

ATTACHMENT 2

TINOSORB[®] S

Bis-Ethylhexyloxyphenol Methoxyphenol Triazine (BEMT)

Next Generation UV Filter for Sun Protection

**Information Package
Submitted with Citizen's Petition**

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Submitted by

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1. INTRODUCTION

1.1 BACKGROUND

This document summarizes the physical/chemical and toxicological data for a new sunscreen additive produced by Ciba Specialty Chemicals Corporation ("Ciba"), Consumer Care Division. Ciba's Consumer Care Division (formally Ciba-Geigy) is one of the world's leading developers and producers of technical UV-absorbers and is well known for its competence in UV-Protection.

The brand name for the product is Tinosorb™ S. Bis-Ethylhexyloxyphenol Methoxyphenol Triazine ("BEMT") is the official International Nomenclature Cosmetic Ingredient (INCI) name for a substance chemically known as 2,4-Bis-[[4-(2-ethyl-hexyloxy)-2-hydroxy]-phenyl]-6-(4-methoxyphenyl)-(1,3,5)-triazine (CASRN: 187393-00-6). The material is currently under patent and Ciba is the sole manufacturer. Tinosorb S is the first "true" broadband-type UV-absorber on the market. It provides overall protection by fully covering the UVA spectrum in contrast to Oxybenzone, which only covers part of the spectrum. Also, Tinosorb S exhibits extremely high photostability in contrast to Avobenzone, which is photolabile. Tinosorb S is extremely easy to formulate in oil or water emulsions. The critical wavelength of *Tinosorb S* (λ_c) = 370 nm. The UVA/UVB-ratio of Tinosorb S is 0.73. Test data indicate that Tinosorb S has a higher efficacy than other UV-absorbers on the market.

1.2 SUMMARY OF EXISTING SAFETY DATA

Tinosorb S exhibits low toxicity by dermal and oral routes of exposure. Acute rat dermal and oral LD₅₀ values were >2,000 mg/kg. Tinosorb S caused zero to minimal irritation when applied to rabbit eyes and skin. Tinosorb S did not cause sensitization, photoirritation, or photosensitization when applied to the skin of guinea pigs or humans. Tinosorb S is not genotoxic in several different assays with and without UV activation. In an *in vitro* assay, Tinosorb S exhibited low penetration (<0.1%) across human skin. In a 90-day subchronic oral gavage study in the rat, the No-Observable-Effect-Level (NOEL) was 1,000 mg/kg/day, which was the highest dose tested. In a developmental toxicity study, the maternal and fetal NOELs were 1,000 mg/kg, which were the highest doses tested. Detailed summaries of each study can be found in Section 3.

1.3 RISK ASSESSMENT

As summarized in the previous section, Tinosorb S exhibited very low toxicity by the dermal and oral routes of exposure. In addition, Tinosorb S did not exhibit enhanced toxicity upon exposure to UV radiation, which is critical for a UV-protectant. Tinosorb S did not exhibit compound or dose-related toxicity in subchronic and developmental toxicity studies. Based on this information, Tinosorb S is safe for use as a human skin UV-protectant since it is unlikely to cause any toxic effects after dermal exposure. A safety factor of >40,000 exists between NOELs in animal toxicity studies and estimated human exposures.

As with any compound that is applied repeatedly to the skin, the potential for inducing cancer should be assessed. Tinosorb S is considered very unlikely to induce cancer and/or enhance UV-induced cancer for several reasons. First, Tinosorb S was not genotoxic in two different assays with and without UV activation. Second, Tinosorb S is very photostable (see Section 2.6) indicating that Tinosorb S is unlikely to degrade into compounds that pose an unknown hazard. Third, Tinosorb S was not phototoxic or photoallergenic when applied to human or guinea pig skin. Fourth, Tinosorb S diminished the effects of UV irradiation on human skin compared to controls in a human phototoxicity study (see Section 3.6.1) and a human photoallergenicity study (see Section 3.6.2). Taken together, these data clearly indicate that Tinosorb S is unlikely to either induce cancer by itself or enhance UV-induced cancer.

In conclusion, the safety data clearly indicate that Tinosorb S is unlikely to pose a health hazard when applied to human skin in sunscreen formulations.

