceral, and skeletal abnormalities, sex ratios, or body weights. The maternal and fetal No Observed Effect Levels (NOELs) were determined to be 1000 mg/kg. (RCC No. 681491, 1998)

#### 2.4.4.6.3 Prenatal Developmental Toxicity Study (Segment II) In Rabbits

Bemotrizinol in vehicle (0.5% carboxymethylcellulose in 0.1% aqueous Tween 80) was administered by oral gavage to groups of 20 timed-pregnant female rabbits (Hra:NZW)SPF). Daily dosing was administered as a single treatment at 10 mL/kg b.wt., adjusted daily for individual bodyweight, at dosages of 0 (vehicle), 100, 300, or 1000 mg/kg b.wt./day. These dosages were selected based on absence of adverse findings in a preceding dose-range finding study. The dosing period included gestation days (GD) 6 through 19 and terminal sacrifice occurred on GD 29 with Cesarean section and necropsy. The uterine contents were evaluated, and all fetuses were examined for external, visceral, and skeletal changes.

No deaths occurred at any dose level, and the observed clinical signs were not considered related to BEMT. The test article did not affect maternal body weight, body weight gain, or food consumption. Additionally, uterine and litter parameters were not adversely affected, and fetuses did not exhibit gross external, soft tissue, or skeletal malformations or variations at any dosage of Bemotrizinol. Based on the absence of effects on maternal and developmental parameters, the No Observed Effect Level (NOEL) for both maternal and developmental outcomes is at least 1000 mg/kg/day. (Charles River Labs No. 203-014, 2004)

#### 2.4.4.6.4 Reproduction (Segment III) Toxicity Study in Rats

This GLP-compliant study evaluated stages C through F of the reproductive process (Segment III) according to the ICH Harmonised Tripartite Guideline. However, it did not include an evaluation of Caesarean-delivered fetuses (stages C and D; Segment II), which are reported separately. The study assessed the potential adverse effects of Bemotrizinol treatment on female rats from implantation through lactation and weaning of the F1 generation offspring. Parameters evaluated included gestation, parturition, lactation, and maternal behavior in the female rats. The development of the F1 offspring was observed through sexual maturity that was confirmed by a mating trial. Male rats and the F1 generation were not dosed or treated as

part of this study; the F1 offspring were exposed to BEMT only in utero during gestation or via maternal milk during lactation.

Each study group consisted of 18-20 pregnant female rats (Crj:CD® (SD)IGS SPF strain), administered a dose volume of 10 mL/kg b.wt. of Bemotrizinol in PEG 400 as the vehicle. The doses of 0 (vehicle), 100, 300, or 1000 mg/kg body weight per day were given daily via oral gavage from gestation day (GD) 6 through day 20 of lactation (DL 20). Rats that did not deliver a litter were dosed through GD 24. Dosing was paused for females in the process of delivering their litter, resulting in each dam missing only one dose. On postnatal day 4 (DL 4), four male and four female pups from each study group were randomly selected and observed until weaning (DL 21). At weaning, one male and one female from each group were selected for further assessment of markers of morphological growth, sexual maturation, behavioral and learning indicators, and reproductive capacity in a mating trial.

Results indicated that, under the conditions of this test, BEMT did not adversely affect any of the standard parameters evaluated in the female rats or in the F1 offspring, including developmental and reproductive parameters. The No Observed Effect Level (NOEL) was determined to be 1000 mg/kg/day for all evaluated endpoints, including parental adult toxicity, reproductive function in dams, and pre- and postnatal development of embryos and offspring. (Hashima Nihon Laboratory No. 300721, 2002)

