Ciba

rec'd 8/10/07

February 28, 2006

Division of Dockets Management
Food and Drug Administration
Department of Health and Human Services
5630 Fishers Lane, Room 1061
Rockville, MD 20852

Via Next Day Courier

Re: DOCKET No. 2005N-0446: Request for Safety and Efficacy Data supporting the Inclusion of bemotrizinol in the OTC Monograph for Sunscreen Drug Products for Over-the-Counter Human Use: Docket No. 78N-0038.

Ciba Specialty Chemicals Corporation ("Ciba"), Business Line Home & Personal Care, respectfully submits these data to the U.S. Food and Drug Administration (FDA) as a demonstration of the safety and effectiveness of bemotrizinol[1]. Based on the sum of the data provided in this submission and the marketing history submitted in the Time and Extent Application[2] Ciba Specialty Chemicals requests that bemotrizinol be determined generally recognized as safe and effective (GRAS/E) and included in the list of active ingredients under FDA's OTC monograph for Sunscreen Drug Products for Over-the-Counter Human Use; *(64 FR 27666-27693, as amended by 66 FR 67485-67487, Docket No. 78N-0038)*.

Ciba submits this data in compliance with the provisions of 21 CFR Part 330.14(f)(1), Procedures for classifying OTC drugs as generally recognized as safe and effective and not misbranded, and for establishing monographs. According to the data presented in our TEA, it is estimated that about one hundred twenty million units of bemotrizinol have been sold continuously during that past five years in several countries (e.g., Brazil, Germany, Greece, France, Spain, and Switzerland). Additionally, the total number of countries where bemotrizinol is sold in sunscreen formulations is thirty-one. To Ciba's best knowledge, these exposures have not resulted in any reported adverse events.

Attached please find two paper and two electronic copies of the safety and efficacy data supporting the inclusion of bemotrizinol in the aforementioned monograph. Ciba affirms that the data cited within are both accurate and current and fully meet the criteria cited in 21 CFR 330.14(f)(1). Please contact the undersigned at (336) 801-2037 or via e-mail (lisa.navarro@cibasc.com) if there are any further questions regarding this submission.

Sincerely,

Lisa Navarro, Ph.D., D.A.B.T.
Product Safety and Regulatory Affairs Director
Ciba Specialty Chemicals Corporation
Business Line Home and Personal Care
4090 Premier Drive
High Point, NC 27261-2444

Desk Copy on CD: Sent to Michael Koenig, Ph.D., FDA Division of OTC Drug Products, via overnight courier
Attachments

[1] 2,4- Bis-{[4-(2-ethyl-hexyloxy)-2-hydroxy]-phenyl}-6-(4-methoxyphenyl)-(1,3,5)-triazine, CAS RN: 187393-00-6.
[2] See Time and Extent Application (TEA) for bisoctrizole, Docket No. 2005N-0446 (December 5, 2005), filed by Ciba Specialty Chemicals on April 11, 2005.

2005N-0446 SUP 2

Ciba

Safety & Efficacy Data

Bemotrizinol

Prepared to support the Inclusion of Bemotrizinol into FDA's Monograph for Sunscreen Drug Products for Over-the-Counter Human Use; *(64 FR 27666-27693, as amended by 66 FR 67485-67487, Docket No. 78N-0038)*.

Submitted to:
DOCKET 2005N-0446

Submitted on:
February 28, 2006

Submitted by:
Ciba Specialty Chemicals Corporation
Home and Personal Care Business Line
4090 Premier Drive
High Point, NC 27265

EXECUTIVE SUMMARY

Since the publication of the Sunscreen Tentative Final Monograph on May 12, 1993 (*58 FR 28194*), FDA medical groups and consumer interest groups have continued to document the significance of, and expressed increasing concern about, exposure to UVA radiation. Exposure to UVA radiation has been causally linked with the high incidence of skin cancer in the United States[3].

UVA radiation has also been demonstrated to contribute to both acute and chronic skin damage such as erythema, abnormal melanogenesis, carcinogenesis, drug-induced photosensitivity, photoaging, and morphological alterations of Langerhans cells (*58 FR 28233*). Moreover, the large amount of UVA radiation present in the solar spectrum at the earth's surface also results in a significant contribution to the generation of erythema.

According to public health experts, skin carcinoma and melanoma rates have reached epidemic levels in the U.S. and are expected to rise. The most recent estimates of the American Cancer Society (2005) now predict over 1 million new cases of basal cell and squamous cell carcinomas occur every year and are predicted to increase annually by 5%.[4] Currently, 1 in 4 new melanomas are found in people under age 40, making it the most costly cancer in terms of years of life lost and lost productivity.[5]

In 1999 the FDA recognized the significance of the need for more effective ultraviolet A radiation (UVA, 320-400nm) filters that provide protection from the deleterious effects of UVA exposure.

> *"protection against UVA radiation is much more important than previously realized. Protection against UVA radiation may be as important to consumers' well-being as protection against UVB radiation" (*Baker, D.E., *FDA Response to CTFA Petition, Docket No. 78N-0038/CP11,* October 1, 1999*).*

Since then, concerns regarding the U.S. public health risks from UVA radiation have continued to mount. Although it is widely accepted that safe and efficacious broad-spectrum photostable sunscreen active ingredients provide a mechanism for helping people protect themselves against the harmful effects of the sun; the new broad-spectrum and photostable UVA protective sunscreen actives on the global market, have not been approved for the US market.

It is our firm belief that the availability of photostable and effective UVA absorbers is extremely limited under the existing FDA OTC Sunscreen Final Monograph. Therefore, Ciba would like to applaud the FDA for creating the Time and Extent Application program, which essentially establishes a vehicle for including "foreign" ingredients that have been used for a material extent and time into FDA OTC monographs. With the establishment of this vehicle, new superior, photostable and safe UVA radiation protection ingredients may now become available for consumer use in the United States.

Thus, we are herein requesting that the FDA take action under the TEA process to include bemotrizinol in the final sunscreen monograph for UVA protection.

Bemotrizinol is a photostable molecule and in the presence of UV radiation does not produce activated moieties such as singlet oxygen, and does not degrade to substituents of the parent structure. This ensures that the available non-clinical and clinical data are representative of the safety of bemotrizinol used in topically applied sunscreens, therapeutic articles, and cosmetic products. The robust toxicological profile plus the good clinical experiences in over 5 years' of consumers' use of sunscreens containing bemotrizinol provide evidence of safety and effectiveness for this drug substance.

The non-clinical toxicology profile for local tolerance of bemotrizinol does not indicate adverse effects in single oral and topical applications, the substance is not genotoxic with or without exposure to UV irradiation, it is not phototoxic topically, and it is not a skin contact sensitizer with or without UV

[3] Nelson, C.G. Photoprotection. In: Shaath N.A ed. Sunscreens, Regulations and Commercial Development, 3rd ed. Boca Raton, FL: Taylor and Francis, 2005: 19-43
[4] Ibid
[5] Kirsner R, Parker D, Brathwaite N, Thomas A, Tejada F, and Trapida E. Sun Protection Policies in Miami-Dade County Public Schools: Opportunities for Skin Cancer Prevention. Ped. Derm. 2005; Vol. 22 No. 6 513-519.

irradiation. Importantly, repeated oral dosing through the full reproductive cycle of rodent did not reveal adverse effects and rabbits did not show effects to key developmental parameters (Segment II study). Bemotrizinol is not considered a toxicologically active substance.

The full body of non-clinical testing with bemotrizinol indicates that it is not readily absorbed, orally or topically (the AUC could not be determined), and it is not metabolized to toxic intermediates, based on ADME testing. Chronic topical dosing to minipig, a recognized non-clinical surrogate for human dermal studies, did not reveal dermal or systemic toxicity at a highest achievable dosage. In addition, with prolonged administration by oral (up to 13 weeks) or dermal routes (up to 2 years) it does not produce any indications for an increased carcinogenic response. Prolonged topical dosing of bemotrizinol for 40 weeks to hairless mice also exposed to daily doses of UV radiation demonstrated a protective effect in that bemotrizinol did not increase the UV-induced carcinogenic response in the mice, and in fact, increased the time to tumor onset and decreased the potency of the UV radiation.

The large body of non-clinical evidence together with the summarized clinical evidence available for bemotrizinol in finished drug products does not indicate a concern for human adverse effects from prolonged topical use by any age category. We find these results as fully supportive of the inclusion of bemotrizinol at levels up to 10% into the FDA's Monograph for Sunscreen Drug Products for Over-the-Counter Human Use without any other restrictions.

Ciba truly believes that it is in the best interest of public health for FDA to add bemotrizinol to the sunscreen monograph under this TEA. The addition of bemotrizinol to the monograph will assure the availability of superior, state-of-the-art, photostable and safe UVA radiation protection products for the American public. Moreover, it will also provide American consumers with a more effective means for protecting themselves against the deleterious effects of overexposure to solar radiation.

GLOSSARY OF TERMS AND ABBREVIATIONS

Term	Synonym, Explanation	
Active's names • Bemotrizinol • bis-ethylhexyl oxyphenol methoxyphenyl triazine • BEMT • TINOSORB® S	• Generic drug name (USAN; Pronunciation: be moe' trye zi nol) • Cosmetic name (INCI) • Abbreviation of INCI name • Tradename	
Active's names used in non-clinical testing:	Test Article names and codes used in study reports 1. FAT 70'884 or FAT 70884 2. CGF-C1607 3. RM 60 4. Tinosorb® S	
Abbreviations of sunscreen actives:	Drug name (USAN)	Cosmetic name (INCI)
BEMT	Bemotrizinol	Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine
BMBM (BMDBM)	Avobenzone	Butyl Methoxydibenzoylmethane
BP3	Oxybenzone	Benzophenone-3
DBT	-	Diethylhexyl Butamido Triazone
EHMC (OMC)	Octinoxate	Ethylhexyl Methoxycinnamate
EHS (OS)	Octisalate	Ethylhexyl Salicylate
EHT (OT)	-	Ethylhexyl Triazone
HMS	Homosalate	Homosalate
IMC	Amiloxate	Isoamyl p-Methoxycinnamate
MBBT	Bisoctrizole	Methylene Bis-Benzotriazolyl Tetramethylbutylphenol
MBC	Enzacamene	4-Methylbenzylidene Camphor
OCR	Octocrylene	Octocrylene
TiO2	Titanium Dioxide	Titanium Dioxide
ZnO	Zinc Oxide	Zinc Oxide
PBSA	Ensulizole	Phenylbenzimidazole Sulfonic Acid
SPF	Sun Protection Factor (in vivo protocol by FDA, COLIPA or Intl. Harmonized Method)	
UVA protection Parameters:	More detailed description in Appendix 3	
UVA-PF	UVA Protection Factor (In vivo protocol: PPD or IPD) P/IPD: Persistent/Immediate Pigment Darkening)	
Lambda crit.	critical Wavelength in vitro method (relative)	
UVA/UVB ratio	in vitro method (relative)	
Australian UVA Standard	in vitro method (absolute)	
Sunscreen Active	UV absorber, UV Filter	
UV Absorber	Sunscreen active	
UV Filter	Sunscreen active	

Ciba

OUTLINE OF CONTENTS

Ciba

OTC DRUG REVIEW INFORMATION

Pursuant to 21 CFR §330.14(f)(1), the safety and effectiveness submissions shall include all data and information listed in 21 CFR §330.10(a)(2) under the outline "OTC Drug Review Information," items III through VII.

I. Labels: Data Not Required as Indicated in 21 CFR §330.14(f)(1)
II. Quantities of Active Ingredients in the Drug: Data Not Required as Indicated in 21 CFR §330.14(f)(1)
III. Animal Safety Data: As Required by 21 CFR §330.10(a)(2)

Based on the data for TINOSORB® S (Bemotrizinol) Ciba Specialty Chemicals considers this product safe for use in human adult or children's topical products when prepared into sunscreen formulations and leave-on or rinse-off daily wear cosmetic products at the recommended concentrations.

Bemotrizinol did not show adverse effects in pre-clinical tests with and without UV irradiation, as described in the attached studies (outlined below). Bemotrizinol, at concentrations up to 10% in leave-on and rinse-off cosmetics, is the approved safe use level for this UV filter, as established by the European Commission Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP, 1999) and the Australian Therapeutic Goods Administration (2004)

TINOSORB® S

Components		CAS RN	Purpose	USP
Bemotrizinol	100%	187393-00-6	Active ingredient	Pharmacopeial Forum (PF32(1)) January-February 2006; USP 30-NF25 (2007)

a) Table of Animal Safety Data

Table begins on following page.

The topical doses of bemotrizinol in the following non-clinical studies are expressed in several ways (e.g., mg/kg bodyweight/day or mg a.i./cm^2) and then the NO(A)EL is compared to the highest estimated human topical daily dose. The estimation of this human dose is based on the information shown in the nearby table 1.

Table 1. Parameter	Value
Human body weight	60 Kg
Skin surface area 'dosed'	18,000 cm^2
Sunscreen application rate	2 mg product/cm^2
Total topical amount applied	3600 mg a.i./day
Daily topical dose	60 mg a.i./kg b wt
Topical area dose rate	0.2 mg a.i./cm^2

The assumption uses 10% bemotrizinol in a topical product, which will be about 4 times higher than the actual maximum human exposure. The usual incorporation rate is 5% bemotrizinol because of the bemotrizinol physical/chemical characteristics and formulation limitations. Typical use rate of topical products, such as sunscreens, is 1 mg/cm^2 skin area. Thus, in Table 1 we show a summary of the 'worst case' calculation for human exposure based on 10% bemotrizinol (a.i.) in a sunscreen.

The animal topical doses reported in the summary of toxicology studies below are compared to the 0.2 mg a.i./cm^2 human dose from this worst case exposure scenario and therefore incorporates another 4-fold safety factor for exposure.

Ciba

SUMMARY TABLE of TOXICOLOGY STUDIES with BEMOTRIZINOL

STUDY	TEST ARTICLE	Test System	DOSES (mg bemotrizinol/kg body wt/day)	FINDINGS	NOEL (mg/kg/day) Active Ingredient, bemotrizinol
ACUTE STUDIES					
Acute Dermal Toxicity	Bemotrizinol in PEG 400 (0.5 g/ml)	Rat	One dose level, 4 ml/kg body weight	LD50 > 2000 mg/kg	
Acute Oral Toxicity	Bemotrizinol in PEG 400 (0.2 g/ml)	Rat	One dose level, 10 ml/kg body weight	LD50 > 2000 mg/kg	
Skin Irritation	Bemotrizinol (neat)	Rabbit	0.5 g test article; moistened (bi-distilled water) to 6cm^2 per rabbit; semi-occlusive 4 hr.	All evaluation scores were zero for each animal at every observation time. Not irritating.	
Eye Irritation	Bemotrizinol (neat)	Rabbit	0.1 g bemotrizinol powder; not rinsed	Cumulative irritation score 0.44 (13 max.) from conjunctival erythema and chemosis that resolved by the 72-h reading. Iris, cornea were not affected. Minimally irritating	
Dermal Sensitization	Bemotrizinol (a.i.) at 3% (w/v) in PEG 400 and at 3% in emulsion with 1:1 (v/v) FCA:saline as intradermal injection. Topical induction and challenge with 30% bemotrizinol in PEG 400.	Guinea Pig	30% bemotrizinol to 4cm^2 topical (50 mg a.i./cm^2)	Not a sensitizer in Guinea Pig Maximization Assay	
GENOTOXICITY STUDIES					
Ames Assay	Bemotrizinol, dissolved in DMSO	*Salmonella* TA98, TA100, TA1535, & TA 1537 ; *E. coli* strain WP2uvrA	33, 100, 333, 1000, 2500, or 5000 µg/plate with and without rat-S9 mix	Bemotrizinol did not induce base pair or frame shift mutations in any bacteria strain used.	
In vitro CHO Chromosome Aberration Assay	Bemotrizinol, dissolved in acetone	Chinese Hamster V79 cells	Doses were 3.3 up to 210 µg/ml without or with rat-S9 mix.	Precipitation of test material was noted at ≥52.5 µg/ml with and without S9 mix. Metaphase analysis at 18h and 28h harvest interval for doses 13 up to 210 µg/ml indicated bemotrizinol did not induce structural chromosome	

Ciba

SUMMARY TABLE of TOXICOLOGY STUDIES with BEMOTRIZINOL					
STUDY	**TEST ARTICLE**	**Test System**	**DOSES (mg bemotrizinol/kg body wt/day)**	**FINDINGS**	**NOEL (mg/kg/day) Active Ingredient, bemotrizinol**
				aberrations; polyploidy did not occur.	
In vivo Mouse Micronucleus Test	Bemotrizinol in corn oil	Mouse	Intraperitoneal injection of 500, 1000, or 2000 mg/kg body wt/d for 2 days.	Bemotrizinol did not induce damage to chromosomes or mitotic apparatus of mouse bone marrow cells after IP administration.	
In vivo Unscheduled DNA Synthesis Assay	Bemotrizinol, suspended in 0.5% w/v carboxymethylcellulose in distilled water (CMC 0.5%) at 200 mg/mL	Rat	1000 or 2000 mg a.i./kg body wt. In one dose by oral gavage	Bemotrizinol did not induce a proliferative effect in rat liver and did not reveal any genotoxic activity.	
GENOTOXICITY - PHOTOMUTAGENICITY STUDIES					
Photo-mutagenicity: Ames Assay	Bemotrizinol dissolved in acetone. Irradiation: UV B/A at 4/80 mJ/cm^2 for TA102 and 1/20 mJ/cm^2 to WP2	*S. typhimurium* TA102 and *E. coli* WP2	33, 100, 333, 1000, 2500 & 5000 µg/plate;	Bemotrizinol did not induce base pair mutations after exposure to UVA/UVB irradiation.	
Photo-genotoxicity: In vitro Chromosomal Aberration Assay	Bemotrizinol dissolved in acetone; dosing in PBS solution; culture medium contained max 1% (v/v) acetone. Irradiation: UV B/A at 200/22 or 300/33 mJ/cm^2	Chinese Hamster V79 cells	6.25 up to 100µg/ml scored at 18h or 28h harvest interval.	Bemotrizinol did not induce structural chromosome aberrations in the presence or absence of UVA/UVB irradiation	
PHOTOTOXICITY STUDIES – DERMAL					
Phototoxicity	Bemotrizinol in PEG 400 Irradiation: 20 J/cm^2 UVA	Guinea Pig	10, 15, 25, and 30%. Applied as a thin layer to a 2 cm^2 test site.	No evidence of phototoxicity.	
Photoallergenicity	Bemotrizinol in PEG 400 Irradiation: Induction: 1.8 J/cm^2 UV-B + 10 J/cm UV-A Challenge: 10 J/cm^2 UV-A	Guinea Pig	0.1 ml 30% in PEG given intradermally; then 4 topical induction by 0.0125 ml/cm^2 of 10, 15, 25, and 30%; Challenge at same doses.	No evidence of photosensitization up to 30% topical challenge dose.	

Ciba

SUMMARY TABLE of TOXICOLOGY STUDIES with BEMOTRIZINOL					
STUDY	**TEST ARTICLE**	**Test System**	**DOSES (mg bemotrizinol/kg body wt/day)**	**FINDINGS**	**NOEL (mg/kg/day) Active Ingredient, bemotrizinol**
SPF-like Evaluation	Bemotrizinol, formulated in hydrated, hydrophilic Base Ointment. Irradiation: One UV (B & A) exposure at 6 different MEDs per mouse.	Hairless mice	Single topical dose of 0 (vehicle), 50, or 200 mg a.i./g formulation; equivalent to 5% or 20% a.i./cm^2 and about 160 and 650 mg a.i./kg/d, respectively.	Administration of bemotrizinol showed a dose-related increase in cutaneous protection against a single UVR exposure.	
13-week Dermal Phototoxicity (Dose Range Finder for 12-month Photo-carcinogenesis Study)	Bemotrizinol formulated in hydrated, hydrophilic Base Ointment. Irradiation: 1200 RBU	Hairless mice	Dosed as % a.i./d: 2.5, 5.0, 10, or 20 in 100 µl/mouse to 25 cm^2 skin daily	13-weeks' topical dosing at 2.5%, 5%, 10% or 20% a.i. daily, before or after UV irradiation, mice did not show effects different from the respective control groups. Doses of bemotrizinol selected for Photocarc study: 50 mg/g (0.2 mg a.i./cm^2) and 200 mg/g (0.8 mg a.i./cm^2). The highest topical dose was about 4 times higher than expected human topical daily doses.	200 mg/g (20% a.i), or 571 (male) to 714 (female) mg a.i./kg body wt/d]
12- Month Dermal Photo-carcinogenicity	Bemotrizinol formulated in hydrated, hydrophilic Base Ointment Irradiation: 600 or 1200 RBU	Mice	0, vehicle, 50, or 200 mg/g before or after UVR for 5 days/week.	The photocarcinogenic response was delayed by bemotrizinol showing a protective effect against photo-co-carcinogenesis. The highest topical dosage was about 4 times higher than expected human topical daily doses.	**20% a.i. (0.8 mg a.i./cm^2**
PHARMACOKINETICS					
ADME - Oral	[^{14}C]-Bemotrizinol	Rat	50 mg orally	A single oral dose of 50 mg [^{14}C]-bemotrizinol/kg to male and female rats showed excretion was rapid in both sexes, with 94-97% of the administered dose excreted directly in feces within 96 hours as unchanged bemotrizinol. The low absorption did not permit AUC calculation. Quantitatively, no sex difference was observed. Urinary excretion was 0.1-0.3% of dose; residual radioactivity in tissues and carcass accounted for 0.1-0.3% of the dose. Residues in individual tissues were all <0.01% of the dose. Orally, bemotrizinol is considered as not bioavailable.	