2. PHYSICAL-CHEMICAL DATA

2.1 CHEMICAL NAME

2,4-Bis-([4-(2-ethyl-hexyloxy)-2-hydroxy]-phenyl)-6-(4-methoxyphenyl)-(1,3,5)-triazin

2.2 MOLECULAR FORMULA

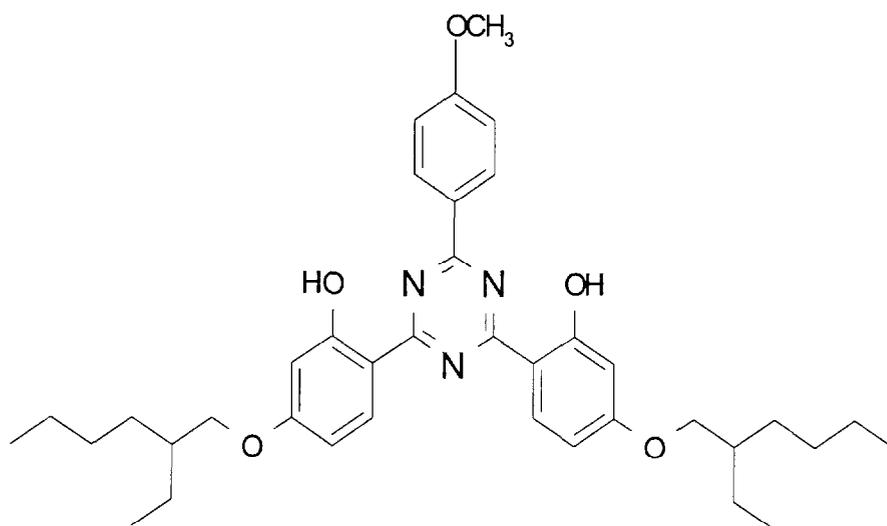
C₃₈H₄₉N₃O₅

2.3 MOLECULAR MASS

627.80 g/mol

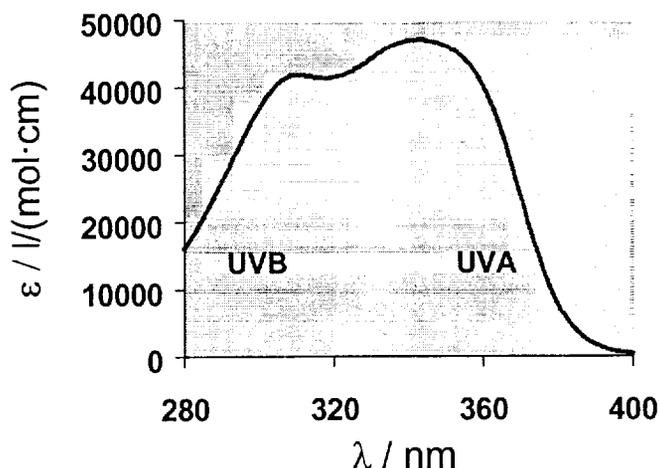
2.4 STRUCTURE

Figure 1. Structure of Tinosorb S



2.5 UV-SPECTRUM (IN ETHANOL)

Figure 2. UV-Spectrum of Tinosorb S (in ethanol)



$$\begin{aligned} \epsilon_{343 \text{ nm}} &= 46750 \text{ M}^{-1}\text{cm}^{-1} \\ E_{343 \text{ nm}} (1\%, 1\text{cm}) &= 745 \end{aligned}$$

2.6 PHOTOSTABILITY

The photostability of Tinosorb S was measured in terms of recovery of the substance after application of different doses of UV-light. The testing was performed using two independent methods, each employing defined irradiation and adapted analysis procedures (Method A was similar to the procedure suggested by Berset, G. et al., *Int. J. Cosmet. Sci.* **18** (1996) 167 - 177; and Method B was based on the irradiation of a highly diluted UV-filter). Doses of UV-light were varied between 0 and 50 MED (minimal erythemal doses) and the samples were analyzed afterwards using UV-spectroscopy and high performance liquid chromatography (HPLC), respectively.

The table below summarizes the recoveries of Tinosorb S as obtained from UV-spectroscopic analysis. As seen in Table 1, even after a UV-dose of 50 MED, recoveries of >98% were detected using the different methods, indicating that Tinosorb S is an extremely photostable UV-filter.