### 2.4.4.7 Local Tolerance Studies

#### 2.4.4.7.1 In vitro Skin Irritation

The skin irritation potential of Bemotrizinol was tested in a human three-dimensional epidermal skin model (EPISKIN Small Model (EPISKIN-SMTM)) according to OECD TG 439 and under GLP compliance. Three moistened skin membranes (with 5 µl of Milli-Q water each) were dosed with approximately 10 mg of the powder-form test item for 15 minutes at room temperature. After exposure, the membranes were rinsed with phosphate-buffered saline (PBS), dried, and then incubated at 37°C for 42 hours. A set of three membranes dosed with 25 µl PBS (phosphate buffered saline) served as the negative control, while another set dosed with 25 µl of 5% sodium dodecyl sulfate (SDS) served as the positive control. Irritancy, represented by cytotoxicity measured as mitochondrial dehydrogenase activity and expressed as remaining cell viability, was 92% for the test item compared to the negative control. The SDS group showed 27% remaining cell viability. Based on these results, the test item is considered non-irritant. (WIL Research No. 512975, 2016)

#### 2.4.4.7.2 In-Chemico Determination of Skin sensitization- DPRA

In this direct peptide reactivity assay, PARSOL® Shield was tested for its reactivity towards model synthetic peptides containing either cysteine (SPCC) or lysine (SPCL). The assay followed OECD Guideline No. 442C and was conducted under GLP compliance. This assay is based on the first key event in the skin sensitization Adverse Outcome Pathway, where electrophilic chemicals covalently bind to skin proteins. The protein reactivity is determined by measuring the depletion of the model peptides' concentrations in the reaction mixtures after a 24-hour exposure. Bemotrizinol was dissolved in acetone, which was deemed the appropriate solvent for the assay due to its ability to form a clear solution compared to other potential solvents.

The results of the lysine reactivity assay were rejected due to precipitation observed in the test item samples. However, the results from the cysteine reactivity assay were valid, showing a 0.6% ± 1.1% depletion with BEMT. The cinnamic aldehyde positive control samples produced the expected results. While BEMT tested negative in the cysteine assay, the results from the lysine peptide assay suggest an uncertain negative outcome that should be interpreted with caution. (Charles River Labs No. 517122, 2017)

#### 2.4.4.7.3 In vitro Skin Sensitization: KeratinoSens™ Assay

Bemotrizinol, suspended in DMSO (25 mM as a stock mixture), was tested for a sensitization response via interaction with the keratinocyte antioxidant/electrophile response element (ARE)-dependent signaling pathway, representing key event 2 in the skin sensitization Adverse Outcome Pathway. The test system utilized keratinocytes modified with an ARE-Nuclear response factor 2 luciferase reporter gene, in accordance with OECD TG 442D, and was conducted under GLP compliance. The assay was deemed acceptable based on the positive control group results and appropriate negative control responses.

For Bemotrizinol, cell viability remained above 70% (ranging from 97-116%) across all test concentrations, and the luminescence data were below the 1.5-fold induction threshold at each concentration. However, a key guideline criterion for concluding a definitive negative result requires dosing at concentrations exceeding 1000 µM. Due to the limited solubility of the test item, all concentrations tested were below this threshold, rendering the results inconclusive. (WIL Research No. 512976, 2016)

#### 2.4.4.7.4 Skin Irritation in Rabbit

The in vivo skin irritation potential of Bemotrizinol was tested according to OECD TG 404 and under GLP compliance using three New Zealand rabbits. A semi-occlusive application of 0.5 g BEMT to 6 $cm^2$ of intact dorsal skin for 4 hours was followed by evaluations of the skin reactions at 1, 24, 48, and 72 hours after dressing removal. Slight yellow staining was observed at the dose site, and all erythema, eschar or edema scores were zero for each animal at every observation time, and no corrosion occurred. Therefore, BEMT was found to be non-irritating when applied undiluted. (RCC No. 651431, 1997)

