



Scientific Committee on Consumer Safety

SCCS

OPINION ON
Bis(butylbenzoate) diaminotriazine
aminopropyltrisiloxane

COLIPA n° S85



The SCCS adopted this opinion at its 10th plenary meeting
of 22 March 2011

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Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

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SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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This opinion has been subject to a commenting period of four weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged. Revised opinions carry the date of revision.

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Opinion on bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane

1. BACKGROUND

The substance with the chemical name Bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane and CAS number 207562-42-3 and the abbreviation BBDAPT is a new UV-filter for use in sunscreen products.

The first submission for this substance was received from COLIPA in March 2010.

2. TERMS OF REFERENCE

1. *Does SCCS consider that the use of Bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane as an UV-filter in cosmetic products in a concentration up to maximum 10.0% is safe for the consumers taken into account the scientific data provided?*
2. *Does SCCS have any other scientific concerns for the safe use of the new UV-filter Bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane in finished cosmetic products?*

Opinion on bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane

3. OPINION**3.1. Chemical and Physical Specifications**

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane (INCI name)

3.1.1.2. Chemical names

Butyl 4-({4-{{4-(butoxycarbonyl)phenyl}amino}-6-[[3-{{1,3,3,3-tetramethyl-1-[[trimethylsilyl]oxy]disiloxanyl} propyl)amino]-1,3,5-triazin-2yl}amino)benzoate

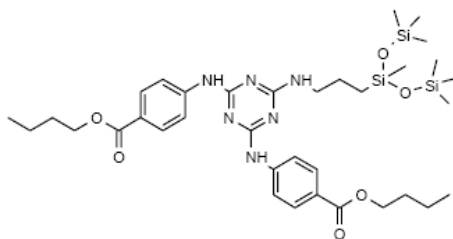
3.1.1.3. Trade names and abbreviations

Mexoryl SBS
 BBDAPT
 R0027603A
 COLIPA S85

3.1.1.4. CAS / EC number

CAS: 207562-42-3
 EC: /

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: C₃₅H₅₆N₆O₆Si₃

3.1.2. Physical form

White powder

3.1.3. Molecular weight

Molecular weight: 741.12 g/mol

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3.1.4. Purity, composition and substance codes

Chemical characterisation was performed by NMR, MS, IR and UV spectroscopy. Data from four different batches of BBDAPT (Batch Pilote 2, Batch Pilote 7, Batch Pilote 9 and Mexoryl SBS Batch 054 D001) were provided. Purities were determined by UV spectrometry, HPLC (UV- detection) and GC. Purities of the four batches of BBDAPT are given in table 1:

Table 1: HPLC and UV-purities of four different batches of BBDAPT

Batch	Titre by HPLC [% w/w]*)	UV purity [%]
Pilote 2	98.2	> 95
Pilote 7	97.3	> 95
Pilote 9	97.5	> 95
Mexoryl SBS Batch 054 D001	98.3	> 95

*) against Mexoryl SBS reference standard batches 056 L001 and MM02-33 considered as 100% pure

3.1.5. Impurities / accompanying contaminants

Impurities (determined by HPLC (UV-detection) and GC) of the four different batches of BBDAPT are presented in table 2.

For the determination of the most important impurities, the applicant used reference standard materials.