Ciba

SUMMARY TABLE of TOXICOLOGY STUDIES with BEMOTRIZINOL					
STUDY	**TEST ARTICLE**	**Test System**	**DOSES (mg bemotrizinol/kg body wt/day)**	**FINDINGS**	**NOEL (mg/kg/day) Active Ingredient, bemotrizinol**
ADME - Dermal	4%[^{14}C]-Bemotrizinol in a representative sunscreen formulation	Rat	2 mg or 0.8 mg [^{14}C]-bemotrizinol applied to 10 cm^2 skin; is about 0.2mg/cm^2 at top dose	After a single topical 6-hour exposure to either 2 mg or 0.8 mg [^{14}C]-bemotrizinol applied as a 4% bemotrizinol formulation, the *in vivo* dermal absorption accounted for 0.2% and 0.3% of the dose, respectively, over 24 hours. The low absorption did not permit AUC calculation. Means of 90% and 96% of the applied dose of 2 mg or 0.8 mg [^{14}C]-bemotrizinol §were readily washed from the skin surface after the 6h exposure. By dermal dosing the absorption of [^{14}C]- bemotrizinol was very low and the substance is not readily bioavailable by dermal absorption. The topical dose is the same as the expected human application rate.	
SUB-ACUTE/SUB-CHRONIC ORAL STUDIES					
14-day Oral Gavage-Dose Range Finder (DRF)	Bemotrizinol in PEG 400	Rat	50, 200, 800, or 2000 in constant volume 10 ml/kg body wt/d	Test article-related effects did not occur in any of the observed parameters in any dose group except for pale feces in the high dose group from day 12 until termination. This finding was considered to be due to the yellow color of the test article and to be of no toxicological relevance in the absence of any abnormal clinical laboratory parameters and histopathology findings.	**2000**
13-Week Oral Gavage	Bemotrizinol in PEG 400	Rat	Vehicle, 100, 500, or 1000	Test article-related effects did not occur on 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 statistically significant differences in a few clinical chemistry and urinalysis values noted between treated groups and controls at various times were considered as not test article-related for lack of clear evidence to the contrary.	**1000**
SUB-ACUTE/SUB-CHRONIC DERMAL STUDIES					
14-day Dermal – Dose Range Finder	Bemotrizinol in PEG 400	Rat	1000 at 2.5ml/kg body wt/d or about 6 mg a.i./cm^2 daily	Clinical signs included scabs on the back of one treated male. Slight desquamation was noted at the dose site in one control female and the majority of the treated females. Body weight, body weight gain, and food consumption did not show	**1000**

Ciba

SUMMARY TABLE of TOXICOLOGY STUDIES with BEMOTRIZINOL					
STUDY	**TEST ARTICLE**	**Test System**	**DOSES (mg bemotrizinol/kg body wt/day)**	**FINDINGS**	**NOEL (mg/kg/day) Active Ingredient, bemotrizinol**
				relevant differences between control and treated animals. Necropsy did not reveal test article-related findings in any of the animals. Microscopic examinations were not conducted. Subsequent experience with the test article in the vehicle (13-Wk study) indicated the observed desquamation in the bemotrizinol-dosed animals was likely dried residual test article resembling desquamated skin.	
13-Week Dermal	Bemotrizinol in PEG 400	Rat	Vehicle, 0, 250, 500, or 1000 in 2 groups with or without protective collar. Topical dosages at 2.5ml/kg body wt/d were about 1.5, 5.0, and 6 mg a.i./cm^2 once daily	Effects of toxicological significance did not occur in any parameter recorded in any of the dose groups. Plasma levels at day 8 and weeks 6 &13 indicated the test item was not bioavailable trans-dermally. NOEL is about 30 times higher than the expected human topical application rate.	**1000**
TERATOLOGY & REPRODUCTION STUDIES					
Segment I Reproduction – Oral Gavage	Bemotrizinol in PEG 400	Rat	10 mL/kg bw as single daily oral gavage doses of 0 (vehicle), 100, 300, or 1000	Test article-related effects did not occur in any parameter.	**1000**
Segment II Developmental – Oral Gavage	Bemotrizinol in PEG 400	Rat	0, 100, 300, 1000	Treatment-related soft feces were considered a vehicle-related effect since it was observed in all groups. The statistically significant differences in reproductive parameters, an increased post-implantation loss and reduced number of fetuses at 100 and 1000 mg/kg/d, were not considered test article-related in the absence of a dose-relationship and their values being within the ranges of historical control data. Test article-related effects did not occur for any other clinical or reproductive maternal parameters or for fetal effects.	**Maternal and Fetal** **1000**
Segment II	Bemotrizinol in vehicle (0.5%	Rabbit	10 mL/kg bw as	No treatment-related effects were noted	

Ciba

SUMMARY TABLE of TOXICOLOGY STUDIES with BEMOTRIZINOL					
STUDY	**TEST ARTICLE**	**Test System**	**DOSES (mg bemotrizinol/kg body wt/day)**	**FINDINGS**	**NOEL (mg/kg/day) Active Ingredient, bemotrizinol**
Developmental Study – Oral Gavage	carboxymethylcellulose in 0.1% aqueous Tween 80		single daily oral gavage doses of 0 (vehicle), 100, 300, or 1000		**Maternal and Fetal** **1000**
Segment III – Reproduction – Oral Gavage	Bemotrizinol in PEG 400	Rat	10 mL/kg bw as single daily oral gavage doses of 0 (vehicle), 100, 300, or 1000	Test article-related effects did not occur in any endpoint for maternal parameters or for pre- and post-natal development parameters of fetuses and offspring.	**1000 for all endpoints**
Endocrine Effects Testing	Bemotrizinol	In vitro Estrogen & Androgen receptors; Immature female rat	ER & AR: $5x10^{-10}$ to $5x10^{-4}$ Molar separately Rat: 3 doses at 250, 500, 1000 mg/kg body wt/d	In the receptor binding assays, bemotrizinol was negative and did not show binding affinity or competitive inhibition of natural hormone binding to either the estrogen or androgen binding sites. The *in vivo* immature rat assay showed bemotrizinol given subcutaneously did not adversely affect maturation of the rats and does not have intrinsic estrogenic properties.	
CHRONIC STUDIES - DERMAL					
39-Week	Bemotrizinol in PEG 400	Göttingen minipig	100, 500, 1250 single topical doses as 10%, 20%, 50% a.i . for 39 weeks; dose volume 2.5 mL/kg body wt/d; equivalent to 7, 14, 35 mg a.i./cm^2 daily	The high dose was the highest technically achievable. Dose site did not show observable tumors or signs of test article intolerance. Systemic toxicity did not occur based on clinical signs, hematology, clinical chemistry, and ophthalmological endpoints. Plasma showed the test item in all dose groups (LOQ= 2 ng/ml), but at levels below 16 ng/ml (only 4 values exceeded 10ng/mL) and without relationship to dose level or weeks of exposure. Microscopic examination of dose-site and control site skin did not reveal test article-related changes. The NOEL is about 175 times higher than expected human daily topical application.	1250 mg/kg/d
Lifetime Dermal Carcinogenicity	Bemotrizinol in PEG 400	Rat	100, 500 or 1000 by single cutaneous doses for 104-105 weeks; dose volume 2.5 mL/kg body	The high dose was the highest technically achievable. The dose site did not show observable tumors; at week 105 all groups had 44-68% survival. Palpable masses onset had mean of week 88 (range week 33 to 105) and on first analysis does not appear to be different from the control groups. Clinical signs, hematology, clinical chemistry, and ophthalmological endpoints did not show test article- or	**Report Completion in progress.** In-Life phase: 100 females and 500 in

Ciba

SUMMARY TABLE of TOXICOLOGY STUDIES with BEMOTRIZINOL					
STUDY	**TEST ARTICLE**	**Test System**	**DOSES (mg bemotrizinol/kg body wt/day)**	**FINDINGS**	**NOEL (mg/kg/day) Active Ingredient, bemotrizinol**
			wt/day; topical at 1, 5, or 10 mg a.i./cm^2 daily	treatment-related affects. Plasma showed the test item in all dose groups (LOQ= 2 ng/ml), but at minimal levels (2.20 to 81.2 ng/mL) and without relationship to dose level or weeks of exposure. Microscopic examination of dose-site and control site skin indicated the MTD was used and pre-neoplastic and neoplastic changes in skin did not occur.	females. Histopathology review is in progress; final conclusion is pending.

A. Individual Active Component, Bemotrizinol.

1. Study results from controlled studies. Data developed by Ciba Specialty Chemicals that meet this definition are referenced below in items a through dd.

a) Acute Dermal Toxicity in Rats (Tab III.A. 1)

Bemotrizinol was applied to the shaved skin of five male and five female Hanlbm:WIST (SPF) rats at a dose of 2000 mg/kg body weight and covered with a semi-occlusive dressing (Arcelin 1997a). Bemotrizinol was suspended in vehicle (PEG 400) at a concentration of 0.5 g/ml and administered at a volume of 4 ml/kg body weight. After 24 hours of exposure, the dressing was removed and the treated skin washed with water. No deaths occurred during the study. Neither clinical signs of systemic toxicity nor local effects of the test article on the skin at the application site were observed during the subsequent 15 days observation period. The body weights of the animals were within the range of physiological variability known for rats of this strain and age. No macroscopic organ findings were observed at necropsy. Since no deaths occurred during the study, the dermal dose LD_{50} is > 2000 mg/kg.

b) Acute Oral Toxicity in Rats (Tab III.A. 2)

Bemotrizinol was administered by oral gavage to five male and five female Hanlbm:WIST (SPF) rats at a dose of 2000 mg/kg body weight (Arcelin 1997b). Bemotrizinol was suspended in vehicle (PEG 400) at a concentration of 0.2 g/ml and administered at a volume of 10 ml/kg body weight. The animals were observed for a period of 15 days. No deaths occurred during the study and no clinical signs of toxicity were observed during the study. The body weights of the animals were within the range of physiological variability known for rats of this strain and age. No macroscopic organ findings were observed at necropsy. Since no deaths occurred during the study, the oral LD50 was > 2000 mg/kg.

IRRITATION/SENSITIZATION STUDIES

c) Primary Skin Irritation in Rabbits (Tab III.A. 3)

Bemotrizinol was applied to the shaved skin of three young adult New Zealand rabbits for four hours using a semi-occlusive exposure (Braun 1997a). A single dose of 500 mg bemotrizinol, moistened with bi-distilled water, was applied to 6 cm^2 intact dorsal skin and after four hours, the dressing was removed and the application site washed with water. The scoring of skin reactions was performed 1, 24, 48 and 72 hours after removal of the dressing. No effects on the skin, including erythema and edema, were noted at any observation time with the exception of reversible light yellow staining of the treated skin at the one-hour observation time. The primary irritation score was calculated by totaling the individual cumulative scores at 24, 48, and 72 hours and then dividing by the number of figures. The primary irritation score was 0.00 (max. 8.0). Based on the referred classification criteria (EEC Commission Directive 93/21/EEC of April 27, 1993), the test article was considered to be "not irritating" to rabbit skin.

d) Primary Eye Irritation in Rabbits (Tab III.A. 4)

Bemotrizinol (0.1 g) was instilled into one eye of each of three young adult New Zealand rabbits. The treated eyes were not rinsed after application. Scoring of irritation effects was performed approximately 1, 24, 48 and 72 hours after application. The primary irritation score (PIS) was calculated by totaling the individual cumulative scores at 24, 48 and 72 hours and then dividing the resulting total by the number of figures. The primary irritation score was 0.44 (max 13). The test article did not stain the cornea, sclera, or conjunctivae of the treated eyes. Based on the PIS, bemotrizinol was considered minimally irritating to the eye; based on EEC criteria bemotrizinol is classified as not irritant. (Braun 1997b)

e) Skin Sensitization (Guinea Pig Maximization Test) (Tab III.A. 5)

Bemotrizinol was administered to prepared skin of 10 male Albino guinea pigs according to a Maximization-Test protocol. Five guinea pigs served as controls. Induction occurred over the first 10 days. On test day one, the animals received three intradermal injections (0.1 ml/site) in separate areas of the dorsal skin in the scapular region. The injections consisted of 1) 1:1 (v/v) mixture of Freund's Complete Adjuvant (FCA) and physiological saline, 2) 3% bemotrizinol in PEG 400, and 3) 3% bemotrizinol in an emulsion with a 1:1 (v/v) mixture of FCA and physiological saline. Control animals also received the same three injections without bemotrizinol. On test day 7, the injection sites were treated with a 10% solution of sodium-lauryl-sulfate to enhance sensitization by provoking a mild inflammatory reaction. On test day 8, a single topical induction dose of about 0.3 g of a mixture of 30% bemotrizinol in PEG 400 was applied over the injection sites using an occlusive dressing for 48 hours. Control animals were treated with PEG 400 only. Challenge occurred two weeks after completion of the topical induction. About 200 mg of 30% percent bemotrizinol in PEG 400 was topically applied to the shaved skin on the left flank using a 24-hour occlusive exposure. The shaved skin on the right flank received 200 µl PEG 400 only. Control animals received the same challenge treatment. Skin reactions were evaluated 24 and 48 hours after removal of the challenge exposure patch. Slight erythema was noted in several animals of both the treated and control groups after the topical induction exposure. After challenge, erythema or edema did not occur in any animals 24 or 48 hours after challenge. In conclusion, under the test conditions, bemotrizinol was not a skin sensitizer. (Arcelin 1997c)

f) Phototoxicity in Guinea Pigs (Tab III.A. 6)

Bemotrizinol was tested in a phototoxicity study according to the Cosmetic, Toiletry, and Fragrance Association (CTFA) Safety Testing Guidelines. Bemotrizinol in PEG 400 was applied to four separate 2 cm^2 sites on the shaved skin of the left flank of 10 female Dunkin Hartley guinea pigs at the following concentrations: 10, 15, 25, and 30%. Due to the high viscosity of the test material, a fixed volume could not be applied to each site. Instead, a thin layer of the test article was applied to saturate each test site. Five control female guinea pigs received PEG 400 only. Thirty to 50 minutes prior to test article application, the test sites were pretreated with 2% DMSO diluted in ethanol (0.0125 ml/cm^2) to enhance skin penetration of the test article. Thirty minutes after application of the test material, the left flank of each animal in the control and treatment groups was exposed to 20 J/cm^2 UVA irradiation. After irradiation, the right flank received the same test-material applications as the left flank, but the sites were not exposed to UVA irradiation. Skin reactions were observed 24, 48, and 72 hours after application.
A positive control test with 8-methoxypsorolene was run separately (October 1996) and the results indicated the test system was sensitive and the protocol appropriate for demonstrating phototoxicity. Bemotrizinol dosed animals did not show skin reactions, including erythema and edema, during the experiment. In conclusion, under the test conditions, bemotrizinol was not phototoxic. (Arcelin 1997e)

g) Photoallergenicity in Guinea Pigs (Tab III.A. 7)

Bemotrizinol was tested in a photoallergenicity study according to the CTFA Safety Testing Guidelines. Induction occurred over the first 11 days. On test day one, each of 20 Dunkin Hartley guinea pigs received four intradermal injections (0.1 ml/site) of a 1:1 (v/v) mixture of Freund's Complete Adjuvant (FCA) and physiological saline in the four corners of the 6-8 cm^2 test site located on the dorsal skin. After injection, 0.1 ml of 30% bemotrizinol in PEG 400 was topically applied to the test site. The site was then exposed to 1.8 J/cm^2 UVB and 10 J/cm^2 UVA irradiation. The topical application followed by irradiation was repeated four times within two weeks on days 3, 7, 9, and 11. Control animals only received the four intradermal FCA injections without any further treatment during the induction phase. The challenge phase started on day 22. For both control and treatment groups, bemotrizinol in PEG 400 was applied to four separate 2 cm^2 sites on the shaved skin of the left flank at the concentrations of 10, 15, 25, and 30%. A dose of 0.0125 ml/cm^2 was applied to each site. After application, the left flank was exposed to 10 J/cm^2 UVA irradiation only. After irradiation, the right flank was treated like the left flank, but without UVA irradiation. Skin reactions were assessed after 24, 48, and 72 hours of exposure.
A positive control test with 3,3',4', 5-tetrachlorosalicylanilide was run separately (May 1997) and the results indicated the test system was sensitive and the protocol appropriate for demonstrating photosensitization response.

After topical induction applications with bemotrizinol, erythema and edema were observed from test day 9 to 15 with the repeated application of 30% bemotrizinol. After the challenge dose, effects did not occur on the skin of any of the animals. In conclusion, under the test conditions, bemotrizinol was not a photosensitizer. (Arcelin 1997d).

SUBCHRONIC and CHRONIC STUDIES

h) 14-Day Oral Gavage Range-Finding Study in Rats (Tab III.A. 8)

Bemotrizinol in PEG 400 (vehicle) was administered to groups of Wistar rats (SPF), 5 rats/ sex/group, by oral gavage at daily doses of 50, 200, 800, and 2000 mg/kg for 14 days (Schmid et al. 1998). Controls received vehicle only. No treatment-related effects on survival, food consumption, body weights, ophthalmoscopy findings, hematology and clinical chemistry values, organ weights, and macroscopic or microscopic findings were noted. The only clinical sign noted was pale feces at 2000 mg/kg from day 12 of the study until termination. This finding is considered to be due to the yellow color of the test article and to be of no toxicological relevance in the absence of any abnormal clinical laboratory parameters and histopathology findings. In conclusion, under the test conditions, the No-Observable-Adverse-Effect-Level (NOAEL) for this study was 2000 mg/kg.

i) 14-Day Dermal Toxicity Study in Rats (Tab III.A. 9)

The objective of this study (Gaou 2003a) was to evaluate the potential toxicity of bemotrizinol following daily cutaneous application to rats for 2 weeks and to aid the selection of dose-levels for a subsequent 13-week dermal toxicity study in this species. Based on information from the 13-week oral dosing study in rats, a single limit-dose approach was used for this dermal study. One treated group of five male and five female Wistar Han rats received bemotrizinol daily by cutaneous application at the dose-level of 1000 mg/kg/day for 2 weeks. An additional group of five males and five females received the vehicle, polyethylene glycol 400, alone under the same experimental conditions and served as the control group. No mortality occurred during the study. Clinical signs included scabs on the back of one treated male. Slight desquamation was noted at the dose site in one control female and the majority of the treated females. Body weight, body weight gain, and food consumption did not show relevant differences between control and treated animals. Necropsy did not reveal treatment related findings in any of the animals. Microscopic examinations were not conducted. The study indicated doses up to 1000 mg/kg/day would be well tolerated in a 13-week study. Subsequent experience with the test article in the vehicle indicated that the observed higher incidence of desquamation in the bemotrizinol-dosed animals might have been the dried residual test article resembling desquamated skin.

j) 90-Day Oral Gavage Toxicity Study in Rats (Tab III.A. 10)

Bemotrizinol in PEG 400 as vehicle was administered to groups of Wistar rats (SPF) (20 sex/group) by oral gavage at daily doses of 100, 500, and 1000 mg/kg body weight for at least 92 days (Hamann et al., 1998). Controls received vehicle only. No treatment-related effects on 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 were noted. Statistically significant differences in a few clinical chemistry and urinalysis values were noted between treated groups and controls at various times. However, the differences were not considered toxicologically significant since they were sporadic, were well within the range of normal background data encountered in rats of the strain and age used, were slight in magnitude and/or displayed no dose-response relationship, and were not correlated with any morphological changes. In conclusion, under the test conditions, the No-Observable-Effect-Level (NOEL) for this study is 1000 mg/kg.

k) 90-Day Dermal Toxicity Study in Rats (Tab III.A. 11)

The objective of this study was to evaluate the potential toxicity of bemotrizinol following daily cutaneous application to rats for 13 weeks (Gaou 2003b). Four treated groups of 13 male and 13

female Wistar Han rats (including three satellite males and three satellite females) received the test item daily by cutaneous application at the dose-levels of 250, 500, 1000 (without protective collar) or 1000 mg/kg/day (with protective collar) for 13 weeks. An additional group of 10 males and 10 females received the vehicle alone (polyethylene glycol 400) under the same experimental conditions and acted as a control group. To assess any differences in effects from grooming access to the dose site and the subsequent oral ingestion of test substance, animals of the second high-dose group given 1000 mg/kg/day (group 6) wore a protective plastic collar over at least a 6-hour period after each application. The application site of these animals was then cleaned with tap water and dried with absorbent paper. I n addition to the standard parameters for a 13-week study, the estrous cycle stage was determined for 14 consecutive days during weeks 11 and 12 for each female. Satellite animals were allocated for the determination of the test item plasma levels and were sampled before dosing on day 8 and in weeks 6 and 13. On completion of the treatment period, the animals were killed and submitted to a full macroscopic *post-mortem* examination. Designated organs were weighed and selected tissue specimens were preserved. A microscopic examination was performed on selected tissues from animals of the vehicle control and the two high-dose groups (groups 2, 5 and 6) and on all macroscopic lesions in the low- and mid-dose groups.

Deaths did not occur during the study and clinical signs of cutaneous or systemic effects related to treatment with the test item did not occur in any of the treated animals. Detailed clinical examination and functional observation battery did not show treatment-related changes in autonomic, physiological or neurotoxicological parameters. Motor activity was not affected by treatment with the test item. Body weight, body weight gain and food consumption were not affected by dermal treatment with bemotrizinol. No treatment-related ophthalmological findings were seen at the end of the treatment period.

Plasma levels of the test item measured in animals given 1000 mg/kg/day with or without collar were very low and indicated that the test item was not bioavailable by the cutaneous route. Both groups were comparable in results for plasma analysis.

Hematology and blood biochemistry parameters did not show differences of toxicological significance between control and treated animals in any of the dose groups. The estrous cycle stage was not affected at any dose-level. Urinalysis results did not show any relevant differences between control and treated animals for qualitative and quantitative parameters.

Organ weights were not different between control and treated animals. At necropsy, macroscopic changes did not show treatment-related effects. Microscopic examination of designated key tissues did not reveal adverse effects or treatment-related changes. Skin from the dosing site was given particular attention and effects related to bemotrizinol dosing were not revealed.

In conclusion, bemotrizinol administered dermally to Wistar Han rats at the dose-levels of 250, 500 and 1000 mg/kg/day, equivalent to 1.5, 5.0, and 6 mg a.i./cm^2/d, respectively, for 13 weeks did not have effects of toxicological significance in any parameter recorded in any of the dose groups. Plasma levels indicated that the test item was not bioavailable. Consequently, under the experimental conditions of the study, the No Observed Effect Level (NOEL) of the test item is 1000 mg/kg/day. The highest topical dose of 6 mg/cm^2 was about 30 times higher than expected human topical daily doses.

l) Lifetime Dermal Carcinogenicity Study-Rats (Tab III.A. 12)

The objective of this study was to evaluate the potential carcinogenicity of the test item, bemotrizinol, following daily cutaneous application to rats for 104 weeks. (Fisch, in preparation)

Three dosed groups of 100 Wistar Han rats (50 males and 50 females) received single daily topical doses of bemotrizinol at 100, 500 or 1000 mg/kg body weight/day by 104 weeks applied at constant dosage volume of 2.5 mL/kg body weight/day. Another group of 50 males and 50 females received no treatment and acted as an untreated control group. An additional group of 50 males and 50 females received the vehicle alone (polyethylene glycol 400) under the same experimental conditions and acted as vehicle control group.

The animals were checked daily for mortality, clinical signs and possible signs of skin irritation. The animals were palpated every 2 weeks after 6 months of treatment in order to monitor the onset and

progression of any masses. Body weight and food consumption were recorded at designated intervals (weekly during the first 3 months of treatment and then monthly). Ophthalmologic examinations were performed on all the animals before the beginning of the treatment period and on 10 animals/sex in the vehicle control and high-dose groups in weeks 26, 52, 78 and 103.

The differential white cell count was determined in weeks 52 and 78 for the animals in the vehicle control and high-dose groups. Blood samples for the determination of plasma levels of the test item were taken in weeks 13, 26, 52, 78 and 104. On completion of the treatment period, blood samples were taken from 10 animals/sex and group for hematology and blood biochemistry investigations. All surviving animals were euthanized and submitted to a macroscopic post-mortem examination. Designated organs and any masses or lesions were sampled from each animal and retained for microscopic examination (except for untreated animals).

The in-life phase was terminated on 4 October 2005 and an interim draft report has been prepared to include with this GRAS/E submission package. Final conclusions and interpretations must await completion of micropathology evaluations for all groups. A first statement for dose-site skin and control site (not treated) skin has been prepared by the study pathologist and is included with this summary and in the report documents submitted under this Tab number.

The following statements are based on results available at time of this submission.