Table 1. Recoveries of Tinosorb S as obtained from UV-spectroscopic analysis including 95% confidence intervals

Dose (MED)	Mean Recovery ± 95% CI (%)
0	100.0 ± 0.2
5	99.8 ± 0.2
10	99.7 ± 0.2
20	99.4 ± 0.2
50	98.4 ± 0.2

Notes: MED = minimal erythemal dose; CI = confidence interval

2.7 SPF

4% *Tinosorb S* in o/w- emulsion, measured *in vitro* by the method of Diffey and Robson (*J. Soc. Cosmet. Chem.*, 40, 1989, 127 - 133) using an Optometrix SPF290-analyzer: 9.4

2.8 UVA/UVB-RATIO

The UVA/UVB-ratio is the ratio of the areas under the extinction curve in the UVA-range (320 - 400 nm) and the UVB-range (290 - 320 nm), each area divided by the range of wavelengths involved.

UVA/UVB-ratio of *Tinosorb S* = 0.73

In the case of a UVA-filter with very weak UVB-absorption the UVA/UVB-ratio may be of a value higher than 1. The lower ratio of *Tinosorb S* is due to its rather strong absorption in the UVB-range.

2.9 CRITICAL WAVELENGTH

The critical wavelength (λ_c) is the wavelength up to which from 290 nm on, the area under the extinction curve is 90% of the area of the extinction curve between 290 and 400 nm [5].

Critical wavelength of *Tinosorb S* (λ_c) = 370 nm

Like the UVA/UVB-ratio, the critical wavelength depends not only on UVA- but also on UVB-absorption. UVA-filters with very weak UVB-absorption approach a λ_c of 380 nm. Again, the somewhat lower value of *Tinosorb S* is caused by its relatively strong UVB-absorption.

2.10 SOLUBILITY

in Miglyol 812:	14 %
in Finsolve TN:	25 %
in Sesame oil:	10 %
in H ₂ O:	<10 ⁻⁷ g/l

2.11 PARTICLE SIZE DISTRIBUTION

Determination of Particle Size Distribution (based on OECD Guideline for testing of chemicals, No. 110, "Particle Size Distribution/Fiber Length and Diameter," adopted May 12, 1981).

About 0.17 wt% of the particles in *Tinosorb S* showed a particle size lower than 10µm. About 50 wt% were determined by sieve analysis to be smaller than 107µm (median mass diameter).

2.12 BOILING POINT

664°C (using Meissner's method)

2.13 MELTING POINT

80.4 °C ± 0.1 ° C

2.14 OCTANOL/WATER PARTITION COEFFICIENT

Log P_{ow} > 5.7

2.15 WATER SOLUBILITY

<10⁻⁷ g/l

2.16 VAPOR PRESSURE

5.9 × 10⁻²⁰ Pa at 25° C (based on the boiling point calculated and using the Modified Watson Correlation)

2.17 EXPLOSIVE PROPERTIES

The substance is not considered to be explosive, thermally, shock or friction sensitive.