Table 2: Impurities of the four different batches of BBDAPT

Impurity	Pilote 2	Pilote 7	Pilote 9	Mexoryl SBS Batch 054 D001
Impurities determined by HPLC [% w/w]				
n-Butyl-4-aminobenzoate (abbr.: n-Butyl PABA)	0.15	0.07	0.08	0.05
Butyl 4-[(4-{[4-(butoxycarbonyl)phenyl]amino}-6-{[3-(1-hydroxy-1,3,3,3-tetramethyldisiloxanyl) propyl]amino}-1,3,5-triazin-2-yl)amino]benzoate (abbr.: Silanol)	0.20	0.25	0.75	0.63
Butyl 4-[(4,6-bis{[4-(butoxycarbonyl)phenyl]-amino}-1,3,5-triazin-2-yl)amino]benzoate (abbr.: Tri n-butyl-PABA)	0.19	0.21	0.15	0.17
Butyl 4-({4-{[4-(butoxycarbonyl)phenyl]amino}-6-[(2-{1,3,3,3-tetramethyl-1-[(trimethylsilyl)oxy]disiloxanyl}propyl)amino]-1,3,5-triazin-2-yl)amino)benzoate (abbr.: Isomer β)	0.72	0.95	ND	0.56
Butyl 4-[(4-{[3-{3-[(4,6-bis{[4-(butoxycarbonyl)phenyl]amino}-1,3,5-triazin-2-yl)amino]-propyl}-1,3,5,5,5-pentamethyl-1-[(trimethylsilyl)oxy]trisiloxanyl}propyl)amino]-6-{[4-(butoxycarbonyl)phenyl]amino}-1,3,5-triazin-2-yl)amino]benzoate (abbr.: Dimere)	0.19	0.05	0.05	0.32
Impurities determined by HPLC [% UV area]				
Butyl 4-({4,6-bis{[3-{1,3,3,3-tetramethyl-1-[(trimethylsilyl)oxy]disiloxanyl}propyl)amino]-1,3,5-triazin-2-yl)amino)benzoate (abbr.: n-Butyl-PABA disiloxane)	< 0.1%	-	-	-
Dibutyl 4,4'-({6-[(3-{3,3,3-trimethyl-1,1-bis[(trimethylsilyl)oxy]disiloxanyl}propyl)amino]-1,3,5-			0.01	

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Impurity	Pilote 2	Pilote 7	Pilote 9	Mexoryl SBS Batch 054 D001
triazine-2,4-diyl}diimino)dibenzoate (homolog of Mexoryl SBS : the methyl group on the siliconed amine is substituted by a trimethyl silyloxy group)				
Impurity NI (homolog of Mexoryl SBS: a supplementary methyl group is present on the siliconed amine chain)			0.10	
Impurities content by GC [% w/w]				
Siliconed amine	0.03	0.03	0.06	0.22
Residual solvents by GC [% w/w]				
Ethyl acetate	Not applicable ^{*)}	Not applicable ^{*)}	Not applicable ^{*)}	ND<0.10
Heptane	D < 0.15	D < 0.15	D < 0.15	D < 0.10
Methyl ethyl ketone	ND < 0.15	ND < 0.15	ND < 0.15	Not applicable ^{*)}

^{*)} Ethyl Acetate was replaced by methyl ethyl ketone in batches Pilote 2, 7, and 9.

3.1.6. Solubility

a) Solubility [g/l] (at 24°C after 24 h) in water and organic solvents

- Water: 0.0001 mg/l^{*)}
- Ethanol: ≥ 200 g/l^{**)}
- DMSO: ≥ 200 g/l^{**)}
- Methanol: 214 mg/l^{*)}
- in receptor fluid for dermal absorption study 0.082 mg/l^{*)}
(bovine serum albumin (approximately 5% [w/v] in phosphate-buffered saline (PBS))

^{*)} from ref. 15

^{**)} from the Analytical file provided in the submission documents

b) Solubility [%] in lipophilic cosmetic solvents

Isopropyl lauryl sarcosinate: 40 to 50%

Dimethylisosorbide: 40 to 50%

Ref.: Complement to subm I, 2010

Comment

Apart from the solubility in methanol and receptor fluid for dermal absorption, the methodologies to determine the solubility were not stated.

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: 9.4

Ref.: 15

Comment

This value is given in the in vitro percutaneous absorption study for batch pilote 2; it is not stated, whether this is a measured or a calculated value.

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3.1.8. Additional physical and chemical specifications

Melting point:	Batch Pilote 2: 106.6°C Batch Pilote 7: 106.6°C Batch Pilote 9: 106.8°C
Boiling point:	not given
Flash point:	not given
Vapour pressure:	not given
Density:	not given
Viscosity:	not given
pKa:	not given
Refractive index:	not given
Visible spectrum (400 - 700 nm):	no characteristic absorbance in visible light
UV/Vis spectrum (200 - 800 nm):	λ max at appr. 310 nm