Survival rate: There was no effect of the test item on survival rate during the 104-week treatment period; at week 105 all groups had 44-68% survival. Factors contributing to death will be completed later when the results of microscopic examination become available.
Clinical signs: The incidence, nature and time of onset of the clinical signs observed in test-treated animals during the study period were similar to those observed in control animals.
Palpable masses: The incidence of tumors will be verified after microscopic examination, which will confirm the diagnosis of tumors. The palpable masses observed during the treatment period were considered to be of a spontaneous nature and usual for animals of this strain and age. Dose site did not show observable tumors. Palpable masses onset had mean of week 88 (range week 33 to 105) and on first analysis does not appear to be different from the control groups. Their frequency was low as indicated in the following table. Consequently, the palpable masses observed during the treatment period were considered to be of a spontaneous nature and usual for animals of this strain and age.

The number of animals with palpable masses is presented in the table below:

Group	1		2		3		4		5	
Dose-level (mg/kg/day)	- (untreated)		0 (vehicle)		100		500		1000	
Sex (n=50)	M	F	M	F	M	F	M	F	M	F
Number of palpable masses	2	17	3	15	4	11	3	21	6	19
% animals bearing masses	4	34	6	24	8	2	6	30	12	32

M: male; F: female.

Body weight and food consumption: The body weight and food consumption were unaffected by treatment with the test item at all dose-levels during the study.
Ophthalmology: No ophthalmological findings were attributed to treatment with the test item at any dose-level.
Hematology and blood biochemistry: No hematological or blood biochemical differences were attributed to treatment with the test item.

Plasma levels of the test item: The test item was not detectable (LOQ= 2 ng/ml) in vehicle control animals at any time-point. In bemotrizinol-treated groups, the test item was detectable at all dose-levels, but at minimal levels (2.20 to 81.2 ng/mL) and without relationship to neither the dose-level nor the study week of sampling. No gender effect was seen. The results are shown in the next table.

Mean plasma levels of the test item (expressed in ng/mL)								
Group	2		3		4		5	
Dose-level (mg/kg/day)	0 (vehicle)		100		500		1000	
Sex	M	F	M	F	M	F	M	F
. Week 13	BLQ	BLQ	6.86	3.29	12.0	12.6	23.9	4.63
. Week 26	BLQ	BLQ	24.2	BLQ	48.3	7.22	20.3	9.02
. Week 52	BLQ	BLQ	9.03	3.89	17.1	19.3	28.4	13.0
. Week 78	BLQ	BLQ	22.7	8.82	33.5	25.5	25.5	27.9
. Week 104	BLQ	BLQ	50.5	10.9	34.7	8.81	24.6	21.2
M: male; F: female; BLQ: below limit of quantification (<2 ng/mL)								

Although dermal absorption is not excluded as a source for the test article observed in plasma, the highly variable distribution of these results suggests oral absorption could also be considered as a source of plasma test article. This is based on the study design using daily topical applications that were not protected after application and would allow transfer of test article to cage bars for ingestion by the rats or permit direct oral ingestion by animal's self cleaning. In light of the low bioavailability of bemotrizinol shown in the ADME oral and dermal (single doses) studies it remains that with repeated topical applications some measurable but low amounts of test article can reach systemic circulation.

Organ weights: Data were not available at time of this submission.

Macroscopic post-mortem examination: Data were not available at time of this submission.

Microscopic examination of all tissue and organ samples will be completed later. Dose site skin and control site skin have been examined and the following summary is considered a preliminary conclusion for the effects of bemotrizinol on rat skin. This statement is included in the following summary.

I. Comparison Vehicle Control And Untreated Control

Decedents

At the treated skin area of males and females, treatment with the vehicle alone induced an increased incidence of diffuse sebaceous gland hypertrophy/hyperplasia, when compared to concurrent untreated control animals of the same sex.

At the untreated skin area, an increase in diffuse sebaceous gland hypertrophy/hyperplasia was only seen in vehicle-treated females, but not in males, when compared to concurrent untreated control animals of the same sex.

Terminal sacrifice

At the treated skin area of males and females, vehicle treatment lead to an increased incidence of diffuse sebaceous gland hypertrophy/hyperplasia, when compared to concurrent untreated control animals of the same sex.

At the untreated skin area, no histomorphological differences were seen between vehicle treated and untreated males or females.

II. Test Article-related skin effects:

Comparison between dose groups and vehicle control

A. Decedents

Predominant histopathological changes at the treated skin area were considered to be the consequence of a chronic local skin irritation and inflammation and confirmed macroscopic observations made at necropsy.

Compound-related effects noted at the untreated skin area were mild and restricted to the top dose group males. These observations are interpreted to be most probably the consequence of

an unintentional contamination of the untreated skin area with the compound preparation, e.g., by licking or scratching by the animal.

No neoplastic or pre-neoplastic lesions that were clearly test article-related were observed at the treated or untreated skin area.

1. Treated skin

- An increased incidence and severity of ulcer(s)/scab(s) was seen from 100 mg/kg/day in males and from 500 mg/kg/day in females. The effect was dose–related in males.
- The incidence and severity of multifocal/diffuse epidermal hyperplasia and of subepithelial inflammatory cell infiltrate(s) was increased in males and females from 100 mg/kg/day.
- An increased incidence of multifocal/diffuse hyperkeratosis was observed in males at 1000 mg/kg/day and in females from 500 mg/kg/day. Furthermore, in a low number of males and females at 500 and 1000 mg/kg/day, the formation of rete pegs and/or a diffuse mixed cell dermatitis were observed.
- The incidence and severity of a diffuse sebaceous gland hypertrophy/hyperplasia was increased in males from 100 mg/kg/day and in females from 500 mg/kg/day.

2. Untreated skin

- In males treated at 1000 mg/kg/day, there was an increased incidence of diffuse sebaceous gland hypertrophy/hyperplasia, focal/multifocal folliculitis/peri-folliculitis and subepithelial inflammatory cell infiltrate(s). The effect was very minor.
- No compound-related effects were noted in females of all dose groups.

B. Terminal sacrifice

Histopathological changes at the treated and untreated skin areas generally corresponded to those observed in decedents with some more effects already seen in the low and mid dose groups. No clearly compound-related neoplastic or preneoplastic lesions were observed at the treated or untreated skin area.

1. Treated skin

- An increased incidence and severity of ulcer(s)/scab(s) was seen in males from 500 mg/kg/day. No clear effect was observed in females.
- The incidence and severity of multifocal/diffuse epidermal hyperplasia and of subepithelial inflammatory cell infiltrate(s) was increased in males and females from 500 mg/kg/day.
- An increased incidence of multifocal/diffuse hyperkeratosis was observed in males from 500 mg/kg/day and in females from 100 mg/kg/day.
- The incidence of follicular dilatation was increased in males at 1000 mg/kg/day and in females from 100 mg/kg/day. In a low number of males from 500 mg/kg/day, the occurrence of rete pegs or a diffuse mixed cell dermatitis were observed.
- The incidence and severity of a diffuse sebaceous gland hypertrophy/hyperplasia was increased in males and females from 100 mg/kg/day.
- In one female treated at 1000 mg/kg/day, a focal basal cell hyperplasia was observed and was considered incidental.
- In view of its isolated occurrence, the occurrence of one sebaceous gland adenoma in a male rat at 500 mg/kg/day was not considered to be clearly compound-related.

2. Untreated skin

- There was a higher incidence of diffuse sebaceous gland hypertrophy/hyperplasia in males from 500 mg/kg/day and in females from 100 mg/kg/day.

- An increased incidence of multifocal/diffuse hyperkeratosis in females at 500 mg/kg/day was considered incidental as the incidences of the low and high dose groups did not exceed that of the treated area in the vehicle-treated group.

Overall, based on information available from the interim draft report, the test article, administered daily by cutaneous application at 100, 500 and 1000 mg/kg to rats for 104 weeks did not induce grossly or microscopically observable tumors at the dosing site. The survival rate and occurrence of palpable masses was not adversely affected by treatment with the test item. Clinical signs of toxicity and effects on body weight or food consumption did not occur at any dose-levels. At laboratory investigations, no

differences among hematological and blood biochemical parameters were attributable to treatment with the test item. A dose related effect of the test article in vehicle was apparent suggesting that the maximum tolerable topical dosages were applied in males and females; from the available information the skin tolerance NOAEL appears to be 100 mg/kg/day in females and 500 mg/kg/day in males. Neoplastic and pre-neoplastic changes did not occur and bemotrizinol is, at this point, considered not to be a dermal carcinogen in rat.

The full conclusion will be completed when the results of histological examinations and peer review will be available.

m) Lifetime Oral Carcinogenicity Study-Rats (Waiver Justification)

Waiver Justification for Oral Carcinogenicity Testing with Bemotrizinol

Ciba Specialty Chemicals has prepared the safety dossier of non-clinical and clinical test results for bemotrizinol in support of our petition for its addition to OTC Sunscreen Monograph. Many human-use drug substances with repeated application for prolonged periods of time that are reviewed by US FDA often are expected to show in non-clinical testing an absence of or low risk for carcinogenic effects, usually in two species. It is Ciba's position that the weight of the available evidence indicates an absence of any indications suggesting a need to conduct an oral carcinogenicity testing in rodents. The evidence is based on our non-clinical test results, including a rat dermal carcinogenicity test and a 12-month photo-co-carcinogenicity test in hairless mice, plus the good clinical experiences in over 5 years of consumers' use of sunscreens containing bemotrizinol. Accordingly, and based on the following additional points, we petition FDA to accept our request for waiver of this additional non-clinical animal test.

1. Bemotrizinol does not present any characteristics indicating oral dosing of animals would result in carcinogenic changes in any tissues or organs. This is based on the very low systemic uptake demonstrated in oral gavage dosing over 13 Weeks in rats and observation of only minimal systemic levels (0.6% of dose applied) of measurable radio-labeled bemotrizinol in rats after a single gavage dose.
2. The non-clinical toxicology profile for local tolerance of bemotrizinol does not indicate adverse effects in single oral and topical applications, the substance is not genotoxic with or without exposure to UV irradiation, it is not phototoxic topically, and it is not a skin contact sensitizer with or without UV irradiation. Importantly, repeated oral dosing through the full reproductive cycle of rodent did not reveal adverse effects and rabbits did not show effects to developmental parameters (Segment II study). Bemotrizinol is not considered a toxicologically active substance.
3. The full body of non-clinical testing with bemotrizinol indicates that it is not readily absorbed, orally or topically, and it is not metabolised to toxic intermediates, based on ADME testing. In addition, with prolonged administration by oral (up to 13 weeks) or dermal routes (up to 2 years) it does not produce any indications for increased carcinogenic response. Prolonged topical dosing of bemotrizinol for 40 weeks to hairless mice also exposed to daily doses of UV radiation demonstrated a protective effect in that bemotrizinol did not increase the UV-induced carcinogenic response in the mice, and in fact, increased the time to tumor onset and decreased the potentcy of the UV radiation.
4. Bemotrizinol is a photostable molecule and in the presence of UV radiation does not produce activated moieties such as singlet oxygen, and does not degrade to substituents of the parent structure. This ensures that the available non-clinical data are representative of the safety of bemotrizinol used in topically applied sunscreens, therapeutic articles, and cosmetic products.
5. The molecular structure of bemotrizinol does not contain any of the structural alerts commonly associated with carcinogenic substances (Ashby and Tennant 1991; Ashby and Paton 1993), and does not present a carcinogenic hazard because it is not genotoxic, is not even moderately systemically available, and any small portion of a topical or oral exposure reaching systemic circulation is rapidly eliminated.

It is our conclusion that bemotrizinol does not require testing for oral carcinogenicity because of the low systemic availability, absence of systemic toxicity, non genotoxicity, and very good topical tolerance in rodents and non-rodent species. Further, the photo-stable molecular structure of bemotrizinol is not characteristic of known carcinogens and in a topical lifetime rodent study

bemotrizinol was shown to be not carcinogenic in the rat. Additionally, the principle route of human exposure to bemotrizinol will be topical application from which only exceedingly low systemic levels could be predicted. The over five years' consumer use in sunscreens gives no indication for increased concern. Therefore, we request that FDA accept this rationale as sufficient evidence to waive the need for oral carcinogenicity testing with bemotrizinol.

References:

Ashby, J and Tennant, R. 1991. Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP. Mutation Research 257:229-306

Ashby, J and Paton, D. 1993. The influence of chemical structure on the extent and sites of carcinogenesis for 522 rodent carcinogens and 55 different human carcinogen exposures. Mutation Research 286: 3-74.

n) Fertility and General Reproduction Toxicity (Segment I) Study in Rats (Tab III.A. 13)

This study evaluates ICH Harmonised Tripartite Guideline stages A and B of the reproductive process to detect effects on the estrous cycle, tubal transport, implantation, and development of preimplantation stages of the embryos of female rats and permits detection of functional effects (e.g., effects on libido or epididymal sperm maturation) that may not be detected by histological examinations of male rat reproductive organs. Bemotrizinol suspended in PEG 400 as vehicle was administered at a dose volume of 10 mL/kg body weight as single daily oral gavage doses of 0 (vehicle), 100, 300, or 1000 mg/kg body weight/day. Each study group consisted of 20 male and 20 female rats (Crj:CD®(SD)IGS SPF).

The dosing of male rats in each group occurred once daily for a total of up to 53 doses, which began 28 days before the planned cohabitation period, through cohabitation (maximum 21 days), and continued through the day before sacrifice, which was the end of the cohabitation period. Female rats were dosed once daily for up to 27 days beginning 15 days before cohabitation (maximum of 21 days), during cohabitation and continuing through day 7 of presumed gestation (GD 7). The estrus cycle was determined by vaginal cytology of all females 14-days before the start of dosing, 14-days after the start of dosing, and then until spermatozoa were observed in the vaginal contents or when a copulatory plug was observed in situ during the cohabitation period (defined as Gestation day 0). The in-life phase parameters for males and females included weekly feed consumption determination and recording of body weights.

Males were sacrificed after completion of the cohabitation period and the reproductive organs weighed (testes, epididymides, seminal vesicles), and sperm evaluated for concentration, survivability, and motility. Females were sacrificed on presumed GD 13 and examined to determine the number of corpora lutea, implantation sites, and of viable and non-viable embryos.

Results indicate bemotrizinol did not adversely affect male or female body weight, food consumption, mating behavior, and fertility; male reproductive organs and sperm parameters and female estrus cycle and numbers of corpora lutea, implantation sites, and numbers of viable and non-viable fetuses were similar to control group values. The No Observed Effect Level is 1000 mg/kg/day for general toxicity to dams, reproductive function of mating animals, and for early embryonic development (Ota 2002a).

o) Developmental Toxicity (Segment II) Study in Rats (Tab III.A. 14)

Bemotrizinol in PEG 400 (vehicle) was administered by oral gavage once daily to groups of pregnant female Wistar rats (22/group) from days 6–17 of gestation at 100, 300, and 1000 mg/kg body weight. Controls received vehicle only. Animals were sacrificed on day 21 of gestation and the fetuses removed by Caesarian section.

Test article-related effects did not occur for survival, food consumption, body weight gain, or macroscopic findings in any dam. Test article-related clinical signs were noted with the exception of soft feces, which were determined to be a vehicle-related effect since it was observed in all groups, including vehicle controls, and it is commonly seen in animals treated with PEG 400. 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, the effects were not considered test article-related since a dose-relationship was not evident and the parameters were within the ranges of historical control data. Test article-related effects did not occur for other reproductive parameters (mean numbers of corpora lutea and implantation sites, and percent of pre-implantation loss) or for fetal effects (external, visceral, and skeletal abnormalities; sex ratios; and body weights). In conclusion, under the test conditions, the maternal and fetal NOELs were 1000 mg/kg. (Becker and Biedermann, 1998)

p) Developmental Toxicity (Segment II) Study in Rabbits (Tab III.A. 15)

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 bodyweight, adjusted daily for individual bodyweight, at dosages of 0 (vehicle), 100, 300, or 1000 mg/kg/day. These dosages were selected based on absence of adverse findings with these dosages 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 Cesearean section and necropsy. The uterine contents were evaluated and all fetuses were examined for external, visceral, and skeletal changes.

Deaths did not occur among the does and the observed clinical signs were considered not related to bemotrizinol. The test article did not affect maternal body weight, body weight gain, or food consumption. Uterine and litter parameters were not adversely affected and fetuses did not show gross external, soft tissue, or skeletal malformations or variations at any bemotrizinol dosage. The maternal and developmental NOEL is at least 1000 mg/kg/day based on the absence of effects on any of the maternal and developmental parameters evaluated in this study. (Hoberman and Foss, 2004)

q) Reproduction (Segment III) Toxicity Study in Rats (Tab III.A. 16)

This study evaluates ICH Harmonised Tripartite Guideline stages C through F of the reproductive process (Segment III), but does not include an evaluation of Caesarean-delivered fetuses (stages C and D; Segment II), because this evaluation was performed in the supplementary studies in rat and rabbit. The parameters evaluated are designed to detect adverse effects of bemotrizinol treatment of female rats from implantation through lactation (DL) and weaning of the F1 generation offspring. Included is the determination of effects on gestation, parturition, lactation and maternal behavior in the female rats and on the development of the F1 offspring with the observations continued through sexual maturity as shown by a mating trial. Male rats and the F1 generation offspring were not dosed or treated as part of this study design; the F1 offspring received any potential exposure to bemotrizinol during gestation (in utero exposure) or via maternal milk during lactation.

After acclimation, virgin female rats were cohabited for a maximum of five days with breeder male rats, one male rat per female rat. Female rats with spermatozoa observed in a smear of the vaginal contents and/or a copulatory plug observed *in situ* were considered to be at day 0 of presumed gestation (GD), were housed individually and assigned to dosage groups based on computer-generated (weight-ordered) randomization procedures. Each study group consisted of 18–20 pregnant female rats (Crj:CD® (SD)IGS SPF) administered a dose volume of 10 mL/kg body weight of bemotrizinol in PEG 400 as vehicle given as single daily oral gavage doses of 0 (vehicle), 100, 300, or 1000 mg/kg body weight/day. Doses were given daily from GD 6 through day 20 of lactation (DL 20); rats that did not deliver a litter were dosed through GD 24. Females were not dosed if in the process of delivering their litter; each dam on test missed only one dose.

Female rats were observed for viability, clinical signs of toxicity, body weight and feed consumption until DL 14 when pups are presumed to start to consume maternal feed. The day of birth was defined as DL 1 (postpartum) and was the first day on which all pups in a litter were individually weighed. Litters were culled on DL 4 by random selection of 4 male and 4 female pups per litter; a total of 8 pups per litter was achieved by filling with available pups when sexed balanced numbers were not possible. These selected F1 generation rats were weaned, based on observed growth and viability of the pups, on DL 21. Observations of all pups included maturational landmarks of pinna unfolding, incisor eruption and eyelid separation. At DL 21, one male and one female per litter were selected for continued observations and the remaining animals sacrificed. The continued observations included:

Maturation: Female rats were evaluated for the age of vaginal patency, beginning on DL 28. Male rats were evaluated for the age of preputial separation, beginning on DL 39.

Passive Avoidance Testing: Each animal was evaluated in a passive avoidance test for learning, short-term retention and long-term retention. Each rat was tested twice and the test sessions were separated by a one-week interval; the criteria are the same for both days of testing.

Watermaze Testing: Beginning at approximately 70 days postpartum, evaluation was made by a water-filled M-maze for overt coordination, swimming ability, learning and memory. Each rat was tested twice and the test sessions were separated by a one-week interval; the correct goal and the criteria are the same for both test sessions.

Reproductive Capacity: At approximately 90 days of age, the F1 generation rats within each dosage group were randomly assigned, one male rat per female rat with the exclusion of sibling matings, to a cohabitation period of 14 days maximum. Female rats with spermatozoa observed in a smear of the vaginal contents and/or a copulatory plug observed *in situ* were considered to be at GD 0 and assigned to individual housing. Female rats that did not mate within the first 14 days of cohabitation were sacrificed. Presumed pregnant female rats were Caesarean-sectioned on GD 20 and the rats examined for number and distribution of corpora lutea, implantation sites, live and dead fetuses, and early and late resorptions. Each fetus was weighed and examined for sex and gross external alterations, and preserved in formalin for possible future evaluation.

Results indicate bemotrizinol under the conditions of this test did not adversely affect any of the parameters evaluated in the female rats or in the F1 offspring, including their developmental and reproductive parameters. The NOEL is 1000 mg/kg/day for each of the endpoints adult toxicity, reproductive function of dams, and for pre- and postnatal development of embryos and offspring (Ota 2002b).

r) Endocrine Effects Testing (Tab III.A. 17)

A separate peer reviewed publication is available describing the tests and results with bemotrizinol (Tinosorb® S or CGF-C1607)) in the *in vitro* Androgen Receptor Binding Assay, *in vitro* Estrogen Receptor Binding Assay, and the *in vivo* rat Uterotrophic assay (Ashby et al. 2001). Lack of Binding to Isolated Estrogen or Androgen Receptors, and Inactivity in the Immature Rat Uterotrophic Assay, of the Ultraviolet Sunscreen Filters Tinosorb M-Active and Tinosorb S. Regulatory Toxicology and Pharmacology 34, 287-291).
In the receptor binding assays, bemotrizinol at doses of $5x10^{-10}$ to $5x10^{-4}$ Molar was negative and did not show binding affinity or competitive inhibition of natural hormone binding to either the estrogen or androgen binding sites. The *in vivo* immature rat assay showed bemotrizinol in Arachis oil given subcutaneously in 3 daily doses each of 250, 500, or 1000mg /kg body weight did not adversely affect maturation of the rats and does not have intrinsic estrogenic properties.

GENOTOXICITY STUDIES

s) *S. typhimurium* and *E. coli* Reverse Mutation (Ames) Assay (Tab III.A. 18)

Bemotrizinol was tested in the Ames assay (*Salmonella typhimurium* reverse mutation assay) to determine if it induces base pair or frameshift mutations in *Salmonella typhimurium* strains TA 98, TA 100, 1535, and TA 1537 (Wollny 1997). The assay was performed using the plate incorporation method (experiment I) and repeated in an independent experiment using the pre-incubation method (experiment II). The test article was tested at the following concentrations in all experiments: 33; 100; 333; 1000; 2500; and 5000 µg/plate. Each concentration, including the controls, was tested in triplicate and was tested with and without exogenous rat liver microsomal (S9 mix) activation. A separate study (Wollny 1998a) was conducted with *Eschericia coli* WP2 *uvr*A by the same protocol and used the same test substance concentrations in each of 2 experiments.