2.18 FLAMMIBILITY

Not Flammable or auto-flammable

2.19 FLASHPOINT

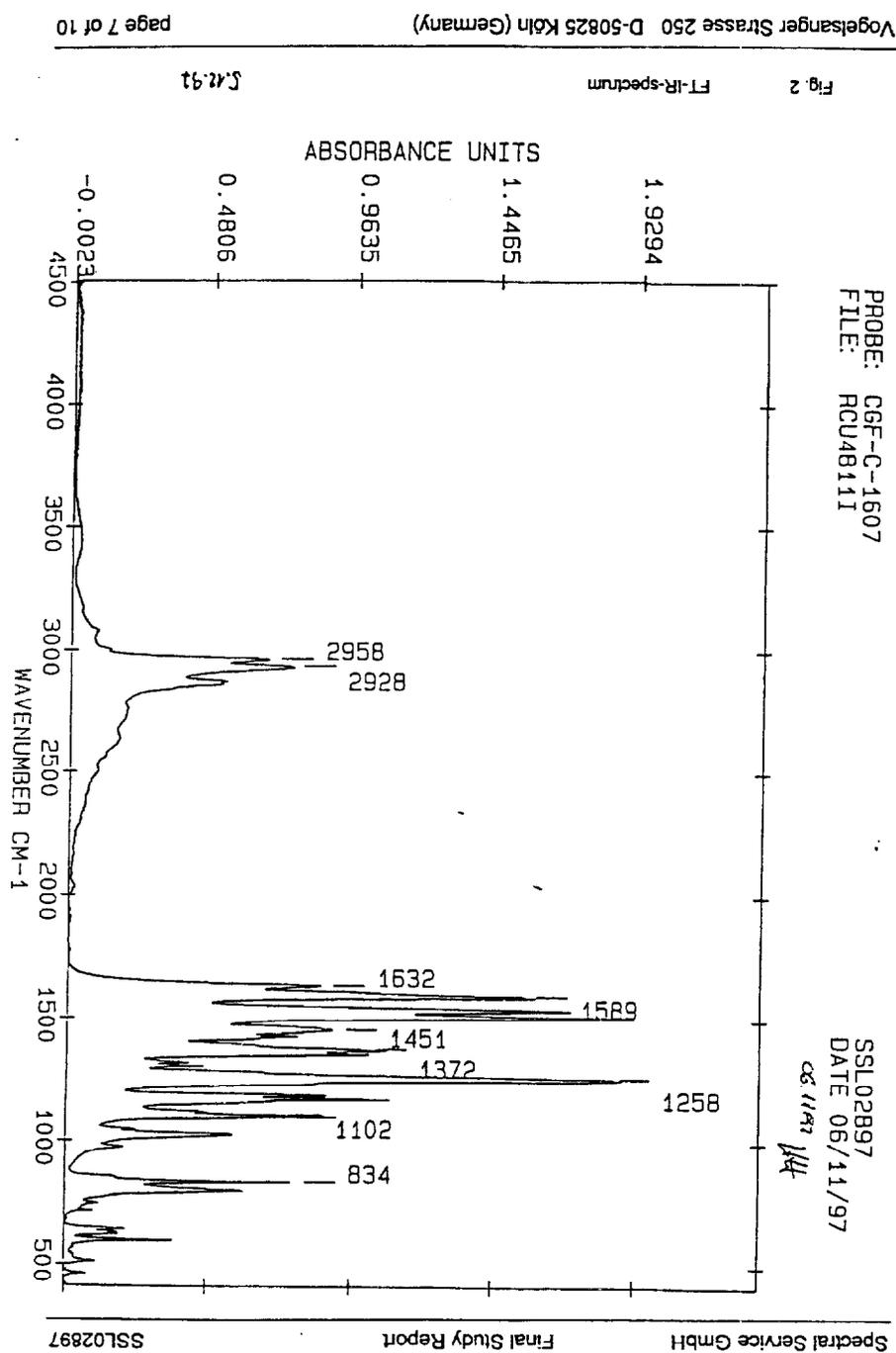
284 °C at 101.3 kPa

2.20 RELATIVE DENSITY

1.17 g/cm³ at 20.4 ° C ± 0.2 ° C

2.21 FT-IR ABSORPTION SPECTRA

Figure 3. FT-IR Spectra for Tinosorb S



3. SUMMARY OF PRE-CLINICAL AND CLINICAL STUDIES

Below is a summary of the various pre-clinical and clinical studies conducted on Tinosorb S (Table 2). Detailed study summaries follow the table.

Table 2. Summary of Tinosorb S Pre-Clinical and Clinical Studies

Study	Results
Acute	
Acute Dermal Toxicity in Rats	LD ₅₀ > 2000 mg/kg
Acute Oral Toxicity in Rats	LD ₅₀ > 2000 mg/kg
Irritation/Sensitization	
Primary Skin Irritation Study in Rabbits	Not Irritating
Primary Eye Irritation Study in Rabbits	Minimally Irritating
Skin Sensitization (Guinea Pig Maximization Test)	Not Sensitizing
Phototoxicity in Guinea Pigs	Not Phototoxic
Photoallergenicity in Guinea Pigs	Not Photoallergenic
Sub-Chronic	
14-Day Oral Gavage Range Finding Study	NOEL = 2000 mg/kg
90-Day Oral Gavage Toxicity Study in the Rat	NOEL = 1000 mg/kg
Range Finding Developmental Study in Rats	NOEL = 1000 mg/kg
Developmental Toxicity Study in Rats	NOEL = 1000 mg/kg
Genotoxicity	
S. typhimurium Reverse Mutation Assay	Negative
In Vitro Chromosome Aberration Assay in Chinese Hamster V79 Cells	Negative
Photomutagenicity: S. typhimurium and E. coli Reverse Mutation Assay	Negative
Photomutagenicity: In Vitro Chromosome Aberration Assay In Chinese Hamster V79 Cells	Negative
Absorption	
In Vitro Human Skin Penetration	<0.1% Penetrated Across Skin
In Vitro Human Skin Penetration and Distribution	<0.1% Penetrated Across Skin
Clinical	
Phototoxicity in Humans	Not Phototoxic
Photoallergenicity in Humans	Not Photoallergenic

3.1 ACUTE STUDIES

3.1.1 Acute Dermal Toxicity in Rats

Tinosorb S was applied to the shaved skin of five male and five female HanIbm:WIST (SPF) rats at a dose of 2000 mg/kg and covered with a semi-occlusive dressing. Tinosorb S was suspended in vehicle (PEG 400) at a concentration of 0.5 g/ml and administered at a volume of 4 ml/kg. 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 observation period of 15 days. The body weight 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 LD₅₀ was > 2000 mg/kg (Arcelin, 1997a).