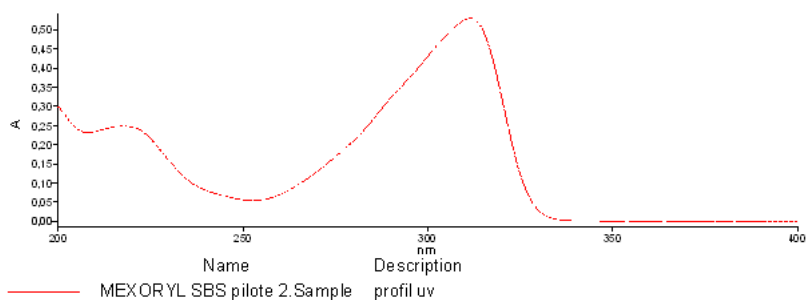


Fig. 2: UV Spectrum of Mexoryl SBS batch Pilote 2

3.1.9. Stability

The stability of BBDAPT (Batch Pilote 2) in 0.5% carboxymethylcellulose at 20 and 200 mg/ml was investigated when stored at ambient temperature (10 days for 20 mg/ml; 11 days for 200 mg/ml). Both concentrations remained homogenous and stable within the observation period.

Ref.: 20

General comments on physico-chemical characterisation

Reference substances have been used for characterisation and identification.

Information on particle size distribution is not given. Although not explicitly demonstrated, the applicant stated that bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane will be fully soluble at a maximum use concentration of 10% in cosmetic formulations available to the consumer.

Information on Log Pow was not provided in the analytical file provided in the submission documents. However, a value of a Log Pow of 9.4 is mentioned in the percutaneous absorption study but it is not stated whether it is a calculated or a measured value.

Bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane is a secondary amine, and thus, it is prone to nitrosation. The nitrosamine content in the test material is not reported.

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3.2. Function and uses

Bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane is a UV filter ($\lambda_{\max} = 310 \text{ nm}$) proposed to be used in sunscreen formulations at a maximum concentration of 10%.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline: OECD TG 420 (Fixed dose procedure) / EC B.1
 Species/strain: rat, Sprague-Dawley (age: between 8 and 12 weeks)
 Group size: 3 female animals in total
 Test substance: BBDAPT; R0027603A
 Batch: 054D001
 Purity: 98.0% (HPLC)
 Dose: 2000 mg/kg bw
 Vehicle: 0.5% carboxymethylcellulose
 Observation: 14 d
 GLP: in compliance
 Study period: 21 Dec 2006 – 18 January 2007

Acute toxicity of BBDAPT was first investigated at a starting limit dose level of 2000 mg/kg bw to one animal (sighting test).

In the main test, three animals (including the animal used for the sighting test) received 2000 mg/kg bw of the test substance as single, oral gavage at a dose volume of 10 ml/kg bw after overnight fasting. All animals were examined clinically on the day of treatment, 30 minutes, 2 hours, 4 hours and 6 hours after administration and thereafter at least once daily for 14 days. Animals were weighed on day 1 (administration), day 7, day 14 and day 15 (euthanasia). After exsanguination on day 15, all animals were subjected to gross necropsy and organs were examined macroscopically.

Results

Individual and mean body weight gains were within the normal range throughout the study period. In both, sighting and main study, no mortalities occurred between day 1 and day 14 and no clinical signs could be observed between day 1 and day 14. At gross necropsy, no organ or tissue gross finding was seen in the sighting and in the main test.

Conclusion

Under the conditions of this test, the oral LD₅₀ of BBDAPT was higher than 2000 mg/kg bw.

Ref.: 1

Comment

The test was performed with three animals only (including the animal used for the sighting study). OECD TG 420 states that normally 5 animals per group should be used.

3.3.1.2. Acute dermal toxicity

Guideline: OECD TG 402 / EC B.3
 Species: rat, Sprague-Dawley Rj:SD
 Group: 5 male and 5 female animals (age: approximately 8 weeks)
 Test substance: R0027603A
 Batch: Pilote 2
 Purity: 98.2%

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Dose: 2000 mg/kg bw
 Route: dermal, semi-occlusive
 Exposure time: 24 hours
 GLP: in compliance
 Study period: Feb – June 2009 (end of in vivo phase: 19 Feb 2009)