Normal background bacterial growth was observed at up to 5000 µg/plate with and without S9 mix. In experiment I, a reduction in the number of revertants was observed in strain TA 1537 at 5000 µg/plate

without S9 mix and at ≥2500 µg/plate with S9 mix and in strain TA 98 at 1000 and 5000 µg/plate with S9 mix. These effects were associated with increased cytotoxicity at these doses. In both experiments with *Salmonella*, no significant increase in revertant colony numbers of any of the four tester strains was observed following treatment with bemotrizinol at any dose level, with or without S9 mix. In both experiments with *E. coli*, a significant increase in revertant colonies did not occur, without or with rat S9 activation. For both species and the respective strains, appropriate reference mutagens were used as positive controls and produced a distinct increase of induced revertant colonies. I

n conclusion, under the test conditions for each bacterial test strain, bemotrizinol did not induce base pair or frame shift mutations.

t) *In vitro* Chromosome Aberration Assay in Chinese Hamster Ovary Cells (Tab III.A. 19)

Bemotrizinol was assessed for its potential to induce structural chromosome aberrations in Chinese hamster V79 cells (Czich 1998a). Two independent experiments were performed with and without exogenous rat liver microsomal (S9 mix) activation. A stock solution was prepared by dissolving bemotrizinol in acetone at a concentration of 21 mg/ml. The stock solution was diluted with culture medium to produce the appropriate exposure concentrations. Based on the limited solubility of the test material in the solvent, the following bemotrizinol concentrations were tested: 6.5, 13.1, 26.3, 52.5, 105.0, and 210.0 µg/ml without S9 mix and 3.3, 6.5, 13.1, 26.3, 52.5, and 210.0 µg/ml with S9 mix. Precipitation of the test material was noted at ≥52.5 µg/ml with and without S9 mix.

In experiment I, duplicate plates of exponentially growing cells were exposed to each concentration of the test material for 4 hours with and without S9 mix. In experiment II, the cells were exposed to the test material for 4 hours with S9 mix and 18 and 28 hours without S9 mix. Approximately 2.5 hours prior to harvesting, colcemid was added to the cultures to arrest the cells in metaphase. The cells exposed for 4 hours were harvested 14 hours after completion of the exposure period. The cells exposed for 18 and 28 hours were harvested upon completion of exposure. The cells from the four highest dose groups were fixed, stained, and analyzed for structural chromosome aberrations. Chromosome gaps and numerical aberrations were recorded, but not included in the analysis. A single significant increase in aberrations of treated cells compared to solvent controls (3.5% aberrant cells exclusive gaps) was observed in experiment II (210 µg/ml, 28 hour exposure, without S9 mix); however, this increase was considered biologically irrelevant since the value was within the historical control range (0.0 – 4.0%). Positive control treatments produced a distinct increase in cells with structural chromosome aberrations in both experiments. In conclusion, under the test conditions, bemotrizinol did not induce structural chromosome aberrations.

u) *In vivo* Mouse Micronucleus Test (Tab III.A. 20)

The objective of this *in vivo* genetic toxicity assay was to evaluate the potential of bemotrizinol to induce structural or numerical damage in bone marrow cells of mice (Haddouk 2003). The study was performed according to the OECD Guideline 474 and was GLP-compliant. A preliminary toxicity test was performed to define the dose-levels to be used for the cytogenetic study. Since no toxic effects were observed, the top dose-level selected for the main test was 2000 mg/kg/day.

In the main study, three groups of five male and five female Swiss Ico: OF1 (JOPS Caw) mice were given daily intraperitoneal administrations of bemotrizinol at dose-levels of 500, 1000 or 2000 mg/kg/day for 2 consecutive days. One group of five males and five females received the vehicle (corn oil) under the same experimental conditions, and acted as control group. The positive control group consisted of five males and five females given cyclophosphamide in distilled water once by the oral route at the dose-level of 50 mg/kg.

The animals of the treated and vehicle control groups were killed 24 hours after the last treatment and the animals of the positive control group were killed 24 hours after the single treatment. Bone marrow smears were then prepared. For each animal, the number of the micronucleated polychromatic erythrocytes (MPE) was counted in 2000 polychromatic erythrocytes. The polychromatic (PE) and normochromatic (NE) erythrocyte ratio was established by scoring a total of 1000 erythrocytes (PE + NE).

Cyclophosphamide induced a highly significant increase in the frequency of MPE, indicating the sensitivity of the test system under the experimental conditions. The study was therefore considered valid. Bemotrizinol in both males and females did not show a difference from the vehicle control group in the mean values of MPE; the PE/NE ratio was also not affected by treatment and was equivalent to those of the vehicle group. Under the experimental conditions, bemotrizinol did not induce damage to the chromosomes or the mitotic apparatus of mice bone marrow cells after two intraperitoneal administrations, with a 24-hour interval, at the dose-levels of 500, 1000 or 2000 mg/kg/day.

v) *In vivo* Unscheduled DNA Synthesis Assay, Rat (Tab III.A. 21)

The potential genotoxic activity of bemotrizinol was tested using the *in vivo* Unscheduled DNA Synthesis (UDS) test in hepatocytes from Fischer rats done in compliance with the OECD guideline No. 486. The oral gavage route was used at the maximum recommended dose of 2000 mg/kg body weight, as well as the lower dose of 1000 mg/kg, in both the 12-16 hours and the 2-4 hours expression times. As the bemotrizinol was not miscible in aqueous medium, it was suspended in 0.5% w/v carboxymethylcellulose in distilled water (CMC 0.5%) at 200 mg/mL and used at a dose volume of 10 mL/kg. Different successive dilutions were also performed with CMC 0.5%. For the positive controls, dimethylhydrazine was dissolved in distilled water and 2-acetamidofluorene was suspended in 0.5% CMC.

The high dose of 2000 mg/kg body weight was used for the main experiments based on results of a preliminary toxicity assay showing an absence of mortality and clinical signs up to 48 hours after dosing 4 male Fischer rats once orally at 2000 mg/kg. A lower dose of 1000 mg/kg was also used for a separate group of rats. No mortality was noted in the animals treated at any dose of bemotrizinol during the main UDS assays.

Concurrently to the main assays, groups of 3 rats received the positive reference compounds dimethylhydrazine (10 mg/kg) for the short expression time and 2-acetamidofluorene (25 mg/kg) for the long expression time. In these positive control groups, mean net nuclear grain count (NNG) values as well as percentage of cells in repair were within the range of the laboratory's historical controls showed the sensitivity and suitability of the rat strain used. Moreover, negative control animals gave a mean NNG value of less than zero with a percentage of cells in repair comparable with historical control data. The validity criteria for the test were thus fulfilled.
At least three male Fischer rats per dose and per expression time were treated by oral gavage with bemotrizinol at 2000 or 1000 mg/kg body weight for the UDS test. Over the two experiments with bemotrizinol, the group mean net nuclear grain count (NNG) values at the two doses used were less than zero, that is, below the threshold value of 0 NNG for a positive response:

At 12-16 h expression time: -2.60 and –3.23 vs –2.74 for negative control
At 2-4 h expression time: -2.24 and –1.96 vs –3.05 for negative control

For cells in repair (NNG ≥ 5), the group mean net nuclear grain count values were not significantly different from controls at any dose of bemotrizinol:

At 12-16 h assay: 5.95 at 2000 mg/kg and 6.15 at 1000 mg/kg vs. 5.91 in control
At 2-4 h assay: 5.51 at 2000 mg/kg and 6.49 at 1000 mg/kg vs. 5.64 in control

In the bemotrizinol-treated rats, the mean percentage of cells in repair was similar to the solvent controls:

At 12-16 h assay: 1.19% at 2000 mg/kg to 0.59% at 1000 mg/kg vs. 1.27% in control
At 2-4 h assay: 1.70% at 2000 mg/kg to 2.0% at 1000 mg/kg vs. 1.32% in control

The frequency of cells in S-phase was low in the bemotrizinol-treated rats:

At 12-16 h assay: 0.14% and 0.07% in 2000 and 1000 mg/kg groups, respectively vs. 0.0% in the respective negative control
At 2-4 h assay: 0.15% and 0.29% in 2000 and 1000 mg/kg rats vs. 00.7% in the respective negative control.

Conclusion: Bemotrizinol investigated at two expression times (2-4 hours and 12-16 hours) in the *in vivo* Unscheduled DNA Synthesis (UDS) test in hepatocytes from male Fischer rats, treated orally once with 2000 or 1000 mg/kg did not induce a proliferative effect in rat liver and did not reveal any genotoxic activity under these test conditions. (Nesslany 2004)

w) Photomutagenicity: Ames Assay (Tab III.A. 22)

Bemotrizinol was tested with photoirradiation in the Ames assay to determine if it induces base pair mutations in *S. typhimurium* strain TA 102 and *E. coli* WP2 (Wollny 1998a, b). These strains were chosen since they tolerate relatively high doses of UV irradiation. The assay was performed using the plate incorporation method (experiment I) and repeated in an independent experiment using the pre-incubation method (experiment II). The test article was tested at the following concentrations in both experiments: 33; 100; 333; 1000; 2500; and 5000 µg/plate. Each concentration, including the controls, was tested in triplicate. Immediately after treating the cells with the test material, the cells were exposed to doses of UVA/UVB irradiation that were determined in preliminary experiments to produce a doubling in the background revertant frequency. TA 102 cells were exposed for 10 seconds to 20 mJ/cm^2 UVA and 1 mJ/cm^2 UVB irradiation. WP2 cells were exposed for 40 seconds to 80 mj/cm^2 UVA and 4 mJ/cm^2 UVB irradiation.

Normal background bacterial growth was observed at up to 5000 µg/plate. Slight toxic effects, evident as a reduction in the number of revertants, occurred in both strains in experiment II. In both experiments, no significant increase in revertant colony numbers of either tester strain was observed following treatment with bemotrizinol at any dose level. Appropriate reference mutagens were used as positive controls and produced a distinct increase of induced revertant colonies. In conclusion, under the test conditions, bemotrizinol did not induce base pair or frame shift mutations after exposure to UVA/UVB irradiation.

x) Photomutagenicity: *In vitro* Chromosomal Aberration Assay (Tab III.A. 23)

Bemotrizinol was assessed for its potential to induce structural chromosome aberrations in Chinese hamster V79 cells with and without UVA/UVB irradiation in two independent experiments (Czich, 1998b). Based on the limited solubility of the test material in the phosphate buffered saline (PBS) solution (containing 1% (v/v) acetone in culture medium), the following bemotrizinol concentrations were tested: 6.25, 12.5, 25.0, 50.0, and 100.0 µg/ml with and without UVA/UVB irradiation. Precipitation of the test material was noted at ≥25.0 µg/ml. In both experiments, duplicate plates of exponentially growing cells were exposed to each concentration of the test material in a PBS solution for 30 minutes followed by irradiation with 200 mJ/cm^2 UVA and 22 mJ/cm^2 UVB for 30 minutes. Additional groups in experiment II were exposed to 300 mJ/cm^2 UVA and 33 mJ/cm^2 UVB for 30 minutes. After irradiation, the PBS solution was replaced with culture medium. Concurrent solvent and positive controls were run in parallel. In experiments I and II, the cells were harvested 18 and 28 hours after the start of the experiments, respectively. Approximately 2 hours prior to harvesting, colcemid was added to the cultures to arrest the cells in metaphase. In experiments I and II, cells were fixed, stained, and analyzed for .structural chromosome aberrations from the 6.25, 12.5, 25.0, and 100.0 µg/ml groups and 12.5, 25.0, 50.0, and 100.0 µg/ml groups, respectively. Chromosome gaps and numerical aberrations were recorded, but not included in the analysis.

In both experiments, with and without UVA/UVB irradiation, the test material did not increase the frequency of cells carrying structural chromosome aberrations. An increase in polyploidy did not occur with the test article. Positive control treatments produced a distinct increase in cells with structural chromosome aberrations in both experiments. In conclusion, under the test conditions, bemotrizinol did not induce structural chromosome aberrations in the presence or absence of UVA/UVB irradiation.

UV IRRADIATION STUDIES- RODENTS

y) Sun Protection Factor (SPF)-Like Evaluation in Hairless Mice (Tab III.A. 24)

The purpose of this study was to determine the protective potential of topically administered bemotrizinol against cutaneous inflammation induced by UVR (UVB and UVA) exposure in Crl:SKH1-*hr*BR hairless mice. The study shared the untreated and vehicle control groups with test groups receiving bisoctrizole or Tinosorb®M.

Each of 3 groups was assigned 10 male albino hairless Crl:SKH1-*hr*BR mice and either no treatment (Group 1) or a treatment with the vehicle hydrated, hydrophilic Base Ointment (Group 2), or

bemotrizinol at 50 mg/g formulation or 200 mg/g formulation (equivalent to 5% or 20% a.i., and 160 mg a.i. or 650 mg a.i./kg body wt., respectively). Formulations were topically administered (100 mcL/mouse) to mice once in each treated group. Approximately 15 minutes after formulation administration each mouse was exposed on six separate sites each given one of six UVR exposures. Group 1 mice were only exposed to UVR. The irradiation source was a Berger Compact Arc high intensity solar simulator with a WG 320 Schott glass filter (1 mm) coupled to an Oriel light pipe. The emission spectrum includes wavelengths in the UVB and UVA portions of the electromagnetic spectrum.

The mean observational MED, standard deviations and medians were calculated for each group and each observational time point. The lowest instrumental UVR dose to cause any cutaneous response at a site of exposure was determined for each mouse. If administration of the formulation had no influence on the UVR dose required to elicit cutaneous responses, a mean calculated observational UVR dose value equivalent to 1.0 would be expected. A mean calculated observational UVR dose value greater than 1.0 would indicate a protective effect of formulation. A mean calculated observational UVR dose value less than 1.0 would indicate a phototoxic effect.

For any mouse that had no skin responses at any of the UVR exposure sites, a MED value of either 4 (Groups 1, 2) or 7 (the bemotrizinol groups) was assigned. Irradiation of skin sites not administered any formulation (Group 1) resulted in mean observational MED values that approximate the instrumental MED of 1.0.

Clinical observations were recorded at least once weekly during acclimation and once before formulation administration and UVR exposure. At approximately 24, 48 and 72 hours after irradiation, the UVR exposure sites on each mouse were examined for signs of inflammation; additionally recorded were erythema, edema, flaking, or any other abnormal finding observed at an irradiation site. Clinical findings were also recorded at these observation times. Body weights were recorded weekly throughout the study and at sacrifice.

Treatment + UVR	Mean Observational MED (± S.D.) after UV Exposure		
	24-hours	48-hours	72 hours
No treatment (Grp 1)	1.2 ± 0.2	0.7 ± 0.0	0.8 ± 0.1
Vehicle (Group 2)	1.6 ± 0.3	1.0 ± 0.2	1.0 ± 0.3
5% bemotrizinol	4.5 ± 0.8	3.6 ± 0.7	3.7 ± 0.9
20% bemotrizinol	5.2 ± 1.1	5.0 ± 1.2	5.0 ± 1.5

Results of the single topical treatments and irradiation are summarized in the nearby table. These values indicate that a single topical administration of the vehicle did not substantively affect the cutaneous response to a single UVR exposure. However, bemotrizinol at a concentration of 50 mg/g (5%) affords a substantial protective effect against acute UVR-induced cutaneous damage. An increase in concentration of bemotrizinol to 200 mg/g (20%) increased this protective effect. The highest topical dose was about 4 times higher than expected human topical daily doses. (Learn et al. 2003a)

z) 13-Week Dermal Phototoxicity Dose Range Study in Hairless Mice (Tab III.A. 25)

The purpose of this study was to provide information for use in evaluating the potential for toxic or possible interactive effects associated with repeated daily administration of bemotrizinol, with or without simulated sunlight, for a period of 13 weeks. The test article vehicle was hydrophilic, hydrated Base Ointment for each test substance. The study shared the untreated and vehicle control groups with separate test groups receiving bisoctrizole or Tinosorb® M. The study results were used to select dosage levels for a 12-month photo-co-carcinogenesis study.

Fourteen groups of 6 male and 6 female albino hairless Crl:SKH1-*hr*BR mice were assigned to groups receiving no treatment, treatments of only UVR at 2 levels, vehicle without or with UVR at 2 levels, or bemotrizinol without or with UVR at 2 levels. The treatments with vehicle and test article were administered topically (100 mcL/mouse) once daily and then the appropriate groups of mice were irradiated once daily, 5 days per week, for 13 weeks. Formulations were applied to the back and sides (approximately 25 cm^2) of the mice before daily UVR exposures on Monday, Wednesday and Friday and after UVR exposures on Tuesday and Thursday. On Monday, Wednesday and Friday, UVR

exposure began approximately one hour after the completion of formulation administration for each group. On Tuesday and Thursday, the duration of time between the completion of UVR exposure and the start of formulation administration for each group of mice was approximately one hour.

Doses, as active ingredient bemotrizinol, are shown in the following table.

mg a.i./g dose formulation	% active	mg a.i. / cm^2 on dose site	Approx. mg a.i./kg body wt./ day
25	2.5	0.1	80
50	5.0	0.2	160
100	10	0.4	325
200	20	0.8	650

The UVR source, a 6.5 kilowatt xenon long arc, water cooled burner was vertically suspended within an octagonal metal frame holding one optical filter on each side. Each filter (15 cm by 15 cm, 1 mm thick; Schott WG 320 doped glass) was held approximately 20 cm from the burner. During exposure, the racks holding the animal cages were located approximately 2.25 meters from the UVR source. Each rack of cages was irradiated through one filter; all racks of cages were irradiated simultaneously from one xenon arc.

Each rack of animal cages was monitored by a customized detector that records both intensity and UVR dose in Robertson-Berger Units (RBU). The lower UVR level was 600 Robertson-Berger Units (RBU) per week, and the higher UVR level was 1200 RBU per week. The RBU is a measure of biological effectiveness for UVR; 400 RBU approximates one minimal erythema dose in previously un-tanned human skin.

In male and female mice no adverse cutaneous or other clinical responses occurred that were related to administration of any dose level of bemotrizinol. Erythema was reduced in some groups of male mice administered test article formulations and exposed to UVR, as compared with the groups administered the vehicle formulation and exposed to UVR. A similar pattern of findings of mean skin-fold thickness also occurred, and significant reductions in mean skin-fold thickness occurred in some groups of mice administered the test article formulations and exposed to UVR, as compared with the groups only administered the vehicle formulation and exposed to UVR.

Mortality during the course of the study (three male and one female mouse sacrificed in moribund condition) was unrelated to test article administration. All other mice survived to scheduled sacrifice. Mean body weights and body weight changes for male and female mice in all study groups were uneventful throughout the course of the study.

In conclusion, clinical observations, including cutaneous observations, skin-fold thickness and body weight did not preclude the use of any of the concentrations of bemotrizinol (25 mg/g to 200 mg/g) in a long-term study. Adverse effects of these formulations did not occur on observed parameters over those seen in the groups of mice administered the vehicle formulation and either not exposed or exposed to UVR. The highest topical dose was about 4 times higher than expected human topical daily doses. (Learn et al. 2003b)

aa) 12-Month Photocarcinogenicity Study in Hairless Mice (Tab III.A. 26)

The purpose of this study was to determine the potential of bemotrizinol to influence the development or growth of skin tumors in hairless Crl:SKH1-*hr*BR mice exposed to simulated solar ultraviolet radiation (UVR). The 5 groups contained 36 male and 36 female mice each, including 2 control groups receiving only UVR at low (600 RBU/week) or high (1200 RBU/week) dosages, a vehicle control group receiving the base ointment vehicle, and 2 groups receiving bemotrizinol at either 50 mg/g formulation or 200 mg/g formulation for five days per week. The dosage equivalents are shown in Table 1.

Table 1. Dosage equivalents of bemotrizinol			
mg a.i./g dose formulation	% active	mg a.i. / cm^2 on dose site	Approx. mg a.i./kg body wt./ day
50	5.0	0.2	160
200	20	0.8	650

For 40 consecutive weeks the UVR exposure was given daily either before application of the treatment formulation (100 µL/mouse) or one hour after treatment with formulation, as shown in Table 2.

In the subsequent 12 weeks mice were not exposed to UVR. Throughout the study, the animals were each observed for external changes in skin and their tumors charted, measured, and tabulated weekly. A full necropsy was performed on all mice and the carcasses were retained in neutral buffered formalin; however, tissue or lesion histopathology was not conducted.

Table 2. Weekly Formulation Administration (mg a.i./gram formulation) and UVR Exposure Regimen during 40 Weeks					
	Monday, Wednesday & Friday		Tuesday & Thursday		Total RBU per Week
Group*	Treatment **Before** UVR	UVR (RBU)	UVR (RBU)	Treatment **After** UVR	
1	Vehicle	100	150	Vehicle	600
2	50 mg/g	200	300	50 mg/g	1200
3	200 mg/g	200	300	200 mg/g	1200
4	None	100	150	None	600
5	None	200	300	None	1200
* Groups 1, 4, and 5 were shared with the TINOSORB® M, bisoctrizole study, reported separately. RBU: Robertson-Berger Units (a measure of UVR effectiveness; 400 RBU approximates one minimal erythema dose in previously untanned human skin).					

The study design specified termination 12 weeks after the last UVR dose, unless animal tumor burden required earlier termination of a study group. Mice were sacrificed when any one tumor reached a diameter of 10 mm. Groups of mice were sacrificed when fewer than 50% of mice survived and more than half of the survivors had at least one tumor of 4 mm or greater in diameter. Statistical comparisons applied 2-tailed probabilities. Comparisons included time to tumor, tumor yield, tumor potency factor, and tumor prevalence by Peto analysis.

As a photo-co-carcinogenicity study, it is designed to determine if a test article influences the formation of UVR-induced skin tumors, assessed as the prevalence of tumors and the time to onset of tumors. A photo-carcinogenic response is enhanced when a higher prevalence of skin tumors or an earlier appearance of tumors in a study group occurs as compared to the control groups.

Results. Adverse effects were not increased by bemotrizinol treatments including survival, clinical signs, skin adverse effects, body weights, and necropsy observations commonly observed in this test system. The protective effect of bemotrizinol was indicated by a reduced mortality in these groups compared to the 1200 RBU/week high UVR calibration group.

Tumor data showed a dose-related protective effect for bemotrizinol. In Table 3 is summarized the unbiased median time for development of the first tumor of ≥1mm diameter.

Table 3. Unbiased Median Week to Tumor for Tumors ≥ 1 mm					
Group	1[a]	2	3	4[a]	5[a]
Formulation	Vehicle	Bemotrizinol 50 mg/g	Bemotrizinol 200 mg/g	None	None
UVR Exposure (RBU/week)	600	1200	1200	600	1200
Sexes combined	33.50	**26.50**	**31.00**	37.00	**22.50**
Males	34.50	**27.50**	**31.50**	37.00	**22.50**
Females	33.00	**25.50**	**31.00**	35.50	**22.50**
a. Group shared with the study investigating bisoctrizole.					

Sacrifice of groups, based on tumor burden criteria, occurred in week 36 for the 1200 RBU/week calibration group, week 42 for the 50 mg/g bemotrizinol group, week 45 for the 200 mg/g bemotrizinol group, week 49 for the vehicle control group, and in week 52 for the 600 RBU/week group.

Tumor yield (average number of tumors per (surviving) mouse) was highest in the 1200 RBU/week group followed by the vehicle treated group, bemotrizinol at 50 mg/g, 600 RBU/week group, and the bemotrizinol 200 mg/g group. The vehicle enhanced the tumor yield compared to the low UVR calibration group (600 RBU/week). The tumor yield data are summarized in Table 4.