#### 2.4.4.7.5 Eye Irritation in Rabbit

The in vivo eye irritation potential of Bemotrizinol was tested according to OECD TG 405 and under GLP compliance with three New Zealand rabbits. One eye of each rabbit received 0.1g of BEMT without rinsing and observations were made at 1, 24, 48, and 72 hours after instillation. The average score over 72 hours for conjunctival erythema was 0.3, 0.3, and 0.6 for each rabbit, respectively. No effects, including staining or corrosion, were observed on the corneal, scleral, or iris tissues. Although the undiluted substance was slightly irritating to the eye, it does not require classification. (RCC No. 651442, 1997)

### 2.4.4.8 Other Toxicity Studies: Endocrine-related effects

Bemotrizinol was demonstrated through separate in vitro assays, conducted under GLP compliance, not to interact with the estrogen receptor (ER) or the androgen receptor (AR). In vivo, BEMT did not influence estrogen-related uterine maturation in rats. These screening assay results were further confirmed in various repeat-dose toxicity studies and developmental and reproduction toxicity studies in intact animals. No macroscopic or microscopic changes

were detected in specific exocrine and endocrine tissues, nor were there any abnormalities in hormone-regulated developmental or reproductive outcomes.

#### 2.4.4.8.1 Estrogen Competitive Binding Assay

The estrogen receptor competitive binding assay, although not GLP-compliant, was validated within the testing laboratory and fully documented as expected for a compliant study. The test system was isolated cytosol from immature rat uteri, incubated with Bemotrizinol at concentrations ranging from $5x10^{-10}$ to $5x10^{-4}$ M in DMSO. Compared to solvent, negative, and positive controls, Bemotrizinol did not interact with the estrogen receptor, indicating an absence of intrinsic agonistic or antagonistic estrogenic activity. (Central Toxicology Lab No. 024529, 2001)

#### 2.4.4.8.2 Androgen Competitive Binding Assay

The androgen receptor competitive binding assay, although not GLP-compliant, was validated within the testing laboratory and fully documented as expected for a compliant study. The test system used cytosol isolated from testosterone-reduced immature rat prostates. Bemotrizinol, at concentrations ranging from $5x10^{-10}$ to $5x10^{-4}$ M in DMSO, did not interact with the androgen receptor when compared to solvent, positive, and negative control substances. This result indicates an absence of intrinsic agonistic or antagonistic androgenic activity. (Central Toxicology Lab No. 024530, 2001)

#### 2.4.4.8.3 Uterotrophic Assay in Immature Rats

The estrogenic activity of Bemotrizinol was tested using the immature rat uterotrophic assay under GLP compliance, following a study design validated within the testing laboratory and now included in OECD Guideline 440. In the assay, groups of ten 19–20-day-old female Wistar-derived rats each received a single subcutaneous injection of BEMT in arachis oil for three consecutive days at doses of 250, 500, or 1000 mg/kg body weight. The rats were sacrificed 24 hours after the third dose. The blotted dry uterine weights of the BEMT-dosed rats, compared to positive controls, did not indicate any estrogen-related effects in this assay. (Central Toxicology Lab No. zr1601, 2002)

### 2.4.4.9 Photo-induced Toxicity

A series of legacy tests with BEMT have been conducted to demonstrate the nonclinical safety of the molecule in standard in vitro and in vivo test systems exposed simultaneously to BEMT and UVR, following study designs current at the time of testing. Although some of these studies are no longer part of current nonclinical approaches for demonstrating photosafety of pharmaceuticals (ICH S10, January 2015), their results have been required and used by other global regulatory agencies for the safety evaluation of sunscreen products in their markets. As these studies were included in the initial data submission to the FDA (Docket No. 2005-N-0453), they are addressed here to ensure completeness of the OMOR BEMT scientific record.