A single dose of 200 mg/kg bw of the test item was placed on a hydrophilic, pre-moistened gauze-pad and applied to a clipped area of skin representing approximately 10% of the total body surface. The test item and the gauze pad were held in contact with the skin for 24 hours using an adhesive hypoallergenic aerated semi-occlusive dressing and a restraining bandage. The animals were observed frequently during the hours following administration of the test item for detection of possible treatment-related clinical signs. Thereafter, observation of the animals was made at least once a day until day 15 (day of sacrifice). Type, time of onset and duration of clinical signs were recorded for each animal individually. All study animals were subjected to a macroscopic examination as soon as possible after death. After opening the thoracic and abdominal cavities, a macroscopic examination of the main organs (digestive tract, heart, kidneys, liver, lungs, pancreas, spleen and any other organs with obvious abnormalities) was performed.

Results

Local cutaneous reactions were recorded from day 2. No deaths occurred during the study. Crusts were noted in 3/5 males and 1/5 females between day 7 and day 11. Crusts persisted in one male and in the female until day 15 (end of the observation period). When compared to historical control animals used in the performing laboratory, the body weight gain of the animals was not affected by treatment with the test item. Macroscopic examination of the main organs of the animals revealed no apparent abnormalities.

Conclusion

Under the conditions of this test, the dermal LD₅₀ of BBDAPT was higher than 2000 mg/kg bw.

Ref.:2

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline: OECD TG 404 / EC B.4
 Species: rabbit, New Zealand White
 Group: 3 male animals in total
 Test substance: R0027603A
 Batch: Pilote 2
 Purity: 98.2%
 Dose: 500 mg
 Route: dermal
 Exposure time: 4 hours
 Application: semi-occlusive
 GLP: in compliance
 Study period: December 2008 – June 2009 (end of in vivo phase: 14 December 2008)

The day before treatment, both flanks of each animal were clipped using electric clippers; the skin of each animal was examined for any signs of skin irritation before treatment. The test item (500 mg) was first evaluated on a single animal whose skin was observed 3

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minutes, 1 hour and 4 hours after administration. Since the test item was neither severely irritant nor corrosive on this first animal, it was applied for 4 hours to two further animals. Doses of 500 mg of the test item were placed on a gauze pad moistened with purified water, which was then applied to an area of approximately 6 cm² of the posterior right flank. The gauze pad was held in contact with the skin by means of an adhesive hypoallergenic aerated semi-occlusive dressing and a restraining bandage. Untreated skin areas served as control. After removal of the dressing, any residual test item was wiped off by means of a moistened cotton pad. The skin was examined approximately 1 hour, 24, 48 and 72 hours after removal of the dressing.

Results

After 4-hour dermal exposure to the test item, slight erythema (grade 1) was noted in 2/3 animals on days 1 and 2 which persisted until day 3 in one of them.

Mean scores over 24, 48 and 72 hours for each animal were 0.0, 0.7 and 0.3 for erythema and 0.0, 0.0 and 0.0 for oedema.

Conclusion

According to the authors, the test item R0027603A should not be classified as irritating to the skin for regulatory purposes.

Ref.: 3

Comment

Slight erythema (grade 1) was noted in 2/3 animals on days 1 and 2 which persisted until day 3 in one of them. The test item is slightly irritating to rabbit skin under the conditions of the experiment.

3.3.2.2. Mucous membrane irritation

Guideline: OECD TG 405 / EC B.5
 Species: rabbit, New Zealand White
 Group: 3 male animals in total
 Test substance: R0027603A at a concentration of 10% in 0.5% carboxymethylcellulose
 Batch: Pilote 2
 Purity: 98.2%
 Dose: 10 µg (0.1 ml of a 10% [w/w] solution; pH 7)
 Route: instillation into conjunctival sac
 Application: single application
 Exposure time:
 GLP: in compliance
 Study period: Dec 2008 – June 2009 (end of in vivo phase: 26 December 2008)

The dosage form was first administered to a single male New Zealand White rabbit. Since the dosage form was not severely irritant on this first animal, it was then evaluated simultaneously in two other animals. A single dose of 0.1 mL of the test item at the concentration of 10% [w/w] in 0.5% carboxymethylcellulose was instilled into the left conjunctival sac. The right eyes served as controls. The eyes were not rinsed after administration of the test item. Ocular reactions were observed approximately 1 hour, 24, 48 and 72 hours after the administration. For the evaluation of corneal opacification (presence or absence, affected area), the eyes were examined under a UV lamp after instillation of one or two drops of 0.5% sodium fluorescein solution (a clear fluorescence is visible in the areas of opacification). The mean values of the scores for chemosis, redness of the conjunctiva, iris lesions and corneal opacity were calculated for each animal.