Table 4. Tumor Yield (tumors ≥ 1mm)

Group	1[a]	2	3	4[a]	5[a]
Formulation	Vehicle	Bemotrizinol 50 mg/g	Bemotrizinol 200 mg/g	None	None
RBU/week	600	1200	1200	600	1200
Sexes Combined	5.74	4.85	2.85	4.26	6.20
Males	5.59	5.18	2.81	4.48	6.06
Females	5.89	4.58	2.88	4.00	6.32

a. Group shared with the study investigating bisoctrizole.

Comparison of tumor relative risk by Peto analysis is summarized in the Table 5. The comparisons show that 50 mg/g or 200 mg/g of bemotrizinol formulation significantly delayed ($p<0.001$) the development of skin tumors in sexes combined compared to 1200 RBU calibration group; this was also the case for male and female mice separately (not shown). Additionally, the dose-related effectiveness of bemotrizinol is seen in the significantly delayed ($p<0.01$ or $p<0.001$) skin tumor onset for 200 mg/g as compared with the 50 mg/g test article formulation.

Table 5. Peto Analysis for Prevalence and Risk for Tumor Onset

		SEXES COMBINED			
Group No.		**1[a]**	**2**	**3**	**4[a]**	**5[a]**
Formulation		Vehicle	Bemotrizinol 50 mg/g	Bemotrizinol 200 mg/g	None	None
Exposure (RBU/Week)		600	1200	1200	600	1200
Group No. being Compared to Successive Groups	1	C	+++	++	– –	+++
	2		C	– – –	– – –	+++
	3			C	– – –	+++
	4				C	+++

C denotes group to which each successive group is compared and their greater (+) risk or lower (-) risk is noted for each group on the same line (row). The statistical significance of any difference (2-tailed p-values) is indicated by the following codes:

(–) = $p<0.1$ – = $p<0.05$ NS = Not Significant

++ or – – = $p<0.01$ +++ or – – – = $p<0.001$

a. Group shared with the study investigating bisoctrizole.

The estimated tumor potency factors (TPF) calculated for the study groups are compared in Table 6 immediately below. Consistent with other comparisons presented, the TPF results also show the vehicle's slight enhanced effect of the photo-carcinogenic response, that bemotrizinol formulations effected a dose-related reduction of TPF compared to the 1200 RBU group. Together this indicates that the test article formulations did not adversely affect the photo-co-carcinogenic response and reduced the potential of the high UVR dose to induce tumors in a dose-dependent manner.

Table 6. Tumor Potency Factors (tumors ≥ 1 mm)

Group	1	2	3	4	5
Formulation	Vehicle	Bemotrizinol 50 mg/g	Bemotrizinol 200 mg/g	None	None
UVR Exposure (RBU/Week)	600	1200	1200	600	1200
Sexes Combined	1.14	1.5964	1.2747	1	2
Males	1.10	1.5163	1.2547	1	2
Females	1.1112	1.6552	1.2243	1	2

Overall, a difference between the sexes was not apparent in any of the tumor and non-tumor-related endpoints of the study. In addition, the study results indicate bemotrizinol did not enhance the photo-carcinogenic response and in fact had a protective effect to reduce the potential of the high UVR dose to induce tumors in a bemotrizinol-dose dependent response.

Conclusions: At the end of UVR exposure (week 40), survival of treated animals was not adversely affected. The tumor prevalence data indicate bemotrizinol delayed the onset of tumors compared to the control groups, and in the bemotrizinol groups, tumors occurred later in the 200 mg/g group than in the 50 mg/g group. Both of these findings achieved statistically significant ($p<0.001$) differences from the high UVR group. Overall, the tumor data indicate bemotrizinol did not have a negative impact on the photo-co-carcinogenic response and in fact, reduced the potential of the high UVR dose to induce tumors in a bemotrizinol-dose dependent response. The highest topical dose was about 4 times higher than expected human topical daily doses. (Learn et al., 2005)

bb) Repeated 39-Week Dermal Dosing in Göttingen Minipig (Tab III.A. 27)

The objective of this study was to evaluate the potential toxicity of bemotrizinol following daily cutaneous application to Göttingen minipigs for 39 weeks (Haag 2006). The study followed the guidance for repeated dose toxicity (CPMP/SWP/1042/99) of the European Agency for Evaluation of Medicinal Products (July 2000).

Three groups of four male and four female Göttingen minipigs received once daily cutaneous application of the test article suspended in the vehicle (PEG 400) at constant dose volume of 2.5 mL/kg body weight/day on closely clipped dorsal skin. The dose-levels used were 250, 500 and 1250 mg bemotrizinol /kg body wt/day and the treatments were maintained for 39 weeks. The dosing equivalents are shown in the nearby table. The highest dose of 1250 mg/kg/day was the highest technically achievable dose based on the physical characteristics (viscosity, density, rheology) of test article in the PEG 400 vehicle that allowed accurate and reproducible preparation and application of the dosages. A control group of four males and four females received the PEG 400 vehicle during the dosing period. The topical dosing area was increased as required to continue dosing about 10% of the body surface area as the animals grew.

Minipig topical daily dosage equivalents.		
mg a.i./kg b wt	**% bemotrizinol**	**mg a.i./cm^2**
250	10	7
500	20	14
1250	50	35

All study animals were checked at least twice daily for mortality and clinical signs. Treatment areas were carefully examined before each application. Body weight was recorded pre-test, on day 1 and then once a week. Daily food consumption was estimated pre-test and until the end of the study. Ophthalmologic examinations were performed on all principal animals before the beginning of the treatment period, and on all the animals in the control and high-dose groups in weeks 12 and 26 and at the end of the treatment period. Hematological and blood biochemical investigations, as well as urinalysis, were performed on all animals pre-test, in weeks 13 and 26, and at the end of the treatment period. Plasma levels of the test item were determined using a LC-MS method on blood samples taken in weeks 13 and 26 and at the end of the treatment period, just before daily dosing (if any).

On completion of the treatment period, all the animals were killed and submitted to a full macroscopic examination. Designated organs were weighed and selected tissue specimens preserved. Histological examinations were performed on selected tissues from all the animals in the control and high-dose groups.

Results.

- Chemical analysis of the dosage formulations met the acceptance criteria for stability, homogeneity and achieved concentrations.
- Mortality by unscheduled deaths did not occur during the study. One mid-dose female was terminated day 20 for reasons not related to the test article; she was replaced with a reserve female.
- Clinical signs at the dose site were similar to those changes noted in the nearby control (not treated) site and did not indicate a test-article related incidence.
- Body weight, body weight gain, feed consumption and ophthalmologic findings did not show test article-related effects.
- Hematological parameters were not adversely affected by the test article.

- Clinical biochemistry indicated some changes in creatinine kinase levels that did not reach statistically significant differences from concurrent control animals and remained within range of historical control values for the parameter. Other endpoints were not affected by bemotrizinol.
- Urinalysis did not show effects that could be attributed to the test article.
- Plasma concentrations of bemotrizinol were detected (LOQ 2 ng/ml), and as shown in the next table, did not show a clear relationship to dose level or duration of dosing. The repeated topical application of high doses of bemotrizinol did not induce a consistent or clear systemic exposure.

Mean plasma concentrations of FAT 70'884 (ng/mL) following cutaneous application of the test item to Göttingen minipigs

Dose-level (mg/kg/day)	250		500		1250	
Sex	M	F	M	F	M	F
Week 12	BLQ	11.4	BLQ	2.16	3.78	4.8
Week 26	BLQ	BLQ	2.85	4.11	5.13	6.66
Week 38	10.1	15.3	5.42	9.42	2.96	11.8

BLQ: < 2.0 ng/mL

- Organ weights and grossly observable changes at necropsy did not show an effect of bemotrizinol.
- Histopathology showed minimal to slight hyperkeratosis, parakeratosis and acanthosis in the treated skin areas of a few individuals from control and test item-treated groups. These changes were considered to be due to mechanical manipulation (clipping, application, cleaning) of the dose site skin. Consequently, these changes were considered not to be of toxicological importance. Other changes reported were determined to be not related to the test article.

Conclusion:
The test item applied topically once daily for 39 consecutive weeks, at the dose-levels of 0, 250,500 or 1250 mg/kg/day, as a suspension in PEG 400, was clinically well tolerated. No local or systemic toxic effects or target organs were identified at any dose-level. Although the test item was detected in the plasma of all the dosed animals, their systemic exposure was minimal, as the test item plasma concentrations were lower than 10 ng/mL in most animals.

Consequently, under the experimental conditions of this study, the No Observed Effect Level (NOEL) is considered to be higher than 1250 mg/kg/day applied as a 50% bemotrizinol formulation. This highest topical dose was about 175 times higher than expected human topical daily doses of bemotrizinol and this study result is pivotal for our estimated human systemic exposure and risk assessment as presented later in this document.

ABSORPTION STUDIES

cc) Oral Adsorption, Distribution, Metabolism, Elimination Study in Rat (Tab III.A. 28)

Groups of 4 male and 4 female rats were each given a single oral dose of 50 mg [^{14}C]-bemotrizinol /kg to investigate systemic exposure, tissue distribution and metabolite profiles (Silcock 2002a). The excretion of radioactivity in urine and feces was monitored for 4 days after dosing when the rats were killed and residual radioactivity was measured in blood, selected tissues and the remaining carcasses. An additional group of nine male and nine female rats were each given a single oral dose of 50 mg [^{14}C]-bemotrizinol/kg and radioactivity was measured in blood and plasma over a 24 hour time course after dosing.

After the single oral dose of radio-labeled test article, excretion was rapid and extensive in both male and female rats. Urinary excretion accounted for mean totals of 0.1% and 0.2% of the dose and fecal excretion accounted for mean totals of 94% and 97% of the dose for males and females, respectively. Only one component was found in the fecal extracts and this was identified as parent bemotrizinol. Unchanged bemotrizinol therefore represented 100% of the dose excreted in feces in both male and female rats. Residues in tissues accounted for <0.01% of the dose in total in both males and females

and were not associated with any specific tissues. The radioactivity remaining in the residual carcass accounted for 0.3% of the dose for males and 0.1% for females. The total recoveries of administered radioactivity were approximately 95% for males and 97% for females. The concentration of radioactivity in blood and plasma was below the limit of detection at all time points up to 24 hours after dosing. The mean limits of detection were <0.038 µg/g and <0.019 µg/g in blood and plasma, respectively.

Following a single oral dose the absorption of [^{14}C]-bemotrizinol was very low and the substance is considered as not orally bioavailable.

dd) Dermal Adsorption, Distribution, Metabolism, Elimination Study in Rat (Tab III.A. 29)

A representative sunscreen formulation, containing 4% bemotrizinol as the only UV absorber (active ingredient), was used as the dermal dosing vehicle (Silcock 2002b) to investigate dermal absorption of labeled test article applied at 2 different dose volumes to the male rat. A measured amount (50 µl or 20 µl) of each formulated dose was applied to 10 cm^2 of shaved skin per rat, corresponding to doses of 2 mg and 0.8 mg bemotrizinol per rat. These applications were designed to simulate potential human dermal exposure to the formulation during normal use.

Thirty-two rats were each given a single dermal dose of either 2 mg or 0.8 mg bemotrizinol and the application sites were protected, but not occluded, using O-rings incorporating a nylon gauze cover. A strip of non-occlusive elastic bandage was wrapped around the rat and over the application devices to help to hold them in place. Rats were housed individually in metabolism cages for the collection of urine and feces. After a 6-hour exposure, the first two groups were terminated as described below and the application sites of all the remaining rats were washed to remove the unabsorbed dose. Urine, feces and cage wash were collected from each cage after the 6-hour skin wash, and then at daily intervals after dosing for the duration of each experiment. Groups of four rats were terminated at 6, 24, 72 and 120 hours after dosing. The skin was washed to remove unabsorbed residual dose, before exsanguination under terminal anesthesia. The protective covers were removed, the skin under the O-rings washed to remove any unabsorbed residual dose and the application site skin was then tape-stripped to remove the *stratum corneum*. All samples, including selected tissues and residual carcasses were analyzed for radioactivity.

After the dermal exposure to 2 mg [^{14}C]- bemotrizinol formulation for 6-hours, a mean of about 90% of the applied radioactivity was washed from the skin surface using soap solution and water. About 2.0% of the dose remained associated with the application site following the 6-hour skin-wash and some of this was available for absorption. The residue associated with the application site declined slightly at later time-points. The amount of dose absorbed was 0.2% after 6- and 24-hours. The absorbed dose was not associated with any specific tissues.

After dermal exposure to 0.8 mg [^{14}C]-bemotrizinol formulation for 6-hours, a mean of approximately 96% of the applied radioactivity was washed from the skin surface using soap solution and water. About 2.1% of the dose remained associated with the application site and some of this was available for absorption. The residue associated with the application site declined slightly at later time-points and the amount of dose absorbed after 6 and 24 hours was 0.1% - 0.4%. The absorbed dose was not associated with any specific tissues.

Conclusions. A 6-hour exposure period to 2 mg or 0.8 mg [^{14}C]- bemotrizinol applied as a 4% bemotrizinol formulation, indicated an *in vivo* dermal absorption of about 0.2% and 0.3% of the dose, respectively, over 24 hours. Means of 90% and 96% of the applied dose of 2 mg or 0.8 mg [^{14}C]-bemotrizinol, respectively, were readily washed from the skin surface by mild soap and water skin washes. Following dermal dosing, it is considered that absorption of bemotrizinol was very low and the substance is not bioavailable by dermal absorption. The highest dose equaled the expected human topical daily application rate of bemotrizinol.

2. Study Results from Partially Controlled or Uncontrolled Studies. No data were developed by Ciba Specialty Chemicals that meet the definition of partially controlled or uncontrolled studies.

B. Combinations of the Individual Active Components (*Not Applicable*).

1. Study results from controlled studies. No data developed by Ciba Specialty Chemicals that meet this definition. TINOSORB® S contains only a single active ingredient, which is bemotrizinol.
2. Study Results from Partially Controlled or Uncontrolled Studies. No data were developed by Ciba Specialty Chemicals that meet the definition of partially controlled or uncontrolled studies.

C. Finished Drug Product (*Not Applicable*).

1. Study results from controlled studies. No data developed by Ciba Specialty Chemicals that meet this definition.
2. Study Results from Partially Controlled or Uncontrolled Studies. No data were developed by Ciba Specialty Chemicals that meet the definition of partially controlled or uncontrolled studies.

IV. Human safety data

A. Individual Active Ingredients, Bemotrizinol

1. Controlled Studies on Bemotrizinol (Tab IV.A. 1)

a) *In vitro* Human Skin Distribution

An experiment to determine the degree of percutaneous absorption was carried out using human skin *in vitro* (Watkinson 1998). The protocol proposed by Colipa was followed according to GLPs. The skin was derived from 3 females undergoing plastic surgery, and was stored at -20 °C until required. It was then immersed in water at 60° for 45 seconds. The epidermis was removed and mounted in Franz type cells exposing a surface area of about 1 cm^2 (the exact area was measured later in each case). The cells were placed in a bath at 37 °C that maintained a skin temperature of 32 °C. The receptor fluid was 6% Oleth 20 in phosphate buffered saline and had solubility of 16µg bemotrizinol/ml receptor fluid The integrity of the membranes was first measured by placing 10 µCi ^3H_2O in the chamber and measuring the radioactivity in the receptor solution after 1 hour. Those cells giving permeability values greater than 1.5 mg/cm^2/hour were noted. There was some correlation between these skin samples and the cells which contained active ingredient at the end of the experiment; the author points out that in the literature a value of this level is "conservative", and a value twice this has been proposed as an acceptable limit.

A cream formulation containing 10% of bemotrizinol was placed in 12 chambers, and a vehicle control in 2 chambers. The target dose was 2.0 mg/cm^2; the mean application rate was 2.1 mg/cm^2 with a range 1.7 to 3.0 mg/ cm^2. Small (200µl) samples were taken from the receiving chamber at 2, 4 and 6 hours, with replacement. At 12 and 24 hours larger samples (1.5 ml) were taken with replacement. The latter samples were freeze-dried and later analyzed for presence of the active ingredient. The first-taken smaller samples were analyzed if test article was found at the 12 hours' sampling.

After 24 hours, the amount of test formulation remaining on the stratum corneum was removed and analyzed. The membranes were then stripped 20 times, and groups of 3 strippings were analyzed for active ingredient. The detection limit (HPLC) was about 25 ng/ml. Samples of the receiving fluid and of the stripped membranes were spiked and the analytical method checked; the recoveries ranged from 94 to 106%.

Results. Of the 12 cells treated with active ingredient, 6 showed no bemotrizinol in the recovery fluid. The values from one cell were rejected because of anomalously high and early penetration. Overall, it was found that after 12 hours the amounts found in the receptor cells plateaued; the variability was high, because the amounts in the receptor cells were close to the minimum detectable concentration.

The value for the 24-hour sampling was estimated by extrapolating the line on a graph representing the permeation rate for the first 12 hours. This procedure gave a "worst case" value for permeation over 24 hours. The permeation rate was calculated to be about 0.004 µg/cm^2/hour. The overall balance showed a recovery of 99%; the average amounts recovered were from the skin surface 65.6%; from strips 1 to 4, 15.5%, and from all other strippings 17.5%. For a 24-hour period the permeation was calculated to be, in the worst case, 0.08 µg/ cm^2 or less than 0.08% of applied bemotrizinol.

b) Phototoxicity in Humans

Bemotrizinol, formulated as a 10% O/W Lotion, was topically applied to 26 human volunteers (Parisse 1998b). Two hundred microliters of the test material, vehicle control (O/W Lotion base), and saline were topically applied to separate sites of each volunteer on one side of the spine. Duplicate applications were made on the opposite side of the spine. The treatment sites were covered with an occlusive dressing. After 24 hours of exposure, the patches and excess test material from the left paraspinal region were removed. The test sites were then exposed to 16 J/cm$_2$ UVA irradiation followed by exposure to 0.75 times the volunteer's minimum erythemal dose (MED) of UVB irradiation. The patches from the right paraspinal region were then removed. Skin reactions were assessed 1, 24, 48, and 72 hours following irradiation and patch removal. Only one adverse reaction was reported that was determined to be not treatment-related. On a scale of 0-3 (0 representing no reaction and 3 representing strong erythema), grade 1 reactions were noted at 1, 24, 48, and 72 hours for the irradiated sites in 7, 1, 0, and 0 volunteers for the test material treatment; 8, 3, 1 and 0 volunteers for the vehicle control treatment; and 9, 7, 3, and 2 volunteers for the saline treatment, respectively. The remaining skin reactions were all less than grade 1. No skin reactions greater than or equal to grade 1 were noted for the nonirradiated test material sites. On average, the irradiated test material-treated sites exhibited lower skin reactions than the irradiated vehicle control and saline treatment sites. In conclusion, under the test conditions, the test material was not phototoxic and was not an irritant to human skin.

c) Photoallerginicity in Humans

Bemotrizinol was tested for photoallergencitiy using a human repeated insult patch test (Parisse 1998a) following the method of Kaidbey [1991- The evaluation of photoallergic contact sensitizers in humans. Dermatotoxicology, 4th edition, edited by Marzulli and Maibach. Hemisphere Publishing Corp.].

The induction phase consisted of two topical applications per week over a 3-week period (total of six topical applications over weeks 1-3) of 200 µl of the test material (10% bemotrizinol in an O/W Lotion), vehicle control (O/W Lotion base), and saline to separate sites on each of 33 volunteers. The treatment sites were covered with an occlusive dressing. Twenty-four hours after each induction exposure, the patches were removed and exposed to 2 times the volunteer's UVA/UVB minimum erythemal dose (MED). For a given induction treatment, the same site was used for each exposure unless unacceptable reactions were noted. In that case, the next induction exposure used a naïve site. After the last induction exposure, volunteers were not treated for two weeks (weeks 4-5). On week 6, duplicate topical applications of 200 µl of the test material, vehicle control, or saline were made to naive sites on both sides of each volunteer's spine. The test sites were covered with an occlusive dressing. After 24 hours of exposure, the patches and excess test material from one side of the spine were removed. The test sites were then exposed to 16 J/cm^2 UVA irradiation followed by exposure to 0.75 times the volunteer's MED of UVB irradiation. The remaining patches were then removed. Skin reactions were assessed 1, 24, 48, and 72 hours following irradiation and patch removal. Only one adverse reaction was reported that was determined to be not treatment related. Skin reactions were graded on a scale of 0-3 (0 representing no reaction and 3 representing strong erythema).

After the challenge phase, grade 1 reactions were noted at 1, 24, 48, and 72 hours for the irradiated sites in 10, 1, 1, and 0 volunteers for the test material treatment; 13, 12, 6, and 2 volunteers for the vehicle control treatment; and 15, 10, 6, and 3 volunteers for the saline treatment, respectively. Grade 2 reactions were noted for two volunteers after one hour for all three treatments. The remaining skin reactions were less than grade 1. The average skin reactions for the non-irradiated sites were lower than the irradiated sites for all three treatments. On average, the irradiated test

article-treated sites exhibited lower skin reactions than the irradiated vehicle control and saline treatment sites.

In conclusion, under the test conditions, the test material was not a photosensitizer or sensitizer to human skin.

2. Partially Controlled or Uncontrolled Studies (*Not Applicable*)

3. Documented Case Reports. Side effects (*Not Applicable*)

4. Pertinent marketing experience of Bemotrizinol (Refer to TEA)

Pertinent market experience has been extensively illustrated in the Time and Extent Application for bemotrizinol submitted on April 11, 2005 (Docket No. 2005N-0446). Prepared to support the Inclusion of bemotrizinol into FDA's Monograph for Sunscreen Drug Products for Over-the-Counter Human Use; (64 FR 27666-27693, as amended by 66 FR 67485-67487, Docket No. 78N-0038).

Conclusion:

The analysis of the marketing experience in 31 countries comes to the conclusion that there were over **2 billion** applications of bemotrizinol containing sunscreen over the first five years.

5. Pertinent medical and scientific literature on Bemotrizinol

Refer to the "Medical Rational" (see section VI)

B. Combinations of the Individual Active Components (*Not Applicable*)

No data developed by Ciba Specialty Chemicals that meet this definition. Bemotrizinol is a single active ingredient.

C. Finished Drug Products

1. Controlled Studies

Controlled studies with finished drug products containing bemotrizinol in combination with other UV filters have been sponsored by Ciba's customers that formulate these consumer products. We have received human safety study reports from four customers. An overview of the studies received is shown in the following table.

In summary: The controlled studies involved panels of 10 to 104 volunteers exposed in separate test protocols to different finished drug products. Each product contained up to 3% (w/w) bemotrizinol in combination with various other UV filters to modify the SPF; the finished drug products also varied in their several formulation factors to modify cosmetic or performance aspects of a product.

Human skin irritation, without or with UV irradiation did not occur in volunteers tested in all but one of the various studies. Skin contact allergenicity, without or with UV irradiation did not occur in the volunteers participating in any of the various studies.

From the controlled studies in human volunteers we reviewed, bemotrizinol in combination with other UV filters is considered as safe for topical use in finished drug products. This conclusion is significantly strengthened by the good market experience with such products that have been applied over 2 billion times by the public in the first 5 years of market use.