3.1.2 Acute Oral Toxicity in Rats

Tinosorb S was administered to five male and five female Hanlbm:WIST (SPF) rats at a dose of 2000 mg/kg by oral gavage. Tinosorb S was suspended in vehicle (PEG 400) at a concentration of 0.2 g/ml and administered at a volume of 10 ml/kg. The animals were observed for a period of 15 days. No deaths occurred and no clinical signs of toxicity were observed during the study. The body weight 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 LD₅₀ was > 2000 mg/kg (Arcelin, 1997b).

3.2 IRRITATION/SENSITIZATION STUDIES

3.2.1 Primary Skin Irritation in Rabbits

Tinosorb S was applied to the shaved skin of three young adult New Zealand rabbits for four hours using a semi-occlusive exposure. Five hundred milligrams of Tinosorb S was applied to 6 cm² intact dorsal skin. 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 (PIS) was calculated by adding the mean erythema to the mean edema 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 PIS, Tinosorb S was considered non-irritating to skin (Braun, 1997b).

3.2.2 Primary Eye Irritation in Rabbits

Tinosorb S (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). No staining of the cornea, sclera, or conjunctivae of the treated eyes by the test article was observed. Based on the PIS, Tinosorb S was considered minimally irritating to the eye (Braun, 1997a).

3.2.3 Skin Sensitization (Guinea Pig Maximization Test)

Tinosorb S was administered to 10 male Albino guinea pigs using a skin 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% Tinosorb S in PEG 400, and 3) 3% Tinosorb S in an emulsion with a 1:1 (v/v) mixture of FCA and physiological saline. Control animals also received the same three injections without Tinosorb S. 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, approximately 0.3 g of a mixture of 30% Tinosorb S in PEG 400 was topically applied over the injection sites using an occlusive exposure for 48 hours. Control animals were treated with PEG 400 only. Challenge occurred two weeks after the topical induction. Two hundred milligrams of 30% percent Tinosorb S 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 μ l 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. During the induction phase, slight erythema was noted in several animals of both the treated and control groups after the topical induction exposure. During the challenge phase, no erythema or edema was noted in any animal after the challenge exposure. In conclusion, under the test conditions, Tinosorb S was not a skin sensitizer (Arcelin, 1997c).

3.2.4 Phototoxicity in Guinea Pigs

Tinosorb S was tested in a phototoxicity study according to the Cosmetic, Toiletry, and Fragrance Association (CTFA) Safety Testing Guidelines. Tinosorb S 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. No skin reactions, including erythema and edema, were observed during the experiment. In conclusion, under the test conditions, Tinosorb S was not phototoxic (Arcelin, 1997e).

3.2.5 Photoallergenicity in Guinea Pigs

Tinosorb S 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% Tinosorb S 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, Tinosorb S in PEG 400 was applied to four separate 2 cm^2 sites on the shaved skin of the left flank at the following concentrations: 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. During the topical induction phase, erythema and edema were observed from test day 9 to 15 in relationship with the repeated application of 30% Tinosorb S. During the challenge phase, no effects on the skin, including erythema and edema, were noted. In conclusion, under the test conditions, Tinosorb S was not a photosensitizer (Arcelin, 1997d).

3.3 SUBCHRONIC STUDIES

3.3.1 14-Day Oral Gavage Range-Finding Study in Rats

Tinosorb S in PEG 400 (vehicle) was administered to groups of Wistar rats (SPF) (5 animals/sex/group) by oral gavage at daily doses of 50, 200, 800, and 2000 mg/kg for 14 days. 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 (Schmid et al., 1998).

3.3.2 90-Day Oral Gavage Toxicity Study in Rats

Tinosorb S in PEG 400 (vehicle) was administered to groups of Wistar rats (SPF) (20 animals/sex/group) by oral gavage at daily doses of 100, 500, and 1000 mg/kg for at least 92 days. 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. Any significant differences in the various parameters were not considered treatment-related since they were not correlated with any morphological changes and they were within the range of normal biological variability for the strain and age of rat used and/or did not exhibit a dose-response. In conclusion, under the test conditions, the No-Observable-Effect-Level (NOEL) for this study was 1000 mg/kg (Harmann et al., 1998).