Results

Slight chemosis and redness of the conjunctiva (grade 1) were noted in all animals one hour after administration. Mean scores calculated for each animal over 24, 48 and 72 hours were 0.0, 0.0 and 0.0 for chemosis, 0.0, 0.0 and 0.0 for redness of the conjunctiva, 0.0, 0.0 and

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0.0 for iris lesions and 0.0, 0.0 and 0.0 for corneal opacity. At termination of the study on day 4, no ocular reactions remained.

Conclusion

The study authors concluded that the test item R0027603A should not be classified as irritating to mucous membrane for regulatory purposes, because the substance was not irritant to the eyes of rabbits when administered at a 10% in 0.5% carboxymethylcellulose.

Ref.: 4

Comment

Slight eye irritation was observed 1 hour after administration when the substance had been applied in a concentration intended for use. The test item is slightly irritating to the rabbit eye under the conditions of the experiment.

3.3.3. Skin sensitisation

Local Lymph node assay (LLNA)

Guideline: OECD TG 429 / EC B.42
 Species: Mouse, CBA/J
 Group: one single female animal per dose (preliminary test)
 4 females per dose (main test)
 Test Substance: R0027603A
 Batch: Pilote 2
 Purity: 98.2%
 Dose levels: Preliminary test: 5, 10, 25 and 50% [w/v] of test item in acetone/olive oil (4:1 [v/v] (AOO))
 Main test: 1, 2.5, 5, 10, 25% [w/v] of test item in AOO
 Controls: negative control: vehicle only (AOO)
 positive control: 25% [v/v] α -hexylcinnamaldehyde (HCA) in AOO
 Route: application to the dorsal surface of both ears on days 1, 2 and 3
 GLP: in compliance
 Study period: December 2008 – June 2009 (end of *in vivo* phase: 23 December 2008)

The study consisted of a preliminary test and a main test. For the preliminary test (performed in order to select doses for the main study), 25 μ l of test item at concentrations of 5, 10, 25 and 50% in vehicle were applied to the external surface of both ears for three consecutive days. Ear thickness was determined using a micrometer the day before treatment, during each treatment day and 72 hrs after the last application. As an increase in ear thickness was noted on day 6 at the 50% concentration, a concentration of 25% was selected as the highest dose for the main study.

In the main test, 25 μ l of control or test item preparations were applied to the dorsal surface of both ears on days 1, 2 and 3 of the study. Ear thickness was determined as in the preliminary study. On day 6, all animals of all groups received a single intravenous injection of 250 μ L of 0.9% NaCl containing 20 μ Ci of [³H]-methyl-thymidine (³H-TdR; specific activity of 25 Ci/mmol) via the tail vein. 5 hours later, the animals were sacrificed; auricular lymph nodes were excised and pooled for each experimental group. Cell pellets were prepared for numeration of lymphocytes (cellularity) and determination of viability. After precipitation, incorporation of ³H-TdR was determined by β -scintillation counting and stimulation indices (SI) were calculated.

Results

No mortality and no clinical signs were observed during the study. Among the different concentrations of test item applied, the highest concentration (25%) revealed to be slightly irritant based on an increase in ear thickness. For the different test concentrations of 1%, 2.5%, 5%, 10% and 25%, SI-values of 0.81, 1.06, 0.97, 1.12, and 2.32 were derived, respectively, whereas the positive control yielded a SI of 14.71.

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Thus, stimulation indices of the test item did not exceed 3 for any of the concentrations tested.

Conclusion

Under the experimental conditions of this study, the test item R0027603A did not induce contact sensitization in the murine Local Lymph Node Assay.