Ciba

	Product Code	Composition	In Vivo				In Vitro			Others	
1	Redacted	**BEMT 6%** **MBBT 6%/2** EHMC 10% ZnO 0.1% TiO2 0.1%	24h OPT TAB_IV.C.1.1a. & TR	HRIPT TAB_IV.C.1.1 b.	HCOM TAB_IV.C.1.1 c.		InVitro Irr Skin TAB_IV.C.1.1 d. & TR	InVitro Irr Cells TAB_IV.C.1.1 e. & TR	InVitro Irr Egg TAB_IV.C.1.1f. & TR	*Summ. Tox Exp. TAB_IV.C.1.1 g. & TR	
			Not irritant	Non primary irritant & non primary sensitizer	Non comedogenic		Moderately irritating to irritating Not included with this submission	Negligible cytotoxicity	Mild irritant	In vitro- mild irritant, not cytotoxic; Human-not irritant, not a sensitizer	*minor formula modification
2	Redacted	**BEMT 1%** **MBBT 0.5%?** EHMC 10% ZnO 0.5% TiO2 0.8%	24h OPT TAB_IV.C.1.2a. & TR	HRIPT TAB_IV.C.1.2 b.	PT TAB_IV.C.1.2 c. & TR	HCOM TAB_IV.C.1.2d .	InVitro Irr Skin TAB_IV.C.1.2 e. & TR	InVitro Irr Cells TAB_IV.C.1.2f . & TR		Summ. Tox. Exp. TAB_IV.C.1.2 g. & TR	Safety Eval TAB_IV.C.1.2 h. & TR
			Not irritant	Non primary irritant & non primary sensitizer	Not irritant	Non comedogenic	Non irritant	Not irritant		In vitro- mild irritant, not cytotoxic; Human-not irritant, not a sensitizer	
3	Redacted	**BEMT 5.0%** EHS 3.0% EHMC 2.5%	H Irr. & HRIPT TAB_IV.C.1.3a.	HRIPT TAB_IV.C.1.3 b. & TR.	Photo H Irr. TAB_IV.C.1.3 c. & TR.						
			Weak irritant; not sensitzer	Not photo-sensitizer	Not photo-irritant						
3^{bis}	Redacted	EHMC 7.51% OCR 5.0% EHS 3.0% **BEMT 2.0%**	H Irr. & HRIPT TAB_IV.C.1.3^{bis}a	HRIPT TAB_IV.C. 1.3^{bis}b	Photo H Irr. TAB_IV.C. 1.3^{bis}c.						
			Non irritant; not sensitzer	Not photo-sensitizer	Not photo-irritant						
4	Redacted	EHMC 10.0% EHT 5.0% HS 5.0% EHS 5.0% **EMT 5.0%**	H Irr. & HRIPT TAB_IV.C.1.4a.	Photo HRIPT TAB_IV.C.1.4 b.	Photo H Irr. TAB_IV.C.1.4 c.						
			Not primary irritant & not primary sensitizer	Not allergenic or photo-allergenic	Not irritant, not photo-irritant						

Ciba

5	Redacted	**BEMT 6.0%** EHS 3.0% EHMC 2.5%	HRIPT TAB_IV.C.1.5a	Photo HRIPT TAB_IV.C.1.5 b	Photo H Irr.TAB_IV.C.1 .5c						
			Not included with this submission	Not included with this submission	Not included with this submission						
6	Redacted	EHMC 10% **BEMT 7.5%** HMS 5% EHS 5%	H Irr. & HRIPT TAB_IV.C.1.6a.	Photo HRIPT TAB_IV.C.1.6 b.	Photo H Irr. TAB_IV.C.1.6 c. & TR.	Photo HRIPT TAB_IV.C.1.6d					
			Moderate irritant (in 104 subj. 3 had grade 1 & 9 grade 2 irrit in 15 days); low potential for sensitization: 1 grade 1 score.	Not a photo sensitizer	Not phototoxic & not photoirritant	Data not available					
6bis	Redacted	EHMC 10% OCR 5.5% HMS 5% EHS 5% **BEMT 7.5%**	H Irr. & HRIPT TAB_IV.C.1.6^{bis}a.	Photo HRIPT TAB_IV.C.1.6^{b} ^{is}b.	Photo H Irr. TAB_IV.C.1.6^{b} ^{is}c.						
			Weak irritant (in 104 subj. 2 had grade 1 & 2 grade 2 irrit in 15 days); low potential for sensitization: all scores zero	Not a sensitizer or photo-sensitizer	Not phototoxic & not photoirritant						
7	Redacted	**BEMT 3%** EHMC 5% IMC 2%	48h OPT TAB_IV.C.1.7a								
	Redacted		Not irritant								
8	Redacted	**BEMT 2%** EHMC> 5% IMC 2% TiO2~ 2.5% ZnO~1.5%	48h OPT TAB_IV.C.1.8a								
			Not irritant								
9	Redacted	**BEMT 3%** EHMC 7.5% ZnO~1.75%	48h OPT TAB_IV.C.1.9a								
			Not irritant								
10	Redacted	**BEMT 3%** EHMC> 7%	48h OPT TAB_IV.C.1.10a								

			Not irritant								
11	Redacted	**BEMT 2,5%** BMBM 4,5% EHMC 9,0% EHT 2,0%	Photo HRIPT TAB_IV.C.1.11a.								
			Is not irritant, photo-irritant; not allergenic or photoallergenic								
12	Redacted	**BEMT 1,0%** BMBM 4,5% EHMC 9,0%	REPT TAB_IV.C.1.12a.								
			Not Irritant								
14	Redacted	**BEMT 1,4%** BMBM 4,5% EHMC 8,0% EHT 1,6% TiO2 3,0%	REPT TAB_IV.C.1.14a.	Photo HRIPT TAB_IV.C.1.1 14b.							
			Not Irritant	Not allergenic or photo-allergenic							
16	Redacted	**BEMT 2,0%** BMBM 4,5% DBT 0,5% EHMC 7,5% OCR 3,5% TiO2 5,0%	REPT TAB_IV.C.1.16a.								
			Not Irritant								
18	Redacted	**BEMT 2,0%** BMBM 4,0% EHMC 9,5% EHT 2,0% TiO2 3,0%	REPT TAB_IV.C.1.18a.								
			Not Irritant								

Ciba

Definition of abbreviations:	
HCOM	Comedogenic potential
Photo H Irr.	Human Photo Patch Test
Photo HRIPT	Photo Allergenicity/Sensitivity
24h/48h OPT	24h/48h Occlusive patch test
Photo H Irr. & RIPT	Human phototoxicity & Phototoallergenicity
H Irr.	Human Patch Test
REPT	Repeated epicutaneous Patch Test
InVitro Irr Skin	In Vitro Irritation potential tested on reconstituted skin
InVitro Irr cells	In Vitro Irritation potential tested by "neutral red uptake"
InVitro Irr Egg	In Vitro Irritation potential tested on egg membrane

2. Partially Controlled or Uncontrolled Studies (*Not Applicable*)

3. Documented Case reports. Side effects (*Not Applicable*)

No adverse effects after 2 billion applications (see 4. pertinent marketing experience)

4. Pertinent marketing experience

Pertinent market experience has been extensively illustrated in the Time and Extent Application for bemotrizinol submitted on April 11, 2005 (Docket No. 2005N-0446). Prepared to support the Inclusion of bemotrizinol into FDA's Monograph for Sunscreen Drug Products for Over-the-Counter Human Use; (64 FR 27666-27693, as amended by 66 FR 67485-67487, Docket No. 78N-0038).

Conclusion:

The analysis of the marketing experience in 31 countries comes to the conclusion that there were over **2 billion** applications of bemotrizinol containing sunscreen over the first five years.

5. Pertinent medical and scientific literature

Ciba Specialty Chemicals does not develop finished drug products and thus has not conducted or published results in scientific literature. The paucity scientific published literature suggests that safety of bemotrizinol is not an issue of current concern.

V. Efficacy data

Preliminary note:
In sunscreens, generally combinations of sunscreen actives are used to achieve a certain SPF and UVA protection. A list of all UV Filters that are available in the major sunscreen countries/regions is given in Tab V. I. Technically bemotrizinol may be combined with all these sunscreen actives

A. Individual active component: bemotrizinol

Studies on the efficacy of bemotrizinol combined with other sunscreen actives are reported a subsequent parts (C. Finished drug products and D. Combinations of bemotrizinol with other UV filters)

1. Controlled Studies

Numerous studies exist that demonstrate the effect/efficacy of bisoctrizole, used alone (or in combination). Most of them have been presented in scientific articles such as the ones quoted below (C.5. and D5. **Pertinent medical and scientific literature).**

The following table shows the results of measurement and calculations by Ciba researchers of bemotrizinol alone[6]. With increasing concentration the protection factors UVA-PF and SPF increase linearly. The UVA/UVB ratio stays high also after irradiation.

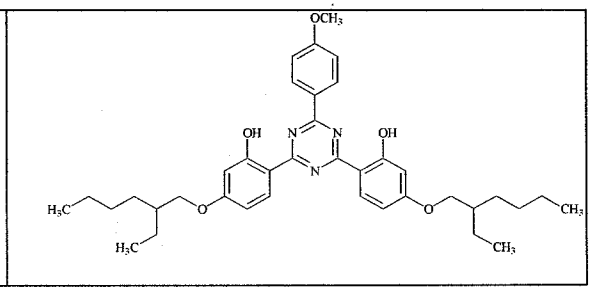
Nr.	Filter Content of Formulation	*in vivo* UVA-PF	Calc. Chardon Spectrum	UVA/UVB-Ratio	UVA/UVB -Ratio (incl. Irr.)	Calc. SPF (Sunscreen Simulator)
35	1% BEMT	3.9	3.4	0.75	0.75	4.1
36	2% BEMT	5.6	4.5	0.78	0.78	5.2
37	3% BEMT	5.9	5.5	0.80	0.80	6.3
38	4% BEMT	9.8	6.5	0.81	0.81	7.6

a) Spectrum of Filters

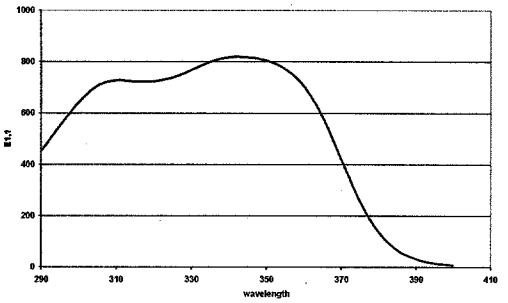
The efficacy of a filter is best illustrated by its UV extinction spectrum, i.e. its protection potential over the whole UV range.

- The larger this spectrum the wider the range of action of the filter.
- The higher the spectrum the more efficient this protection

The particularity of bemotrizinol is to cover the whole UV spectrum, which only very few UV filters on the market do, even less with good efficiency. In the USA there are only 2 broad-spectrum sunscreen actives on the sunscreen monograph, the microfine inorganic filters Titanium dioxide and Zinc Oxide. Both are less efficient than bemotrizinol.



[6] Herzog et al (2002)

	
Extinction Spectrum of Bemotrizinol (E1,1) value	Structure of Bemotrizinol

b) Photostability

Photostability characterizes the property of a product to resist and not to degrade under the influence of sun light. The UV radiation absorbed by sunscreen actives may have the effect to damage chemical bonds of the absorber and hence provoke a decomposition of the molecule.
In UV protection, this lack of photostability can infer[7] concerns form a safety and an efficacy point of view (allergy due to penetration of decomposition products). Secondly, if degraded the protection by a UV filter diminishes.

For this reason, one of the industry's mains focus in now to develop highly photostable filters.
Bemotrizinol has been demonstrated to be very photostable (see literature below).
The photostability of an ingredient can be expressed with its photodegradation constant k[8] .
K for bemotrizinol has been determined as extremely low. (0.001k/MED^{-1})
The table below present k constants for some of the most frequently used UV filters in Europe

UV absorber (sunscreen active)		Photodegradation Constant k (MED^{-1})
	USAN ("INCI")	
MBBT	bisoctrizole	0.0002
BEMT	**bemotrizinol**	**0.001**
OCR	octocrylene	0.001
EHS	octisalate	0.001
EHT	„ethylhexyl triazone"	0.006
DBT	"diethylhexyl butamido triazone"	0.006
PBSA	ensulizole	0.009
MBC	enzacamene	0.04
BMP	"Polysilicone-15"	0.04
EHMC	octinoxate	0.04
BMDBM	avobenzone	0.13

In addition, as shown in the table below[9], bisoctrizole has been shown to have a stabilizing effect on others filters[10]. Avobenzone and Octinoxate can be reasonable stabilized by bemotrizinol as long as they are not combined. As the data show, especially the octinoxate degradation is enhanced when avobenzone is present.

UV absorber combination (constant k referring to the first UV absorber)	Photodegradation Constant k (MED^{-1})	Photodegradation Constant in presence of Bemotrizinol k (MED^{-1})

[7] Lütolf B et al (2004)
[8] Herzog B et al (2004)
[9] Herzog B et al (2000)
[10] Herzog B et al (2002)

EHMC (+ BEMT)	0.04	0.02
BMDBM (+ EHMC)	0.13	0.14
BMDBM (+ BEMT)	0.13	0.05

2. **Partially controlled or uncontrolled studies *(Not applicable)***

3. **Documented case reports *(Not applicable)***

4. **Pertinent marketing experience *(Not applicable)***

5. **Pertinent medical and scientific literature**

The following references describe bemotrizinol, how it was developed and how it performs. References on the efficacy of bemotrizinol combined with other sunscreen actives are reported in subsequent parts (C. Finished Drug Products, D. Combinations of bemotrizinol with other UV filters)

Herzog, B. Hueglin D., Borsos E., Stehlin A., Luther H., **"New UV Absorbers for Cosmetic Sunscreens – A Breakthrough for the Photoprotection of Human Skin"**, BChimia 58, 554 – 559 (2004)

Content:

This article presents the theoretical explanations for photostability of bemotrizinol.

Herzog B, Hueglin D, Osterwalder U, **"New Sunscreen Actives"**, in: "Sunscreens – Regulation and Commercial Development", ed. Nadim Shaath, 3rd ed., Taylor & Francis, Boca Raton 2005, p. 291-320

Content.

Overview of new sunscreen actives. Dertailed discussion of development of bemotrizinol

Hueglin, B. Herzog, S. Mongiat, **"Hydroxphenyltriazines: A new generation of cosmetic UV filters with superior photoprotection"**, D., Proc. 22nd IFSCC Congress, 2002, Edinburgh

Abstract:

Today sunscreens are expected to protect not only against sunburn, but also against long term damaging effects. Therefore UV-A- and broadband filters are becoming increasingly important. Until recently, the offering of such filters was limited. In the year 2000 the first UV-filter based on Hydroxyphenyltriazine technology was added to the positive list of European cosmetic UV filters (INCI: Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine, BEMT; trade name: TinosorbâS, Ciba Specialty Chemicals). BEMT is a new oil-soluble filter with strong broadband protection in the UVA and UV-B regions of the UV spectrum ("full spectrum" performance). Due to its outstanding filter efficacy, combined with inherent photostability and compatibility with all types of cosmetic filters as well as other cosmetic ingredients, BEMT represents a new generation of cosmetic UV filters. This paper presents the molecular design of BEMT that was directed to achieve

- Maximal "full spectrum" absorbance with high molecular extinction in the UV-A and UV-B
- Inherent photostability
- No incompatibility with other UV-filters and cosmetic ingredients
- Boosting effect on photoprotection, when combined with conventional UV-filters
- Stabilization of photolabile sunscreens and cosmetic actives
- Good solubility in cosmetic solvents (e.g. oils)
- Non-toxic and safe to use

Strong broadband absorption with emax = 47500 M-1cm-1 at lmax = 343nm (ethanol) was obtained using hydroxyphenyltriazine chemistry in combination with two electronic transitions exhibiting high dipole moments, polarized perpendicular to each other (UV-A and UV-B). BEMT contains two intramolecular hydrogen bridges that lead to an excited-state intramolecular proton transfer (phototautomerism) after photoexcitation. This is followed by rapid radiationless energy dissipation (internal conversion), which ensures that the UV radiation, efficiently absorbed by the compound owing to the high extinction coefficient, is almost completely transformed into

harmless vibrational energy. Population of the triplet state and subsequent photoreactions do not take place at all, resulting in inherent photostability.

B. Combinations of the individual active components (*Not Applicable*):

Bemotrizinol is the only active ingredient applied for inclusion in the sunscreen monograph discussed here

C. Finished drug product

1. Controlled Studies (customers and Ciba Specialty Chemicals)

Ciba customers routinely analyze sunscreen products containing bemotrizinol and determine the SPF and UVA protection potential of a product. Bemotrizinol is used in sunscreens all over the world since March 2000. Details on the marketing experience were provided in the TEA application (Docket No. 2005N-0446). We estimated that there were over one billion applications of bemotrizinol containing sunscreen in the first 5 years of marketing.

Ciba customers routinely analyze sunscreen products containing bemotrizinol, and determine the SPF and UVA protection potential of their products. To substantiate certain claims, e.g. sensory aspects, other tests may also have been conducted.

The results of measurements from a selection of customers from different countries that are serving different market segments are summarized in the tables below.

Overview/Characterization of customer data

Customers from Market Segment	Countries Manufactured (sales)	UV filters combined with bemotrizinol	Tested performance parameters		
			SPF	UVA-protection	Others
Pharmacy Mass Market	Australia France South Korea Switzerland Sales in Europe, Asia, South America	EHMC TiO2 ZnO HMS EHS EHT BMBM 4-MBC PBSA DBT IMC	COLIPA FDA Intl Method	In vivo UVA-PF (PPD) In vitro lambda crit. UVA/UVB ratio Australian UVA Standard	Sensory testing

The study results are summarized in the following table. The studies that Ciba's customers provided can be found in the respective Tabs. These data from selected customer products show that bemotrizinol is used in combination with a great variety of other sunscreen actives. Its incorporation level is normally between 2 and 5%. Bemotrizinol influences the SPF as well as the UVA-PF, due to its broad-spectrum character. In the chapter below the contribution of bemotrizinol is estimated by a computer simulation (Section V. D. Combinations of bemotrizinol with other UV filters).

Ciba

Summary of the results from customers of Ciba Specialty Chemicals

No.	Product Code	Composition	SPF &WR		UVA		Others	
1	Redacted	**BEMT 6%** MBBT 3% EHMC 10% ZnO 0.1% TiO2 0.1%	SPF & WR (FDA) TAB V.C.1.1a.		UVA-PF (IPD) TAB V.C.1.1b. & TR.	Austr. Std TAB V.C.1.1c.	Sens. Profiles TAB V.C.1.1d. & TR.	
			SPF= 60.84		UVA-PF= 75.1	fulfilled	- white residue, + transparent vs. competition	
2	Redacted	**BEMT 1%** MBBT~ 3% EHMC 10% ZnO 0.5% TiO2 0.8%	SPF & WR (COLIPA) TAB V.C.1.2a.	SPF Intl TAB V.C.1.2b.	UVA-PF (IPD) TAB V.C.1.2c. & TR.	UVA prot. Colipa ring test method TAB V.C.1.2d.	Sens. Profile TAB V.C.1.2e. & TR.	Test in use TAB V.C.1.2f. & TR.
			SPF= 73.5 WR OK	SPF= 66.7	UVA-PF= 66.2	Lambda Crit.= 370nm	- white residue, + transparent vs. current product	
3	Redacted	**BEMT 5.0%** EHS 3.0% EHMC 2.5%	SPF & WR (FDAmod) TAB V.C.1.3a. & TR.				Labsph. & Spectro TAB V.C.1.3b.	
			SPF= 17.8; WR OK					
3bis	Redacted	EHMC 7.51% OCR 5.0% EHS 3.0% **BEMT 2.0%**	SPF (COLIPA) &WR (FDA) TAB V.C.1.3^{bis}a.					
			SPF= 18.4; WR OK					
4	Redacted	EHMC 10.0% EHT 5.0% HS 5.0% EHS 5.0% **BEMT 5.0%**	SPF & WR (COLIPA) TAB V.C.1.4a.				Labsph. & Spectro TAB V.C.1.4b.	
			SPF= 54.85; WR OK					
5	Redacted	**BEMT 6.0%** EHS 3.0% EHMC 2.5%	SPF & WR (COLIPA) TAB V.C.1.5a.	SPF & WR (FDA) TAB V.C.1.5b.			Labsph. & Spectro TAB V.C.1.5c.	
			SPF= 20.6; WR OK					
6	Redacted	EHMC 10%	SPF & WR				Labsph. & Spectro	

Ciba

No.	Product Code	Composition	SPF &WR		UVA		Others	
		BEMT 7.5% HMS 5% EHS 5%	(FDAmod) TAB V.C.1.6a. & TR.				TAB V.C.1.6d.	
			SPF= 27.4; WR OK					
6[bis]	Redacted	EHMC 10% OCR 5.5% HMS 5% EHS 5% **BEMT 7.5%**	WR (FDA) TAB V.C.1.6[bis]a.					
			SPF= 30.13; WR OK					
7	Redacted	**BEMT 3%** EHMC 5% IMC 2%	SPF (KFDA) TAB V.C.1.7a.		UVA-PF (KFDA) TAB V.C.1.7b.			
			SPF= 36.2± 3.2		UVA-PF= 4.8, PA++			
8	Redacted	**BEMT 2%** EHMC> 5% IMC 2% TiO2~ 2.5% ZnO~1.5%	SPF (KFDA) TAB V.C.1.8a.		UVA-PF (KFDA) TAB V.C.1.8b.			
			SPF= 40.2± 4.3		UVA-PF= 4.7, PA++			
9	Redacted	**BEMT 3%** EHMC 7.5% ZnO~1.75%	SPF (KFDA) TAB V.C.1.9a.		UVA-PF (KFDA) TAB V.C.1.9b.			
			SPF= 36.4 ± 2.4		UVA-PF= 5.8, PA++			
10	Redacted	**BEMT 3%** EHMC> 7% ZnO~0.75%	SPF (KFDA) TAB V.C.1.10a.		UVA-PF (KFDA) TAB V.C.1.7b.			
			SPF= 27,5± 3.9		UVA-PF= 5.5, PA++			
13	Redacted	**BEMT 1,0%** BMBM 4,5% EHMC 9,0% PBSA 1,0%	SPF & WR Intl TAB V.C.1.13a.					
			SPF=24.9; high before and after watering					
15	Redacted	**BEMT 0,5%** BMBM 3,5%	SPF & WR Intl TAB V.C.1.15a.					