As mentioned in the metabolism section, the stability of a molecule is crucial for its performance expectations and safety. For a UV filter, stability to UVR and in its formulation are essential to prevent the release of harmful degradation products and ensure efficacy and human safety. The photo-stability of Bemotrizinol was demonstrated through in vitro testing under a solar simulator, where up to 98% of the active ingredient remained after exposure to

50 MED (minimum erythema dose) equivalent irradiation. (see section 5.4 Herzog, Hueglin, et al., 2004)

Nonclinical guideline and GLP-compliant tests demonstrated that BEMT in combination with UV irradiation, did not cause in vivo skin irritation or phototoxicity (see section 4.2.3.7.7 651475_RCC_phototoxicity-guinea-pig), nor did it induce photosensitization or allergenicity (see section 4.2.3.7.7 651497_RCC_Photoallergenicity_guinea pig). Additionally, in vitro test systems did not reveal mutagenic effects in bacteria (see section 4.2.3 618300_CCR_bact_muta_ecoli, 582800_CCR_bact_muta_salm and 512974_WIL_bact_reverse_muta) and no genotoxic effects on chromosomes in Chinese hamster V79 cells (see section 4.2.3 597700_CCR_chromo_aberr_invitr).

Key in vivo results from hairless Crl:SKH1-hrBR mice dosed topically for 13 weeks (see section 4.2.3.7.7 203006_CRL-Argus_13Wk_Dermal-dosed_phototox_mice) or for 40 weeks followed by 12 weeks without irradiation showed that BEMT was efficacious in reducing erythemal responses and skin thickening compared to control animals. In the 52-weeks' study, BEMT reduced tumor-related and exposure-related mortality, demonstrated a dose-related skin tumor protective effect against skin tumors, and reduced skin tumor potency factors. Additionally, BEMT delayed the onset of the photocarcinogenic response. (see section 4.2.3.7.7 203008_CRL-Argus_12mo-dermal_photocarc_mice)

The principal conclusions are that Bemotrizinol is a photostable UV filter, effective in reducing the adverse effects from UV irradiation and poses no concerns for long term topical use, whether with or without concomitant UV radiation exposure.

### 2.4.5 Integrated Overview and Conclusions

The overall nonclinical profile of BEMT indicates very low systemic bioavailability, low toxicity, no genotoxicity, and no reproductive, endocrine, or carcinogenic concerns. The extensive nonclinical evidence suggests that BEMT is metabolically stable, not toxicologically active and poses no risk for human adverse effects from prolonged topical use at a maximum concentration of 6% in OTC sunscreen products. This comprehensive assessment supports the safety of BEMT for long-term use in sunscreen products for ages 6-months and older. In Summary, BEMT:

1. Is not toxic by single limit doses by oral or dermal dosing.
2. Shows no genotoxicity in both in vitro and in vivo tests, with or without UV exposure.
3. In animals and humans is not phototoxic topically and is not a skin contact sensitizer without or with irradiation.
4. In a 52-week study, prolonged topical application to hairless mice exposed to UV radiation resulted in fewer tumors compared to control groups, indicating no increase in UV-induced carcinogenicity.
5. Is photo-stable with minimal degradation under high irradiation of up to 50 MED equivalents.
6. Shows no adverse effects in pivotal rodent reproductive studies with repeated oral dosing.
7. Does not affect key developmental parameters in rats and rabbits during guideline Segment II studies.
8. Reveals no endocrine system interactions or modulations in any studies, including DART and repeat-dose studies.

9. Does not affect estrogen or androgen receptors in specific in vitro and in vivo assays.
10. Is without BEMT-related neoplastic or non-neoplastic tissue effects in lifetime topical dosing studies in rats.
11. Is without dermal or systemic carcinogenic or toxic effects in chronic topical dosing studies in minipigs.
12. Does not cause systemic target organ effects in repeat-dose oral (up to 13 weeks) or dermal (up to 2 years) studies, with no signs of increased carcinogenic response.
13. Does not trigger any molecular structural alerts for toxicity by in silico assessments.
14. Does not demonstrate dose-response effects in nonclinical testing.