Ref.: 5

3.3.4. Dermal / percutaneous absorption
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Guideline:	OECD TG 428
Species:	Human
Test system:	Flow-through diffusion cells
N° of samples:	12 samples from 5 different donors
Radiolabelled test substance:	[¹⁴ C]-R0027603A; specific activity: 106.6 µCi/mg; Batch B1 (Code No. CFQ40506), purity 98.0% (HPLC); radioactive label at the central triazin ring
Non-labelled test substance:	R0027603A; batch pilote 2, purity 97.7% (HPLC, Sep 2008); 98.2% (HPLC, Sep 2009)
Test formulation:	10% radiolabelled R0027603A in sunscreen formulation
Applied amount:	5 mg/cm ²
Receptor fluid:	Bovine serum albumin (appr. 5% [w/v] in phosphate-buffered saline (PBS)
Solubility in receptor fluid:	0.082 mg/l
Exposure time:	24 hr
Sampling periods:	0 – 0.5 hr; 0.5 – 1 hr; hourly from 1-6 hr; 2-hourly from 6-24 hr
GLP:	in compliance
Study period:	January – May 2009

In order to determine skin penetration *in vitro*, R0027603A was investigated at a 10% [w/w] concentration in a typical sunscreen lotion test preparation. The 24 hr stability of the test item in the formulation has been demonstrated. Five samples of full-thickness human skin (4 breast and 1 abdomen) were obtained from donors aged 42 to 81 years old from which split-thickness skin membranes (resulting membrane thickness ranged between 370 and 400 µm) were prepared and stored at -20 °C until use. The integrity of the skin was checked by determination of the permeability coefficient for tritiated water. [¹⁴C]-R0027603A in the test preparation (no details on solubility of the test item in the formulation) was applied to human split-thickness skin membranes mounted in twelve flow-through diffusion cells *in vitro* (n= 2 or 3 per donor; target application rate: 5 mg/cm²). The surface area of exposed skin within the cells was 0.64 cm². The receptor chamber volume was 0.25 ml and was set at a flow-through rate of ca. 1.5 ml/hr. Absorption was assessed by collecting receptor fluid samples until 24 hr post dose (flow rate: 1.5 mL/h). At 24 h post dose, the skin was washed with sodium dodecyl sulphate (SDS) solution and water. After removal from the cells, stratum corneum was removed by tape stripping. The remaining skin was divided into exposed and unexposed skin and the exposed epidermis was separated from the dermis by heat separation. Radioactivity of all samples of skin, receptor fluid, tissue and washes was performed by liquid scintillation counting.

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Results

Table 3: Distribution of radioactivity (% of applied dose) at 24 hr post-dose following topical application of [¹⁴C]-R0027603A in a test preparation (10% [w/w] to human split thickness skin).

Cell	1	4	5	6	9	10	14	15	17	18	Mean	SD	Range
Total dislodgeable dose (Skin wash, pipette tips, tissue swabs, cell wash)	90.29	95.22	75.18	78.00	86.78	86.73	86.90	63.55	85.03	68.82	81.65	9.99	63.55 – 95.22
Unabsorbed dose (stratum corneum and unexposed skin)	94.38	101.55	94.78	93.14	99.98	91.13	93.72	93.73	92.04	87.48	94.19	4.06	87.48 – 101.55
Absorbed dose (epidermis, dermis, receptor fluid, receptor rinse)	0.39	0.27	1.53	1.63	0.17	2.04	1.2	2.87	0.07	4.79	1.50	1.48	0.07 – 2.87
Mass balance	94.77	101.82	96.31	94.77	100.15	93.17	94.93	96.60	92.11	92.28	95.69	3.19	92.11 – 101.82

Table 4: Distribution of radioactivity (µg equiv/cm²) at 24 hr post-dose following topical application of [¹⁴C]-R0027603A in a test preparation (10% [w/w] to human split thickness skin).