Ciba

No.	Product Code	Composition	SPF &WR		UVA		Others	
		EHMC 7,0% TiO2 2,0%	SPF=21.5 & 65% WR					
16	Redacted	**BEMT 2,0%** BMBM 4,5% DBT 0,5% EHMC 7,5% OCR 3,5% TiO2 5,0%	SPF (COLIPA) TAB V.C.1.16a.					
			SPF=41.1					
17	Redacted	**BEMT 3,0%** BMBM 3,0% DBT 4,0% TiO2 4,0%	SPF & WR Intl TAB V.C.1.17a.					
			SPF=44.6 & WR OK					
19	Redacted	**BEMT 2,0%** BMBM 4,5% OCR 3,5% TiO2 3,0%	SPF Intl TAB V.C.1.19a.					
			SPF=22.5					

Definition of abbreviations:

	Abbreviation	Definition
SPF	SPF & WR	Sun Protection Factor and Water Resistance measurement
UVA Protection	UVA-PF	UVA protection factor
	Lambda Crit.	Critical Wavelength
	(IPD)	UVA- PF measurement according to the Immediate Pigment Darkening method
	Austr. Std	Australian Standard
Others	Labsph. & Spectro	Labsphere & Spectrophotometer analysis
	Sens. Profiles	Sensorial Profiles
Guidelines	(COLIPA)	measurement following European Toiletry and Perfumery Association
	(KFDA)	measurement following Korean Food & Drug Administration Guidelines
	(FDA)	measurement following American Food & Drug Administration Guidelines
	(FDAmod)	measurement following American Food & Drug Administration modified Guidelines
	Intl	measurement following International Guidelines (CTFA, JCIA & COLIPA)

2. **Partially controlled or uncontrolled studies (*Not Applicable*)**

3. **Documented case reports (*Not Applicable*)**

4. **Pertinent marketing experience**

Pertinent market experience has been extensively illustrated in the Time and Extent Application for bemotrizinol submitted on April 11, 2005 (Docket No. 2005N-0446). Prepared to support the inclusion of bemotrizinol into FDA's Monograph for Sunscreen Drug Products for Over-the-Counter Human Use (64 FR 27666-27693, as amended by 66 FR 67485-67487, Docket No. 78N-0038).

Conclusion:
The analysis of the marketing experience in 31 countries comes to the conclusion that there were over **2 billion** applications of bemotrizinol containing sunscreen over the first five years

Bemotrizinol was designed to meet the needs of the cosmetic industry. Besides the excellent performance as a photostable broad-spectrum UV filter, it is compatible with organic and inorganic filters and shows synergistic effects with UVB filters for high-SPF sunscreens.

Bemotrizinol shows indeed a very strong synergy with the two most efficient UVB filters Ethylhexyl Triazone and Diethylhexyl Butamido Triazone, and a slight synergy in combination with the frequently used UVB filters, 4-Methylbenzylidene Camphor, Ethylhexyl Methoxycinnnamate and Phenylbenzimidazol Sulfonic Acid. Moreover, Bemotrizinol limits efficiently the photo-degradation of Avobenzone[11].

Bemotrizinol, beyond its own efficiency is also an enhancer for the properties of other filters and is thus used in combination with numerous other UV filters. The table below lists these filters The filter combinations observed in Bemotrizinol-containing market samples were provided in ***Appendix III*** of the bemotrizinol TEA application (see reference above)

Filters used in combination with Bemotrizinol

Abbr.	USAN	US Registration Status
MBBT	Bisoctrizole	In the TEA process
BMBM	Avobenzone	US registered
BP3	Oxybenzone	US registered
DBT	Diethylhexyl Butamido Triazone	Not applicable
EHMC	Octinoxate	US registered
EHS	Octisalate	US registered
EHT	Ethylhexyl Triazone	In the TEA process
HS	Homosalate	US registered
IMC	Amiloxate	In the TEA process
MBC	Enzacamene	In the TEA process
OCR	Octocrylene	US registered
PBSA	Enzulizole and salts	US registered
TiO2	Titanium Dioxide	US registered
ZnO	Zinc Oxide	US registered

5. **Pertinent medical and scientific literature**

All reports provided are proprietary product data provided by our customers. These are not publicly published data.

Hauri U., Lütolf B., Schlegel U., Hohl C., "**Determination of photostability of UV filters in sunscreens by HPLC/DAD and HPLC/MS**", Mitt. Lebensm. Hyg. 95, 147 – 161 (2004)
Content:

[11] Brochure TINOSORB® S: The daily broad-spectrum UV Absorber (available on www.cibasc.com)

As addressed earlier, photostability of UV filters in sunscreen formulation is a matter of great importance. It is here subject to a comprehensive study, based on the analysis by HPLC of different ready to *use cosmetic formulations*, that shows in particular the very high stability of bisoctrizole (in combination with OMC, MBC, OS and BMBM.

Abstract:

The photostability of UV filters in sunscreens towards daylight was investigated in 10 products covering most of the currently used sunscreen filters, applying newly developed or adapted LC/MS and LC/DAD methods. The study showed that up to 40% of ethyl-hexylmethoxy cinnamate (EHMC), isoamylmethoxy cinnamate (IMC), and butylmethoxy dibenzoylmethane (BMDBM) were degraded. Photodegradation. also took place in the case of Methyl benzylidene camphor. Besides E/Z isomeric change, intermol. reactions between EHMC, IMC, and BMDBM were also observed EHMC and BMDBM belong to the most often used sunscreen filters. As these filters are used in concns. in the percent range in cosmetics and their application involves large parts of the body's surface, further toxicol. evaluation of the products formed is needed.

Hauri, Urs; Luetolf, Beat; Hohl, Christopher, Determination of organic sunscreen filters in cosmetics with HPLC/DAD, Mitteilungen aus Lebensmitteluntersuchung und Hygiene (2003), 94(1), 80-92

Abstract:

An HPLC method for the screening of 21 sunscreen filters in cosmetics is presented. For quantitation small adaptions of the extraction must be made for some of these substances. A market survey on 47 products revealed that only 14 of over 25 approved organic sunscreen filters are currently used. Total organic sunscreen concns. are very high (until 25%) in some products and do not correlate well with claimed sun protection factors

Monfrecola G.; Fabbrocini G.; Prizio E.; Russo I. [**Evaluation of clinical efficacy of Minesol® SPF 50(+) spray**]. VALUTAZIONE DELL'EFFICACIA CLINICA DI MINESOL® SPF 50(+) SPRAY, Dermatologia Clinica, (2005) 25/2 (69-74)

Abstract:

During the day human skin may be exposed to sunlight ultraviolet radiations. These rays can be of the three types: "A" (from 320 to 400 nm), "B" (from 290 to 320 nm) and "C" (from 200 to 290 nm). It is useful the use of different sunscreens to prevent radiations negative effects on human skin. The aim of solar cosmetic products for skin is not to increase the total number of exposure hours to sunlight, but to reduce the risks derived by ultraviolet radiations exposition. In fact sunlight protection products tend to prevent: erythema, sunburns, dermatosis, photo-aging and cutaneous cancer. Ideal sunscreen product must be: efficacy, pleasant in the cosmetic use, resistant to the water and to the sweat. On the same time, it would be safe and no toxic. Therefore a sunscreen must reduce the total amount of ultraviolet radiations, and it must leave unchanged the quality of the ultraviolet radiations. In this paper, the Authors report the results of photo-protective efficacy of a solar product containing two new filters (**Tinosorb S** and Tinosorb M) and an innovative emulsion RoC patented (Silsoft® Surface System). In this paper, the Authors report the results of photo-protective efficacy of a solar product containing two new filters (**Tinosorb S** and Tinosorb M) and an innovative emulsion RoC patented (Silsoft® Surface System).

D. Combinations of Bemotrizinol with other UV filters

1. Controlled Studies (*in vivo, in vitro* and *in silico*)

Numerous studies exist that demonstrate the effect/efficacy of bemotrizinol, used alone (or in combination). Most of them have been presented in scientific articles such as the ones quoted below. Results from *in vivo* experiments such as *in vivo* Sun Protection Factor (SPF) or Persistent Pigment Darkening (PPD) or *in vitro* experiment are summarized in the table below. Furthermore we are able to predict the performance of any combination of UV absorbers used in sunscreen by a special simulation program called the Ciba Sunscreen Simulator[12]. Such calculated values are also added to

[12] Herzog et all (2002)

the table below. The table shows an extract of the study results. Both the influence on the SPF as well as the various UVA-assessment parameters can be seen.

Summary of efficacy data on bemotrizinol used in combination (with values for other UV filters alone for comparison)

Nr.	Filter Content of Formulation	*in vivo* UVA-PF	Calc. Chardon Spectrum	UVA/UVB-Ratio	UVA/UVB-Ratio (incl. Irr.)	Calc. SPF (Sunscreen Simulator)
19	5% EHMC	2.7	1.5	0.25	0.25	7.1
23	5% EHS	1.8	1.1	0.14	0.14	3.5
39	5% PBSA	2.8	1.1	0.08	0.08	5.5
41	5% EHT 3% BEMT	3.0	1.2	0.14	0.14	8.3
42	+ 5% EHT	8.8	5.7	0.44	0.45	24.1
43	5% Parsol SLX 3% BEMT	2.6	1.3	0.21	0.21	3.7
44	+ 5% Parsol SLX	5.6	5.6	0.72	0.73	7.8
45	5% MBC 3% BEMT	3.0	1.4	0.19	0.19	7.0
46	+ 5% MBC	8.8	5.7	0.51	0.54	16.2
47	5% Uvasorb HEB 3% BEMT	3.0	1.2	0.14	0.14	8.7
48	+ 5% Uvasorb HEB	9.5	5.8	0.43	0.44	26.6
49	5% TiO_2 3% BEMT	5.3	4.8	0.65	0.65	9.1
50	+ 5% TiO_3 1% BEMT	11.0	8.1	0.69	0.69	15.9
51	+ 5% EHMC 2% BEMT	5.3	3.7	0.47	0.48	10.6
52	+ 5% EHMC 3% BEMT	9.4	4.9	0.52	0.54	12.9
53	+ 5% EHMC 4% BEMT	15.7	5.9	0.55	0.57	15.5
54	+ 5% EHMC	15.1	7.1	0.58	0.60	18.6

In the table below the contribution of bemotrizinol in 3 commercial sunscreen examples is determined. The calculations (*in silico*) demonstrate the effect of bemotrizinol on the SPF as well as the UVA-PF. Example 1 (SPF 20) and example 2 (SPF 30) are typical mass market sunscreens, whereas example 3 (SPF 60 is pharmacy brand. The contribution of bemotrizinol in these examples is about 20% per 1 % incorporated bemotrizinol on the SPF and 30-40% per 1 % on the UVA-PF. A description and discussion of the current UVA assessment method is given in Appendix 3.

Contribution of bemotrizinol to SPF and UVA-PF (*in silico* experiments)

	Example 1	Example 2	Example 3
Product code **Composition**	**Redacted** SPF20	**Redacted** SPF40	**Redacted** SPF60
BEMT (Bemotrizinol)	**2**	**3**	**6**
Broad-spectrum UV absorbers			
MBBT (Bisoctrizole)			**3**
ZnO			0.1
UVA absorbers			
BMBM (avobenzone)	4.5	3	
UVB absorbers			
TiO2	3.0	4	0.1
DBT (Butamido Triazine)		4	
OCR (octocrylene)	3.5		

EHMC (octinoxate)				10
	Total amount	13.0	14.0	19.2
SPF				
In vivo (COLIPA)		22.5	44.6	60.8
***in silico* (with/without BEMT)**		**21.4 (14.2)**	**42.7 (29.9)**	**47.7 (23.6)**
Bisotrizole effect	total	51%	43%	102%
	per 1%	**25%**	**21%**	**17%**
UVA-PF		8.8 (6.5)	10.7 (6.9)	24.7 (7.1)
In vivo (PPD)				
***in silico* (with/without BEMT)**				
Bemotrizinol effect	total	35%	41%	248%
	per 1%	**18%**	**20%**	**41%**

2. Partially controlled or uncontrolled studies (*Not Applicable*)

3. Documented case reports (*Not Applicable*)

4. Pertinent marketing experience (*Not Applicable*)

See section V.C. Finished drug products Subsection 4. Pertinent marketing experience

5. Pertinent medical and scientific literature

Chatelain E, Gabard B., **Photostabilization of Butyl methoxydibenzoylmethane (Avobenzone) and Ethylhexyl methoxycinnamate by Bis-ethylhexyloxyphenol methoxyphenyl triazine (Tinosorb S), a New UV Broadband Filter**, Photochem.Photobiol. (2001), 74(3), 401 - 406

Abstract:

It is now well documented that chronic UVA exposure induces damage to human skin. Therefore, modern sunscreens should not only provide protection from both UVB and UVA radiation but also maintain this protection during the entire period of exposure to the sun. UVA filters, however, are rare and not sufficiently photostable. We investigated the effect of the introduction of a new UV filter, bis-ethylhexyloxyphenol methoxyphenyl triazine (**Tinosorb S**), in oil in water sunscreen formulations on the photostability of butylmethoxydibenzoylmethane (Avobenzone [AVB]) after irradiation with an optically filtered Xenon arc source (UV irradiance adjusted at 1 mean effective dose [MED]/min). With spectrophotometrical methods to assess the sun protection factor (SPF) and UVA ratio and chromatographical methods to determine the amount of UV filters recovered after irradiation we showed that **Tinosorb S** prevented the photodegradation of AVB in a concentration-dependent way, leading to a sustained SPF and UVA ratio even after irradiation with doses of up to 30 MED. Since AVB was shown to destabilize ethylhexyl methoxycinnamate (EHM) we tested the effect of **Tinosorb S** in sunscreens containing this UV filter combination. Here too **Tinosorb S** showed photoprotective properties toward both UV filters. Thus, **Tinosorb S** can be used successfully to improve the photostability and efficiency of sunscreens containing AVB and EHM.We investigated the effect of the introduction of a new UV filter, bis-ethylhexyloxyphenol methoxyphenyl triazine (**Tinosorb S**), in oil in water sunscreen formulations on the photostability of butyl methoxydibenzoylmethane (Avobenzone [AVB]) after irradiation with an optically filtered. . .(SPF) and UVA ratio and chromatographical methods to determine the amount of UV filters recovered after irradiation we showed that **Tinosorb S** prevented the photodegradation of AVB in a concentration- dependent way, leading to a sustained SPF and UVA ratio even after irradiation. . . doses of up to 30 MED. Since AVB was shown to destabilize ethylhexyl methoxycinnamate (EHM) we tested the effect of **Tinosorb S** in sunscreens containing this UV filter combination. Here too **Tinosorb S** showed photoprotective properties toward both UV filters. Thus, **Tinosorb S** can be used successfully to improve the photostability and efficiency of sunscreens containing AVB and EHM.

Herzog B.; Mongiat S.; Deshayes C.; Neuhaus M.; Sommer K.; Mantler A.,***In vivo* and *in vitro* assessment of UVA protection by sunscreen formulations containing either butyl methoxy dibenzoyl methane, methylene bis-benzotriazolyl tetramethylbutylphenol, or microfine ZnO**,

Journal of Cosmetic Science (2002), 24(3), 170-185
Content:

This study demonstrates the photostabiliy of bisoctrizole (MBBT) in combination with EHMC and EHS (and alone) by analysing the evolution of results of different assessment methods for UVA protection level (critical wavelength, Australian standard, ppd). It also shows the synergetic effect of bisoctrizole with EHMC

Extract of Abstract:

The UVA-attenuating properties of the three UVA filters Butyl methoxy dibenzoyl methane (BMDBM), methylene bis-benzotriazolyl tetramethylbutylphenol (MBBT), and microfine zinc oxide (ZnO), are compared. For this purpose persistent pigment darkening (PPD) as an *in vivo* method as well as different *in vitro* approaches like the UVA/UVB ratio, the critical wavelength, and the Australian standard have been used. For the case of the UVA/UVB ratio and the critical wavelength the behavior was also assessed after irradiation with 10 minimal erythemal doses (MED). Sunscreen formulations were manufactured containing either one of these UVA filters or combinations of one UVA filter and a constant amount of UVB filter. The concentration of the resp. UVA filter was varied. The UVA-protection factors (UVA-PF) obtained from the *in vivo* studies did increase with the concentration of the UVA filter in the formulations. Formulations, which showed UVA-PFs ≥ 4 in most cases met also the conditions of the Australian Standard An irradiation dose of 2.5 kJ m-2 (10 MED) induced significant decreases of UVA/UVB ratio or critical wavelength only with some BMDBM formulations, indicating a loss of UVA protection in those cases.

Herzog B., Sommer K., "**Investigations on Photostability of UV-Absorbers for Cosmetic Sunscreens**", Proceedings of the XXI. IFSCC International Congress, Berlin 2000
Content:

The photostability of bisoctrizole (MBBT) in combination with EHMC, BMBM and MBC (and alone) was analysed in this study. . *It was demonstrated that* bisoctrizole *ranked higher than all the others on this criterion (with a recovery of which is 98% after 50 MED*). It was also demonstrated that bisoctrizole (MBBT) stabilizes BMBM and EHMC.

Herzog B., Mongiat S., Deshayes C., Neuhaus M., Quass K., Mantler A., Comte C., ***In vivo* and *in vitro* assessment of UVA protection by sunscreen formulations containing either ZnO, butyl methoxy dibenzoyl methane, methylene bis-benzotriazolyl tetramethylbutylphenol, or bis-ethylhexyloxyphenol methoxyphenyl triazine**, Proc. 22nd IFSCC Congress, 2002, Edinburgh
Content:

This study states the performance of bisoctrizole (MBBT) *in combination with EHMC* (*and* alone) according to different assessment methods for UVA protection level (critical wavelength, Australian standard, PPD). It also demonstrates the photostability of bisoctrizole and shows its strong synergetic effect with EHMC

Rudolph T, UV **Filter Systems: Trends and Pesrpectives** (UV Filtersysteme: Trends und Perspektiven), 15th Symposium DGK, Deutsche Gesellschaft für Wissenschaftliche und angewandte Kosmetik e.V., Kosmetischer Lichtschutz: Dermatologische Aspekte – Wirkstoffe – Formulierungen –Pruefmethoden, 12-14 Maerz 2003, Maritim Hotel, Koeln/Cologne, Germany, Proceedings, Paper 11, 80-87 (German)
Extract from Abstract:

The paper discusses actual trends in the formulation of modern sunscreens, starting from the European situation of the year 1997. The reference to the year 1997 is justified by the fact that at that time only Butyl Methoxydibenzoylmethan (BM-DBM) existed as effective UVA-I-filter. Then as additional UVA protection Benzophenone-3 was used as oil-soluble and Terephthalidene Dicamphor Sulfonic Acid (TDSA) as a water-soluble UV filter (exclusively with L'Oreal), as well as the inorganic pigments titanium dioxide and zinc oxide. UV filter systems that besides UVB protection also covers UVA protection today also contain the UVA filter BM-DBM. After expiry of the patent situation in Europe BM-DBM is marketed by several suppliers. Chemical structures and mechanism of action, as well as the photo-stability of BM-DBM are then discussed in detail. Within the discussion for a better stability, Mehtylene Bis-

Benzotriazolyl Tetramethylbutylphenol (MBBT), Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine (**BEMT**) and Drometrizole Trisiloxane (this substance only for L'Oreal) were developed as broad-band UV filters. Structures and efficacy profiles of these substances are further discussed

Literature on the Ciba Sunscreen Simulator

The Ciba Sunscreen Simulator has been developed over the last 5 years, Its major application is in the development of finished drug products (sunscreens). It Based on the UV Filter composition ofa sunscreen and their extinction spectra, the performance expressed as SPF, UVA-PF and various other UVA assessment parameters are determined. The Ciba sunscreen simulator is publicly accessible on the Ciba Specialty Chemicals home page (www.cibasc.com/tinosorb)

Herzog B, "**Prediction of sun protection factors by calculation of transmissions with a calibrated step film model**", Journal of Cosmetic Science (2002), 53(1), 11-26,
Extract from Abstract:

Measurements of *in vitro* sun protection factors (SPFs) are a common way of assessing sunscreen formulations at the stage of screening. The aim of the present investigation is to provide an alternative tool for the estimation of SPF values using a calculation based on the UV spectroscopic properties of the individual UV absorbers. As with *in vitro* measurements, the crucial step is to work out realistic values of transmissions of UV light through a film of the sunscreen formulation in the important spectral range between 290 and 400 nm. Once these transmissions are given, the SPF can be calculated. Since the human skin is an inhomogeneous substrate, a step film model for the calculation of such transmissions had been proposed by J.J. O'Neill. The step film geometry in this model is a function of two parameters that characterize the fraction of the thin and thick parts of the film and their difference in thickness. The transmissions and therefore the SPF are sensitive functions of the step film parameters. In order to use the model for the prediction of realistic SPF values, the step film parameters are calibrated using three sunscreen standard formulations with well-known *in vivo* SPF. A satisfactory correlation of *in vivo* SPF values and SPF values calculated with the calibrated step film model using an additional 36 different sunscreen formulations (*in vivo* SPF values between 3 and 36) is demonstrated.

Herzog B., Mongiat S., Quass K., Deshayes C., "**Prediction of Sun Protection Factors and UVA Parameters by Using a Calibrated Step Film Model**", J. Pharm. Sc. 93, 1780 – 1795 (2004)
Content:

Development of the sunscreen simulator. Calibration against COLIPA standard reference samples and testing with sunscreen formulations that contain bisoctrizole, bemotrizinol and other sunscreen actives.

Osterwalder U, Mueller S, Gril A, Herzog B, **Understanding Sunscreens – New insight into the role of photo-stability through *in vivo, in vitro* and *in silico* experiments**, 64th AAD Meeting, San Francisco, 3-7 March 2006, Poster P415
Content:

Dynamic view on sunscreens. Prediction of sunscreen performance taking into account photostability.

VI. Summary of data and views setting forth the medical rational and purpose for the drug

A summary of data and views setting forth the medical rational and purpose for the drug and its ingredients and the scientific basis for the conclusion that the drug and its ingredients have been proven safe and effective for the intended uses. If there is an absence of controlled studies in the material submitted, an explanation as to why such studies are not considered necessary must be included.

1. Urgent Need for UVA Sunscreen Active Ingredients to Protect U.S. Public Health Against Skin Cancer (TAB VI.1)

2. Risk Assessment: Statement on the Weight of Evidence Supporting Bemotrizinol as Safe for Use in Topical Products.

General Considerations

The non-clinical toxicology profile for local tolerance of bemotrizinol does not indicate adverse effects in single oral and topical applications, the substance is not genotoxic with or without exposure to UV irradiation, it is not phototoxic topically, and it is not a skin contact sensitizer with or without UV irradiation. Importantly, repeated oral dosing through the full reproductive cycle of rodent did not reveal adverse effects and rabbits did not show effects to key developmental parameters (Segment II study). Bemotrizinol is not considered a toxicologically active substance.

The full body of non-clinical testing with bemotrizinol indicates that it is not readily absorbed, orally or topically (the AUC could not be determined), and it is not metabolized to toxic intermediates, based on ADME testing. Chronic topical dosing to minipig, a recognized non-clinical surrogate for human dermal studies, did not reveal dermal or systemic toxicity at a highest achievable dosage. In addition, with prolonged administration by oral (up to 13 weeks) or dermal routes (up to 2 years) it does not produce any indications for an increased carcinogenic response. Prolonged topical dosing of bemotrizinol for 40 weeks to hairless mice also exposed to daily doses of UV radiation demonstrated a protective effect in that bemotrizinol did not increase the UV-induced carcinogenic response in the mice, and in fact, increased the time to tumor onset and decreased the potency of the UV radiation.

Bemotrizinol is a photostable molecule and in the presence of UV radiation does not produce activated moieties such as singlet oxygen, and does not degrade to substituents of the parent structure. This ensures that the available non-clinical and clinical data are representative of the safety of bemotrizinol used in topically applied sunscreens, therapeutic articles, and cosmetic products. The foregoing evidence plus the good clinical experiences in over 5 years' of consumers' use of sunscreens containing bemotrizinol do not indicate issues of concern with this drug substance.