3.3.3 Range Finding Developmental Toxicity Study in Rats

Tinosorb S in PEG 400 (vehicle) was administered by oral gavage to groups of pregnant female Wistar rats (5 animals/group) from days 6 – 17 of gestation at 100, 300, and 1000 mg/kg. Controls received vehicle only. Animals were sacrificed on day 21 of gestation and the fetuses removed by Caesarian section. No treatment-related effects on clinical appearance, survival, food consumption, body weight gain, or macroscopic findings were noted in any dam. No treatment-related reproductive effects (mean numbers of corpora lutea and implantation sites, and percent of pre- and post-implantation loss) were noted. No treatment-related fetal effects (external abnormalities, sex ratios, and body weights) were noted, with the exception of an incidental increase in fetal body weights (on an individual basis) at 1000 mg/kg. In conclusion, under the test conditions, the maternal and fetal NOELs were 1000 mg/kg (Becker, 1998).

3.3.4 Developmental Toxicity Study in Rats

Tinosorb S in PEG 400 (vehicle) was administered by oral gavage to groups of pregnant female Wistar rats (22 animals/group) from days 6 – 17 of gestation at 100, 300, and 1000 mg/kg. Controls received vehicle only. Animals were sacrificed on day 21 of gestation and the fetuses removed by Caesarian section. No treatment-related effects on survival, food consumption, body

weight gain, or macroscopic findings were noted in any dam. No treatment-related clinical signs were noted with the exception of soft feces. However, the soft feces was 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 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 treatment-related since a dose-relationship was not evident and the parameters were within the ranges of historical control data. No treatment-related effects were noted for other reproductive parameters (mean numbers of corpora lutea and implantation sites, and percent of pre-implantation loss). No treatment-related fetal effects (external, visceral, and skeletal abnormalities; sex ratios; and body weights) were noted. In conclusion, under the test conditions, the maternal and fetal NOELs were 1000 mg/kg (Becker and Beidemann, 1998).

3.4 GENOTOXICITY STUDIES

3.4.1 *S. typhimurium* Reverse Mutation Assay

Tinosorb S 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, TA 1535, and TA 1537. 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, dissolved in dimethyl sulfoxide, 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. 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. In both experiments, no significant increase in revertant colony numbers of any of the four tester strains was observed following treatment with Tinosorb S at any dose level, with or without S9 mix. Appropriate reference mutagens were used as positive controls and produced a distinct increase of induced revertant colonies. In conclusion, under the test conditions, Tinosorb S did not induce base pair or frame shift mutations (Wollny, 1997).

3.4.2 *In Vitro* Chromosome Aberration Assay in Chinese Hamster V79 Cells

Tinosorb S was assessed for its potential to induce structural chromosome aberrations in Chinese hamster V79 cells. Two independent experiments were performed with and without exogenous rat liver microsomal (S9 mix) activation. A stock solution was prepared by dissolving Tinosorb S 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 Tinosorb S 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, Tinosorb S did not induce structural chromosome aberrations (Czich, 1998b).

3.4.3 Photomutagenicity: *S. typhimurium* and *E. coli* Reverse Mutation Assay

Tinosorb S was tested in the Ames assay (*Salmonella typhimurium* and *Escherichia coli* reverse mutation assays) to determine if it induces base pair mutations in *S. typhimurium* strain TA 102 and *E. coli* strain WP2 after UV irradiation. 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. WP2 cells were exposed for 10 seconds to 20 mJ/cm² UVA and 1 mJ/cm² UVB irradiation. TA 102 cells were exposed for 40 seconds to 80 mJ/cm² UVA and 4 mJ/cm² 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 Tinosorb S 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, Tinosorb S did not induce base pair mutations after exposure to UVA/UVB irradiation (Wollny, 1998).

3.4.4 Photomutagenicity: *In Vitro* Chrom Ab Assay in Chinese Hamster V79 Cells

Tinosorb S 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. Based on the limited solubility of the test material in the phosphate buffered saline (PBS) solution (containing 1% (v/v) acetone with the test material), the following Tinosorb S concentrations were tested: 6.25, 12.5, 25.0, 50.0, 75.0, 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/cm² UVA and 22 mJ/cm² UVB for 30 minutes. Additional groups in experiment II were exposed to 300 mJ/cm² UVA and 33 mJ/cm² 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. Positive control treatments produced a distinct increase in cells with structural chromosome aberrations in both experiments. In conclusion, under the test conditions, Tinosorb S did not induce structural chromosome aberrations in the presence or absence of UVA/UVB irradiation (Czich, 1997).