Cell	1	4	5	6	9	10	14	15	17	18	Mean	SD	Range
Total dislodgeable dose (Skin wash, pipette tips, tissue swabs, cell wash)	497.95	525.15	414.59	430.14	478.56	478.31	479.23	350.45	468.92	379.55	450.28	55.07	350.4 – 525.15
Unabsorbed dose (stratum corneum and unexposed skin)	520.49	560.02	522.67	513.66	551.37	502.59	516.87	516.91	507.61	482.46	519.47	22.4	482.4 – 560.02
Absorbed dose (epidermis, dermis, receptor fluid, receptor rinse)	2.13	1.50	8.45	9.01	0.92	11.24	6.63	15.81	0.36	26.43	8.25	8.16	0.36 – 26.43
Mass balance	522.62	561.52	531.12	522.67	552.29	513.84	523.50	532.72	507.98	508.89	527.71	17.6	507.9 – 561.52

Results from two cells were excluded from final calculations of mean and SD values, due to low mass balance. Thus, results from 10 skin samples obtained from 5 donors were taken for evaluation. The mean recovery among the remaining 10 samples was $95.69 \pm 3.19\%$. Under the present experimental conditions and excluding two samples from final evaluation, effectively most of the applied dose of [¹⁴C]-R0027603A in the sunscreen lotion test preparation was washed off the skin at 24 h post dose (75.44% of the applied dose). A further 6.21% was removed in the cell wash. Therefore, the dislodgeable dose was 81.65% of the applied dose. The amount recovered from the stratum corneum was 10.78% of the applied dose with 8.57% contained in the first five tape strips. At 24 h post dose, the absorbed dose was $1.5\% \pm 1.48$ of the applied dose (corresponding to $8.25 \pm 8.16 \mu\text{g equiv/cm}^2$).

Ref.: 15

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Comment

The solubility of R0027603A in the receptor fluid was very low (0.082 mg/l). However, based on the available physico-chemical data the solubility of this test item in any physiologically based receptor was anticipated to be low. The high variability between the samples might be due to methodological factors: low solubility of the test compound in general, low penetration through the skin layers and low solubility in the receptor fluid.

Concerning the protocol, the study complies with the basic requirements of SCCS, however, high variability in dermal absorption were observed between the samples as demonstrated by the wide range in absorption values and by high standard deviations. Thus, the mean plus two standard deviations (4.46%; 24.57 µg equiv/cm²) will be taken for MoS calculation.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral toxicity

Guideline:	OECD TG 407
Species/strain:	rat, Sprague-Dawley
Group size:	5 male and 5 female animals
Test substance:	R0027603A
Batch:	054D001
Purity:	98.3%
Dose levels:	100, 300, 1000 mg/kg bw/d
Vehicle:	0.5% carboxymethylcellulose
Route:	oral (gavage)
Controls:	vehicle only
Exposure:	29 days
GLP:	in compliance
Study period:	March 2007 – Dec 2009 (end of in vivo phase: 11 May 2007)

Animals received daily oral gavages of R0027603A, control groups received vehicle only. The animals were checked daily for mortality and clinical signs. Body weight was recorded before the beginning of the treatment period, on the first day of treatment and then once a week. Food consumption was recorded once a week. At the end of the treatment period, a neurotoxicological evaluation, using a Functional Observation Battery (FOB) and haematological, blood biochemistry, thyroid hormone assays and urinary investigations were performed. On completion of the treatment period, all the animals were sacrificed and submitted to a full macroscopic *post-mortem* examination.

Results

There were no treatment-related changes in the autonomic, physiological or neurotoxicological parameters. No differences of toxicological significance were seen in motor activity. There were no treatment-related changes in haematological parameters and in blood biochemistry in treated groups when compared to control mean values. No changes in urinalysis were observed at any dose-level or in either sex.

The test item caused increases in T3 and TSH levels in males and T4 levels in females at all dose-levels, as well as increased T4 levels in males at 1000 mg/kg bw/day. It also caused an increased number of lipophilic vacuoles in the glandular epithelium of the mammary gland in males treated at 1000 mg/kg bw/day. In the absence of abnormal clinical signs, altered thyroid weights and histopathologic evidence of cell proliferation in the thyroid gland, the authors considered changes in thyroid hormone and TSH levels as non-adverse effects. There are no indications in the literature that fat in mammary gland acini represents toxicity. Further, the increased vacuolation observed was of low severity, so this change was also considered to be non-adverse. A relationship, however, between increased numbers of small thyroid follicles (a non-adverse finding) and compound administration could not be ruled out.