Pediatric Considerations

The body of safety date for bemotrizinol does not indicate concern for its use in babies and young and maturing children. Our conclusion is derived from the absence of early on-set toxicity after dermal or oral dosing in animals of a young age (8 weeks old rats; 3-4 months old minipig). Additionally, the reproductive toxicity tests at maximum bemotrizinol doses fully evaluated in-utero development in rats and rabbits, and also in the full reproductive cycle in rats, including trans-placental, lactation, and post weaning exposures to bemotrizinol. All phases of development and maturation were not adversely affected.

Immunotoxicology Considerations

Immunologic changes related to dosing with bemotrizinol were not revealed in any of the non-clinical studies we have conducted. Changes in lymph, thymus and bone marrow did not occur in minipig, rats, or mice exposed to maximally achievable doses for prolonged periods. We do not find basis for concern for bemotrizinol effects on the immune system.

Risk Assessment and Margin of Safety Calculation for Human Use:

Using the available data for bemotrizinol, an estimated Margin of Safety (MoS) can be determined to show the relative safety of human use rates compared to animal test results. Estimating the human systemic dose is based on the measured bemotrizinol in minipig plasma during 39 weeks' dermal dosing and despite the absence of a clear dose and time effect relationship we estimate 0.06% of a topical dose could reach detectable levels systemically in humans. This estimation is summarized in the nearby Table 1.

Table 1. Minipig Parameter	Value
Median body weight	17 Kg
Average blood volume	65 ml/kg body wt.
Total blood volume	1105 ml
Skin area dosed (10% of total area[1] 6000 cm^2)	600 cm^2
Daily topical dosage	1250 mg a.i./kg b.wt
Total topical a.i. applied (1250 mg/kg x 17 kg)	21,250 mg a.i. /day
Dose a.i. per skin area (21,200 mg/ 600 cm^2)	35 mg/cm^2
Average[2] systemic concentration	12 ng/ml
Average Systemic uptake (12 ng/ml x 1105 ml)	13.2 mg
Dermal penetration [(13.2 mg/21,250 mg) x100]	0.06% of dose

1. Total skin area estimated from $A=[KW^{0.75}]/1000$, where K =70; W=17Kg
2. Average of the 4 values (10.1, 11.4, 15.3, 11.8 ng/ml) that were above 10 ng/ml

The similarity of minipig skin anatomy to that of human skin makes it a reliable basis for estimation of human systemic availability. However, the minipig topical dosages applied to the highest dose group were 175 times higher than the highest estimated human topical application rate (35 vs 0.2 mg/cm^2, respectively) and makes the minipig results an over estimation of the human dermal absorption situation. We consider this as further support for the low bioavailability from topical dosing and as adding another level of conservatism to our risk characterization.

Risk characterization using the margin of safety (MoS) calculation, shown in Table 2, integrates the conservative assumptions of the minipig systemic uptake rate for comparison with the over estimation of the human daily topical use rate of final drug products containing bemotrizinol. From this large MoS we conclude that bemotrizinol does not present an increased risk of adverse systemic or dermal toxicity with prolonged repeated daily topical usage.

The forgoing risk characterization based on topical chronic minipig results taken together with the absence of dermal cancer in rodent and absence of photo-carcinogenic effect for the drug product indicates no concern for safe use of bemotrizinol.

Table 2. Margin of Safety Calculation for Bemotrizinol at 10% in Sunscreen	
Human Parameter	**Human Value**
Human body weight	**(B)** 60 Kg
Skin surface area 'dosed'	**(A)** 18,000 cm^2
Product application rate	**(C)** 2 mg/cm^2
Daily topical dosage a.i. [3600 mg / B]	60 mg a.i./kg/day
Total topical a.i. applied [A x C x 10%]/ B	3600 mg a.i./day
Dose a.i. per skin area (2 mg/cm^2 x 10%)	0.2 mg a.i./cm^2
Systemic daily exposure	2.2 mg a.i.
Systemic daily dosage	0.036 mg/kg b wt
Margin of Safety[1]	**34,700[(a)]**

1. MoS = [Minipig NOEL 1250 mg/kg/d] ÷ [Human Systemic dose 0.036 mg/kg/d]
a. Calculations rounded.

The large body of non-clinical evidence together with the summarized clinical evidence available for bemotrizinol in finished drug products does not indicate a concern for human adverse effects from prolonged topical use by any age category. We find these results as fully supportive of the inclusion of bemotrizinol up to 10% into the FDA's Monograph for Sunscreen Drug Products for Over-the-Counter Human Use without any other restrictions.

VII. USP drug monograph: (Tab VII)

The draft monograph for bemotrizinol has been reviewed by the USP expert committee (Summer 2005) and is scheduled for publication in the Pharmacopeial Forum (PF32(4)) July-August 2006. Subsequent to public review and comment, the monograph is scheduled to be published in USP30-NF25, January 1, 2007.

VIII. Appendices

A. Appendix 1. Cross Reference of all UV Filters by INCI Designation-USAN Names[13]

Designation		US	Japan	EU	Australia
INCI	**USAN**				
1-(3,4 Dimethoxyphenyl)4,4-Dimethyl-1,3 Pentanedione			7		
3-Benzylidene Camphor				2	
4-Methylbenzylidene Camphor	Enzacamene			4[14]	4
Benzophenone-1			10		
Benzophenone-2			10		
Benzophenone-3	Oxybenzone	6	5	10	10
Benzophenone-4	Sulisobenzone	10	10	5	10
Benzophenone-5			10	5	10
Benzophenone-6			10		
Benzophenone-8	Dioxybenzone	3			3
Benzophenone-9			10		
Benzylidene Camphor Sulfonic Acid				6	6
Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine	Bemotrizinol			10	10
Butyl Methoxydibenzoylmethane	Avobenzone	3	10	5	5
Camphor Benzalkonium Methosulfate				6	6
Cinoxate	Cinoxate	3	5		6
Diethylamino Hydroxybenzoyl Hexyl Benzoate			10	10	
Diethylhexyl Butamido Triazone				10	
Diisopropyl Methyl Cinnamate			10		
Disodium Phenyl Dibenzimidazole Tetrasulfonate	Bisdisulizole Disodium			10	
Drometrizole Trisiloxane				15	15
Ethyl PABA			4		
Ethylhexyl Dimethoxybenzylidene Dioxoimidazolidine Propionate			3		
Ethylhexyl Dimethyl PABA	Padimate O	8	10	8[15]	8
Ethylhexyl Methoxycinnamate	Octinoxate	7.5	20	10	10
Ethylhexyl Salicylate	Octisalate	5	10	5	5
Ethylhexyl Triazone			3	5	5
Ferulic Acid			10		
Glucopyranoxy Propylhydroxy Benzephenone			5		
Glyceryl Ethylhexanoate Dimethoxycinnamate			10		
Glyceryl PABA			4		
Homosalate	Homosalate	15	10	10	15
Isoamyl p-Methoxycinnamate	Amiloxate			10	10
Isopentyl Trimethoxycinnamate			7.5		

[13] Table as prepared by David Steinberg (David Steinberg & Associates, Inc. 2005)
[14] Currently under EU review for possible delisting
[15] Not being supported in the EU and may be de-listed

Trisiloxane					
Isopropyl Methoxycinnamate			10		
Menthyl Anthranilate	Meradimate	5			5
Methylene Bis-Benzotriazolyl Tetramethylbutylphenol	Bisoctrizole		10	10	10
Octocrylene	Octocrylene	10	10	10	10
PABA	Aminobenzoic Acid	15	4	5[16]	15
PEG-25 PABA				10	10
Pentyl Dimethyl PABA			10		
Phenylbenzimidazole Sulfonic Acid	Ensulizole	4	3	8	4
Polyacrylamidomethyl Benzylidene Camphor				6	
Polysilicone-15			10	10	
TEA-Salicylate	Trolamine Salicylate	12			12
Terephthalylidene Dicamphor Sulfonic Acid	Ecamsule		10	10	10
Titanium Dioxide	Titanium Dioxide	25	no limit	25	25
Zinc Oxide	Zinc Oxide	25	no limit	25[17]	20

[16] Not being supported in the EU and may be de-listed
[17] Currently under SCCP Review

B. Appendix 2: Bibliography of References Cited for Bemotrizinol

1. Supporting Literature

Ashby J. and D. Paton. The influence of chemical structure on the extent and sites of carcinogenesis for 522 rodent carcinogens and 55 different human carcinogen exposures. Mutation Research. (1993). Vol. 286, p. 3-74.

Ashby J. and R. Tennant. Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP. Mutation Research. (1991). Vol. 257, 229-306.

Australian Therapeutic Goods Administration. Australian Regulatory Guidelines for OTC Medicines (ARGOM). (2003). Table of Approved Sunscreen Actives on page 10.

2. Pre-Clinical and Clinical Safety (Toxicology) Studies

Tinosorb® S (INCI name: Bis-Ethylhexyloxyphenol Methoxyphenol Triazine (BEMT)) is also referred to as CGF-C1607 and RM 60. Bemotrizinol is the USAN assigned to Tinosorb® S. Codes used in study reports are FAT 70'884 or FAT 70884.

Arcelin, G. (1997a). Acute dermal toxicity study with CGF-C-1607 in rats. RCC Project 651420. Sponsored by Ciba Chemikalien GmbH. (Tab. III.A.1)

Arcelin, G. (1997b). Acute oral toxicity study with CGF-C-1607 in rats. RCC Project 651407. Sponsored by Ciba Chemikalien GmbH. (Tab.III.A.2)

Arcelin, G. (1997c). Contact hypersensitivity to CGF-C-1607 in albino guinea pigs maximization-test. RCC Project 651453. Sponsored by Ciba Chemikalien GmbH. ((Tab.III.A.5)

Arcelin, G. (1997d). Determination of photoallergenicity with CGF-C-1607 in albino guinea pigs (including information about allergenicity, photoirritation, and irritation). RCC Project 651497. Sponsored by Ciba Chemikalien GmbH. (Tab.III.A.7)

Arcelin, G. (1997e). Determination of phototoxicity with CGF-C-1607 in albino guinea pigs. RCC Project 651475. Sponsored by Ciba Chemikalien GmbH. (Tab.III.A.6)

Ashby J, Tinwell H, Plautz J, Twomey K, Lefevre PA (2001). Lack of binding to isolated estrogen or androgen receptors, and inactivity in the immature rat uterotrophic assay, of the ultraviolet sunscreen filters TINOSORB® M-active and TINOSORB® S. Regul Toxicol Pharmacol. 34: 287-91. (Tab III.A.17)

Braun, W.H. (1997a). Primary skin irritation study with CGF-C-1607 in rabbits (4-hour semi-occlusive application). RCC Project 651431. Sponsored by Ciba Chemikalien GmbH. (Tab.III.A.3)

Braun, W.H. (1997b). Primary eye irritation study with CGF-C-1607 in rabbits. RCC Project 651442. Sponsored by Ciba Chemikalien GmbH. (Tab.III.A.4)

Becker, H. and Biedermann, K. (1998). Prenatal toxicity study with CGF-C-1607 in the rat. RCC Project 681491. Sponsored by Ciba Chemikalien GmbH. (Tab.III.A.14)

Czich, A. (1998a). *In vitro* chromosome aberration assay in Chinese hamster V79 cells with CGF-C-1607. RCC-CCR Project 597700. Project ID of contracting institute RCC 672017. Sponsored by Ciba Chemikalien GmbH. (Tab.III.A.19)

Czich, A. (1998b). Chromosome aberration assay *in vitro*: photomutagenicity in Chinese hamster V79 cells with CGF-C-1607. Project ID of contracting institute RCC 701853. (Tab.III.A.23)

Gaou, I. 2003a. Preliminary 2-Week toxicity study by cutaneous route in rats. CIT Study Number 25455 TSR. (Tab.III.A.9)

Gaou, I. 2003b. 13-Week toxicity study by cutaneous route in rats with FAT 70'884. CIT Study Number 25378 TCR. (Tab.III.A.11)

Haag, V. (2006). 39-week toxicity study by cutanoeus route in Göttingen minipigs with FAT 70'884. CIT Study number 25384 TCN. (Tab.III.A.27)

Haddouk, H. (2003). Bone marrow micronucleus test by intraperitoneal route in mice with test item FAT 70'884/B. CIT Study number 25608 MAS (Tab.III.A.20)

Hamann, H-J., Schmid, H., Luetkemeier, H., Biedermann, K., and Chevalier, H-J. (1998). 13-Week oral toxicity (gavage) study with CGF-C-1607 in the rat. RCC Project 667541. Sponsored by Ciba Chemikalien GmbH. (Tab.III.A.10)

Hoberman, A. and Foss, J. (2005). Oral (stomach tube) developmental toxicity study of Tinosorb S in rabbits. Charles River Discovery and Developmental Services, Argus Division. Study protocol number 203-014. (Tab.III.A.15)

Learn, D., Sambuco, C., and Forbes, D. (2003a). Topical evaluation of the protective potential of Tinosorb M, Tinosorb MBBT and Tinosorb S against ultraviolet radiation (uvr) induced cutaneous inflammation in hairless mice: a Sun Protection Factor (SPF)-like evaluation Charles River Discovery and Developmental Services, Argus Division. Study protocol number 203-018. (Tab.III.A.24)

Learn, D., Sambuco, C., and Forbes, D. (2003b). Thirteen-week topical range-finding study of Tinosorb M, Tinosorb MBBT and Tinosorb S in hairless mice, with or without simulated sunlight. Charles River Discovery and Developmental Services, Argus Division. Study protocol number 203-006. (Tab.III.A.25)

Learn, D., Sambuco, C., and Forbes, D. (2005). Twelve-month topical study to determine the influence of Tinosorb S on Photocarcinogenesis in hairless mice. Argus Research, Charles River Laboratories, Study number 203-008. (Tab.III.A.26)

Lefevre, P. A. (2001a). CGF-C2089 and CGF-C1607:Androgen competitive binding assay. Central Toxicology Laboratory Study No. 024530. (Tab.III.A.17)

Lefevre, P. A. (2001b). CGF-C2089 and CGF-C1607:Estrogen competitive binding assay. Central Toxicology Laboratory Study No. 024529. (Tab.III.A.17)

Nesslany, F. (2004). In vivo/*in vitro* Unscheduled DNA (UDS) synthesis assay in rat hepatocytes with FAT 70'884/C. Institute Pasteur de Lille; study number FSR-IPL 040012. (Tab.III.A.21)

Ota, T. (2002a). Study for effects of oral administration of RM-60 on fertility and early embryonic development to implantation in rats. Hashima Laboratory, Nihon Bioresearch, Inc., Hashima, Gifu, Japan; Study No. 100721. (Tab.III.A.13)

Ota, T. (2002b). Study on effects of oral administration of RM-60 on pre- and postnatal development, including maternal function, in rats. Hashima Laboratory, Nihon Bioresearch, Inc., Hashima, Gifu, Japan; Study No. 300721. (Tab.III.A.16)

Parisse, A.J. (1998a). Evaluation of human photoallergy by repeated insult patch test. HTR Project No. 100243B. Sponsor Number CGF-C-1607/7. Sponsored by Ciba Specialty Chemicals Corporation. (Tab.IV.A.1)

Parisse, A.J. (1998b). Evaluation of phototoxicity in humans. HTR Project No. 100243A. Sponsor Number CGF-C-1607/7. Sponsored by Ciba Specialty Chemicals Corporation. (Tab.IV.A.1)

Schmid, H., Luetkemeier, H., and Wilson, J. (1998) 14-Day dose range-finding (gavage) study with CGF-C-1607 in the rat. RCC Project 667530. Sponsored by Ciba Chemikalien GmbH. (Tab.III.A.8)

Silcock, R. 2002a. CGF-C1607: Metabolic fate following oral administration to rats. Central Toxicology Laboratory Study No. UR0698. (Tab.III.A.28_29)

Silcock, R. 2002b. CGF-C1607: Metabolic fate following *in vivo* dermal administration to rats. Central Toxicology Laboratory Study No. UR0722. (Tab.III.A.28_29)

Twomey, K. (2002). CGF-C-1607: Uterotrophic Assay in immature rats. Central Toxicology Laboratory Study No. ZR1601. (Tab.III.A.17)

Watkinson, A.C. (1998). *In vitro* human skin penetration and distribution of the UV absorber CGF-C1607. An-eX Analytical Services, Ltd. Report Number CSC/8/98. Sponsored by Ciba Spezialitatenchemie Grenzach GmbH. (Tab.IV.A.1)

Wollny, H. (1997). *Salmonella* typhimurium reverse mutation assay with CGF-C-1607. CCR Project 582800. Project ID of the contracting institute- RCC 651508. Sponsored by Ciba Chemikalien GmbH. (Tab.III.A.18)

Wollny, H. (1998a). *Escherichia coli* reverse mutation assay with CGF-C1607. CCR Project 618300; RCC project 704428. (Tab.III.A.18)

Wollny, H.E. (1998b). Photomutagenicity in a *Salmonella* typhimurium and *Escheri*chia coli reverse mutation assay with CGF-C-1607. RCC-CCR Project 609003. Project ID of the contracting institute RCC 689354. Sponsored by Ciba Chemikalien GmbH. (Tab.III.A.22)

3. References Efficacy Data for Bemotrizinol

Chatelain E, Gabard B., Photostabilization of Butyl methoxydibenzoylmethane (Avobenzone) and Ethylhexyl methoxycinnamate by Bis-ethylhexyloxyphenol methoxyphenyl triazine (Tinosorb S), a New UV Broadband Filter, Photochem.Photobiol. (2001), 74(3), 401 - 406

Ciba Sunscreen Simulator Access: www.cibasc.com/tinosorb

Hauri U., Lütolf B., Schlegel U., Hohl C., "Determination of photostability of UV filters in sunscreens by HPLC/DAD and HPLC/MS", Mitt. Lebensm. Hyg. 95, 147 – 161 (2004)

Hauri, Urs; Luetolf, Beat; Hohl, Christopher, Determination of organic sunscreen filters in cosmetics with HPLC/DAD, Mitteilungen aus Lebensmitteluntersuchung und Hygiene (2003), 94(1), 80-92

Herzog B., Sommer K., "Investigations on Photostability of UV-Absorbers for Cosmetic Sunscreens", Proceedings of the XXI. IFSCC International Congress, Berlin 2000

Herzog B.; Mongiat S.; Deshayes C.; Neuhaus M.; Sommer K.; Mantler A.,*In vivo* and *in vitro* assessment of UVA protection by sunscreen formulations containing either butyl methoxy dibenzoyl methane, methylene bis-benzotriazolyl tetramethylbutylphenol, or microfine ZnO, Journal of Cosmetic Science (2002), 24(3), 170-185

Herzog B, "Prediction of sun protection factors by calculation of transmissions with a calibrated step film model", Journal of Cosmetic Science (2002), 53(1), 11-26,

Herzog B., Mongiat S., Deshayes C., Neuhaus M., Quass K., Mantler A., Comte C., *In vivo* and *in vitro* assessment of UVA protection by sunscreen formulations containing either ZnO, butyl methoxy dibenzoyl methane, methylene bis-benzotriazolyl tetramethylbutylphenol, or bis-ethylhexyloxyphenol methoxyphenyl triazine, Proc. 22nd IFSCC Congress, 2002, Edinburgh

Herzog B., Mongiat S., Quass K., Deshayes C., "Prediction of Sun Protection Factors and UVA Parameters by Using a Calibrated Step Film Model", J. Pharm. Sc. 93, 1780 – 1795 (2004)

Herzog B., Hueglin D., Borsos E., Stehlin A., Luther H., "New UV Absorbers for Cosmetic Sunscreens – A Breakthrough for the Photoprotection of Human Skin", BChimia 58, 554 – 559 (2004)

Herzog B., Hueglin D, Osterwalder U, "New Sunscreen Actives", in: "Sunscreens – Regulation and Commercial Development", ed. Nadim Shaath, 3rd ed., Taylor & Francis, Boca Raton 2005, p. 291- 320

Hueglin D. Herzog B, Mongiat S., "Hydroxphenyltriazines: A new generation of cosmetic UV filters with superior photoprotection", D., Proc. 22nd IFSCC Congress, 2002, Edinburgh

Monfrecola G.; Fabbrocini G.; Prizio E.; Russo I. [Evaluation of clinical efficacy of Minesol® SPF 50(+) spray]. VALUTAZIONE DELL'EFFICACIA CLINICA DI MINESOL® SPF 50(+) SPRAY, Dermatologia Clinica, (2005) 25/2 (69-74)

Osterwalder U, Mueller S, Gril A, Herzog B, Understanding Sunscreens – New insight into the role of photo-stability through *in vivo*, *in vitro* and *in silico* experiments, 64th AAD Meeting, San Francisco, 3-7 March 2006, Poster P415

Osterwalder U., and Herzog B., How to Meet Emerging Standards in UVA Protection, 20° CONGRESSO BRASILEIRO DE COSMETOLOGIA, Sao Paulo, 18 a 20 de abril de 2006

Rudolph T, UV Filter Systems: Trends and Pesrpectives (UV Filtersysteme: Trends und Perspektiven), 15th Symposium DGK, Deutsche Gesellschaft für Wissenschaftliche und angewandte Kosmetik e.V., Kosmetischer Lichtschutz: Dermatologische Aspekte – Wirkstoffe –Formulierungen – Pruefmethoden, 12-14 Maerz 2003, Maritim Hotel, Koeln/Cologne, Germany, Proceedings, Paper 11, 80-87 (German)

C. Appendix 3: How to Meet Emerging Standards in UVA Protection

20º CONGRESSO BRASILEIRO DE COSMETOLOGIA
18 a 20 de abril de 2006

HOW TO MEET EMERGING STANDARDS IN UVA PROTECTION

Uli Osterwalder and Bernd Herzog, Ciba Specialty Chemicals, Basel, Switzerland

Summary
The need for UVB and UVA protection during work and leisure is generally recognized. Over the last 5 years new broad-spectrum UV filters such as Methylene Bis-Benzotriazolyl Tetramethylbutylphenol (MBBT, Tinosorb M) or Ethylhexyloxyphenol Methoxyphenyltriazine (BEMT, Tinosorb S) and UVA Filters such as Disodium Phenyl Dibenzimidazole Tetrasulfonate (DPDT, Neoheliopan AP) or Diethylamino Hydroxybenzoyl Hexyl Benzoate (DHHB, Uvinul A Plus) became available. This allows the formulators to create sunscreens with far superior broad-spectrum protection than ever. The open question in most countries still is how to communicate the degree of UVA protection to the consumer. Besides established UVA methods and standards such as Persistent Pigment Darkening (Japan), Australian UVA standard, UVA/UVB ratio (Great Britain) and UVA balance (Germany), new methods, e.g. an improved UVA balance that takes into account photostability, are emerging. This latter method is the only in vitro method that allows the necessary differentiation between high and low protection in the UVA range over the full period of time a sunscreen is expected to be effective. We present how long-lasting UVA protection can be achieved with the new UV filters, but also with the help of photo-stabilizing the classic UVA Filter ButylMethoxyDiBenzylMethane (BMBM, Parsol 1789).