Clinical study results confirm the findings indicated by the nonclinical test systems. Topical tolerance and safety tests did not show any adverse effects, and systemic exposures after maximal topical applications of complete sunscreen formulations with BEMT 6% did not exceed the plasma steady state above the 0.1 ng/mL plasma LLOQ. Only 113 (3%) of the 3722 plasma samples exceeded a 0.5 ng/mL threshold, and detailed analysis showed no consecutive sample concentrations at or above 0.5 ng/mL. Specifically, 3, 4, and 4 pairs of elevated concentrations were observed in different participants across the 3 study arms, with these 11 occurrences representing just 0.47% to 0.66% of all samples measured per treatment group. Across all the plasma concentration results an observable trend or pattern did not emerge for BEMT plasma concentrations at or above the 0.5 ng/mL threshold.

Results with the MUsT's unrealistically high BEMT dermal application rates (111 mg BEMT/kg b.wt./d) indicated it was well tolerated at the topical application sites (2 mg/cm$^2$ to 75% of body surface area) and across the individual participants.

Nonclinical testing's main purpose is to support the safety evaluation of human use through risk assessment. In none of the 12 repeated dose nonclinical studies did a toxic effect arise to indicate a point of departure for the human risk estimation. Thus, a default approach to calculating the BEMT Margin of Safety, independent of human PK results, allows for a straightforward comparison of daily human exposures of BEMT 6% in sunscreen formulations with the nonclinical NOAEL.

The parameters used for the MoS estimation are those set forth in the EU-SCCS Notes of Guidance 12$^{th}$ revision (SCCS 2023) and are as follows: 18 grams of sunscreen applied per day, a 0.53% human skin permeation rate (2$^{nd}$ IVPT, 0.19 % + 2 SD (0.17)), 60 kg body wt., and the rat lifetime dermal carcinogenicity study NOAEL (1000 mg/kg b.wt./d) adjusted for the rat skin IVPT result of 5.0% penetration (+1.54 SD x 2=3.08 + 5.0 = 8.08% absorbed), which yields a nonclinical systemic dose of 80.8 mg/kg b.wt./d for the rat dermal NOAEL.

The amount of 18 grams sunscreen applied daily is a standard exposure value that is supported by data from 75 clinical safety studies conducted between 2006 and 2016 to assess the cutaneous tolerance and efficacy of topically applied commercial products. The subjects were healthy children and adults of both sexes with different skin types and with different Fitzpatrick phototypes (Gomez-Berrada et al. 2017 and 2018).

The Margin of Safety (MoS), then is:

Total sunscreen applied: 18 g

BEMT applied: 1.08 g
BEMT absorbed to systemic: 5.7 mg/person
Systemic estimated human dose (SED): 0.095 mg/kg b.wt./d

**Margin of Safety (80.8 / 0.095) = 851**

The result indicates sufficient difference between human systemic exposure and the indicative animal no observed adverse effect dose, with 100 being commonly accepted as the minimum margin. This MoS is conservatively calculated and likely a minimum value, considering that the nonclinical pivotal studies did not show any adverse effects to establish a point of departure for risk estimation. Additionally, the human pharmacokinetic maximum usage trial showed an estimated systemic exposure of 0.014 mg/kg (study day 4), which is approximately 10 times lower than the 0.19 mg/kg used in the calculation.

The overall safety of BEMT for use in consumer sun protection products has been demonstrated in each of the pivotal nonclinical studies and the clinical safety studies we have conducted and include in this Tier 1 OMOR. The large MoS is considered sufficient for all age categories, including pediatric populations (6 months and older).