3.5 ABSORPTION STUDIES

3.5.1 *In Vitro* Human Skin Penetration

This study was designed to determine the *in vitro* skin penetration and distribution of Tinosorb S (10% w/w in a representative sunscreen formulation) over a 24 hour period after application to epidermal sections of human skin. The sunscreen formulation containing 10% Tinosorb S was applied to human epidermal skin membranes mounted in Franz type diffusion cells at a target dose of 2 mg/cm². The receptor phase consisted of 6% Oleth 20 in phosphate buffered saline (pH 7.4). Of the twelve skin samples treated with Tinosorb S, four showed some permeation of Tinosorb S through the skin and into the receptor phase; however, one of the samples was excluded from further analysis on the basis of anomalously early and high permeation. Overall permeation through the skin was very low (15±8 ng/cm² representing 0.006±0.003% of the applied dose after 24 hours) (Watkinson, 1998).

3.5.2 *In Vitro* Human Skin Penetration and Distribution

This study was designed to determine the *in vitro* skin penetration and distribution of Tinosorb S (10% w/w in a representative sunscreen formulation) over a 24 hour period after application to epidermal sections of human skin. The sunscreen formulation containing 10% Tinosorb S was applied to human epidermal skin membranes mounted in Franz type diffusion cells at a target dose of 2 mg/cm². The receptor phase consisted of 6% Oleth 20 in phosphate buffered saline (pH 7.4). Of the twelve skin samples treated with Tinosorb S, six showed some permeation of Tinosorb S through the skin and into the receptor phase; however, one of the samples was excluded from further analysis on the basis of anomalously early and high permeation. Overall permeation through the skin was very low (40±20 ng/cm² representing 0.02±0.01% of the applied dose after 12 hours and an extrapolated level of 80 ng/cm² representing 0.04±0.01% of the applied dose after 24 hours). Linear extrapolation to 24 hours was necessary since the permeation profile plateaued between 12 and 24 hours. This plateauing effect was due to the large errors associated with the very low levels of permeation that occurred. An assessment of Tinosorb S levels on and in the skin after 24 hours revealed that the majority of the recovered material (>80 % of the applied dose) was found either on the skin surface or in the first three tape strips. The remaining material was recovered in tape strips 4-20 (10.2% of the applied dose), the remaining sample of skin (7.3%), or the receptor phase (<0.1%). In conclusion, under the test conditions, less than 0.1% of the applied Tinosorb S penetrated through epidermal sections of human skin over a 24 hour period (Watkinson, 1998).

3.6 CLINICAL STUDIES

3.6.1 Phototoxicity In Humans

Tinosorb S, formulated as a 10% O/W Lotion, was topically applied to 26 human volunteers. 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/cm² UVA irradiation followed by exposure to 0.75 times the volunteer's minimum erythema 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, 9, 8, 4, and 4 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 (Parisse, 1998b).

3.6.2 Photoallergenicity in Humans

Tinosorb S was tested for photoallergenicity using a human repeated insult patch test (HRIPT). The induction phase consisted of two topical applications per week over a three week period (total of six topical applications over weeks 1-3) of 200 µl of the test material (10% Tinosorb S 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 erythema 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, and saline were made to naïve 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/cm² 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 nonirradiated sites were lower than the irradiated sites for all three treatments. 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 a photosensitizer or sensitizer to human skin (Parrise, 1998a).

4 GLOBAL REGULATORY/REGISTRATION STATUS

4.1 EUROPE

Tinosorb S (as (1,3,5)-Triazine-2,4-bis((4-(2-ethyl-hexyloxy)-2-hydroxy)-phenyl)-6-(4-methoxyphenyl)) was reviewed by the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP) of the European Commission. The SCCNFP concluded Tinosorb S is safe for use without restrictions as a UV absorber in cosmetic products, including sunscreen products, at a concentration of up to 10%. The UV filter is now included for cosmetic under the Twenty-Fourth Commission Directive 2000/6/EC of the Commission of the European Communities on March 1, 2000. A copy of this Directive is enclosed as Attachment 1.

Tinosorb S is also approved for use in Switzerland.

4.2 AUSTRALIAN STANDARD

In Australia, UVA-protection may be claimed when the transmission of the sunscreen, measured at an optical pathlength of 8 μm , is below 10% in the wavelength range between 320 and 360 nm. This an absolute criterion whereas the UVA/UVB-ratio as well as the λ_c -concept are both measured in relation to UVB.

A minimum of 2% of Tinosorb S is necessary to fulfill this requirement.

4.3 OTHER

Tinosorb s is also approved for use in Brazil.

APPENDIX 1

Example of a Formulations with Tinosorb S

O/W-Sunscreen-Lotion (Adaption to a Formulation of Th.Goldschmidt)

	Components	Chemistry	%
A	Tego® Care 450	Polyglyceryl-3 Methylglucose Distearate	2.0
	Tegosoft® DO	Decyl Oleate	5.7
	Tegosoft® P	Isopropyl Palmitate	5.0
	Tegosoft® CT	Caprylic/Capric Triglyceride	6.5
	Tinosorb S		2.0
	Tinosorb OMC	Octyl Methoxycinnamate	5.0
B	Glycerin		3.0
	Phenonip		0.5
	Wasser		62.9
C	Tego® Carbomer 141	Carbomer	0.2
	Tegosoft® P	Isopropyl Palmitate	0.8
D	Tinosorb M®(d.h.4% AS)		8.0
E	NaOH (10%ig)		as required

Formulation procedure :

- Phases A and B are heated separately to 80° C and put together without stirring.
- Addition of C follows intense homogenization.
- Under slight stirring room temperature is to be approached.
- Tinosorb M (D), which has been adjusted to pH 5.5 with citric acid, is added portion by portion under slight stirring. Stirring should be continued for further 15 minutes for the sake of good mixing.
- Eventually, the final pH is adjusted using E.

Just after finishing the formulation the SPF in vitro (SPF290-Analyser, Optometrix) shows a value of about 23 which increases after a storage time of four weeks to values between 40 and 54.

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TWENTY-FOURTH COMMISSION DIRECTIVE 2000/6/EC

of 29 February 2000

adapting to technical progress Annexes II, III, VI and VII to Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products

(Text with EEA relevance)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products ⁽¹⁾, as last amended by Commission Directive 98/62/EC ⁽²⁾, and in particular Article 8(2) thereof,

After consulting the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers,

Whereas:

- (1) Tallow derivatives, such as fatty acids, glycerine, esters of fatty acids and soaps and fatty alcohols, fatty amines and fatty amides derived therefrom, are considered safe for use in the manufacture of cosmetic products with regard to the risk of contracting transmissible spongiform encephalopathies if they are prepared in strict accordance with specific physico-chemical processes in which temperature is the decisive parameter on which the corresponding pressure conditions depend. Annex II to the abovementioned Directive should therefore be amended accordingly.
- (2) Harmful secondary effects have been shown to arise following prolonged use of hydroquinone as skin-lightening cream. This particular use of hydroquinone must not therefore be authorised, meaning that Part I of Annex III to the abovementioned Directive needs to be amended. Studies also show that the concentration of hydroquinone used in hair dyes does not have harmful effects for health if it does not exceed 0,3 %. Part I of Annex III to the abovementioned Directive must be amended accordingly.
- (3) On the basis of new scientific data, benzalkonium chloride, bromide and saccharinate have recently been added to the list of substances which may be used as preservatives in the manufacture of cosmetic products set out in Part 1 of Annex VI to the abovementioned Directive. In the light of experience, it is also acceptable for these benzalkonium salts to be used for other purposes in cosmetic products, according to the length of their carbon chain, provided that the maximum authorised concentrations are observed. These specific characteristics therefore justify their inclusion in the list Part 1 of Annex III.
- (4) The cosmetics industry has supplied new scientific data based on studies of the percutaneous absorption of aqueous solutions of boric acid, borates and tetraborates at various pH numbers and at various concentrations showing that the requirement that pH should be neutral or slightly alkaline in order to minimise the percutaneous absorption of these boron derivatives is not justified. The list of substances which cosmetic products must not contain except subject to the restrictions and conditions laid down, set out in Part 1 of Annex III, should therefore be amended accordingly.
- (5) In the concentrations in which it is normally used as a preservative in cosmetic products intended to be removed by rinsing, benzylhemiformal is not likely to cause harmful effects for human health. Therefore it should be removed from Part 2 of Annex VI to the abovementioned Directive which sets out the list of preservatives provisionally allowed in cosmetic products and included in Part 1 of Annex VI which contains the list of preservatives allowed in cosmetic products.

⁽¹⁾ OJ L 262, 27.9.1976, p. 169.

⁽²⁾ OJ L 253, 15.9.1998, p. 20.