This conclusion is particularly and strongly supported by the absence of adverse effects or indications from of the development and reproductive tests of the complete reproductive cycle in rats, rabbit embryo-fetal developmental testing, and absence of effects to estrogen, androgen, and fertility markers. In none of the nonclinical repeat dose studies, including subchronic and chronic dosing for lifetime of rats and 9 months to minipigs, did any of the animals have behavioral signs or cellular and tissue-organ level changes signaling a hormone-axis related effect. Neoplastic, non-neoplastic, or genotoxic markers were not induced by BEMT through any of the dosing regimens used and thereby do not indicate any cause-for-concern of target tissue effects. The absence of adverse effects clinically and in key nonclinical studies gives clear indication that BEMT can be used in products intended for use in people ages 6 months and older and has been so used and marketed globally since 2000 (see section 1.3.6)

### 2.4.6 List of Literature Citations

AnEx 1998. In vitro human skin penetration and distribution of the UV absorber CGF-C1607. An-Ex analytical services; Final 190698; Report CSD/8/98. https://downloads.regulations.gov/FDA-2005-N-0453-0003/attachment_40.pdf).

Gomez-Berrada, M. P., Ficheux, A. S., Rakotomalala, S., Guillou, S., Bellec, M., De Javel, D., Roudot, A. C., Ferret, 2018. Consumption and exposure assessment to sunscreen products: A key point for safety assessment. In Food and Chemical Toxicology, 114, 170–179. https://doi.org/10.1016/J.FCT.2018.02.035

Gomez-Berrada, M. P., Ficheux, A. S., Rakotomalala, S., Roudot, A. C., & Ferret, P. J. 2017, Probabilistic exposure assessment of sun care products. In Food and Chemical Toxicology, 108, 314–325. https://doi.org/10.1016/J.FCT.2017.07.044

FDA 2005. Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. July 2005. Available at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/estimating-maximum-safe-starting-dose-initial-clinical-trials-therapeutics-adult-healthy-volunteers.

FDA. 2014. Initial Determination and Feedback letter dated 13 November 2014, regarding Time and Extent Application in Docket No. FDA-2005-N-0453 (Legacy Docket No. 2005N-0446) for Bemotrizinol from Dr S. Kweder (FDA CDER) to Ms K. Sanzo (Morgan, Lewis, & Brockius LLP) representing the original Sponsor Ciba Specialty Chemicals Corporation. Available at: https://www.regulations.gov/document?D=FDA-2005-N-0453-0035

FDA 2016. Nonprescription Sunscreen Drug Products - Safety and Effectiveness Data Guidance for Industry, November 2016. Available at: https://www.fda.gov/media/94513/download

FDA 2018. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Veterinary Medicine; May 2018. https://www.fda.gov/media/70858/download

Federal Register; vol. 70, No. 232; Monday, December 5, 2005; 72449

Hermansky, S., Neptun, D., Loughran, K., Leung, H. 1995. Effects of polyethylene glycol 400 (PEG 400) following 13 weeks of gavage treatment in Fischer-344 rats. Food Chem Toxicol. 1995. 33(2):139-49. doi: 10.1016/0278-6915(94)00119-9.

Herzog, B. Hueglin, D., Borsos, E. et al. 2004. New UV Absorbers for Cosmetic Sunscreens: A Breakthrough for the Photoprotection of Human Skin. CHIMIA International Journal for Chemistry 58(7-8) 554-559.

Peto et al. 1980. Long-term and short-term screening assays for carcinogens: a critical appraisal. Guidelines for simple, sensitive significance tests for carcinogenic effects in long-

term animal experiments. In: IARC Monographs on the evaluation of carcinogenic risk of chemicals to humans, Suppl. 2: 311-426.

SCCNFP/0082/98. Concerning 2,4-bis-{[4-(2-Ethyl-hexyloxy)-2-hydroxy]-phenyl}-6-(4-methoxyphenyl)-(1,3,5)-triazine - COLIPA n° S81 adopted by the plenary session of the SCCNFP of 17 February 1999. Available at http://ec.europa.eu/health/archive/ph_risk/committees/sccp/documents/out52_en.pdf

SCCS (Scientific Committee on Consumer Safety), SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation 12th revision, 15 May 2023, SCCS/1647/22.