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Conclusion

Under the experimental conditions of the study, the No Observed Adverse Effect Level (NOAEL) was considered to be 1000 mg/kg bw/day in males and females. A NOEL could not be derived.

Ref.: 24

3.3.5.2. Sub-chronic (90 days) oral toxicity
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Guideline:	OECD TG 408
Species/strain:	Rat, Sprague-Dawley; RjHan:SD
Group size:	10 male and 10 female animals per dose group
Test substance:	R0027603A
Batch:	Pilote 2
Purity:	98.2%
Dose levels:	100, 300 and 1000 mg/kg bw/d
Route:	oral (gavage)
Vehicle:	0.5% carboxymethylcellulose, aqueous solution
Dosing schedule:	daily oral doses (gavage) for 91 days
Dosage volume:	5 ml/kg bw/d
Controls:	10 male and 10 female animals receiving vehicle only
GLP:	in compliance
Study period:	Nov 2008 – December 2009 (end of in vivo phase: 27 February 2009)

Groups of male and female Sprague-Dawley rats received the test item by daily gavage for 13 weeks at dose-levels of 100, 300 or 1000 mg/kg bw/day. Control animals received vehicle only under the same experimental conditions. The test item concentrations in the dosage forms were measured in weeks 1, 4, 8 and 13. Animals were checked daily for mortality and clinical signs. A neurotoxicological evaluation using a Functional Observation Battery (FOB), including detailed observations, the measurement of reactivity to manipulation and different stimuli, and the evaluation of motor activity, was performed in week 11 of the treatment period. Body weight and food consumption were recorded once per week. Ophthalmological examinations were performed on all the animals before the beginning of the treatment period, and on control and high-dose animals at the end of the treatment period. Haematological parameters, blood biochemistry and urine were investigated at the end of the treatment period, where blood was also sampled for the determination of thyroid hormones. At the end of the treatment period, animals were sacrificed and submitted to a full macroscopic *post-mortem* examination. Designated organs/tissues were weighed and selected tissue specimens (in compliance with OECD TG 408) were preserved. A microscopic examination was performed on almost all tissues taken from the control group and animals receiving 1000 mg/kg bw/d sacrificed at the end of the treatment period. Microscopic examination was also performed in one female of the 100 mg/kg bw/d group found dead during the study and on animals from the low- and mid-dose groups in case macroscopic lesions were observed.

Results

No test item-related deaths occurred and no test item treatment-related clinical signs were observed. There were no effects on body weight, body weight gain or food consumption. No changes were noted in the investigated neurotoxicological parameters. There were no test item-related changes in the haematological parameters, blood chemistry or urinalysis. There were no toxic effects on the pituitary/thyroid function (assessed by the measurement of circulating levels of TSH, T4, T3, Free T4 and free T3) and no treatment-related changes were noted in the thyroid glands. There were no test item treatment-related modifications in the haematological parameters when compared to controls. None of the blood biochemistry parameters investigated at the end of the treatment period was affected by the test item treatment. Statistically significant differences from controls were observed (including ASAT and ALAT activities in males given 100 mg/kg bw/day, and protein levels in

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females given 1000 mg/kg bw/day), but these were of low magnitude, and variations did not occur in a dose-related manner. They were therefore considered not to be test item-related. There were no changes in the urinary parameters of any of the test item-treated groups when compared to control mean values at the end of the treatment period. Slight changes in organ weight of test-item-treated animals (e.g. statistically significantly increased kidney weights of high-dose males) were observed. However, these changes were of low magnitude, not dose-related and partially of opposing trend between groups and sexes and thus not considered to be treatment-related. No treatment-related macroscopic and microscopic findings were noted.

Conclusion

Under the experimental conditions of this study, a No Observed Adverse Effect Level (NOAEL) of 1000 mg/kg bw/d was derived.

Ref.: 9

Comment

No explanation was given by the study authors why substance-related (non adverse) effects as observed in the oral 28 day repeat-dose study were not seen in the oral 90 day repeat-dose study.

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted.

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

Bacterial gene mutation assay

Guideline:	OECD TG 471 / EC B.13/14
Species/strain:	Salmonella typhimurium TA 1535, TA 1537, TA 98, TA 100 and TA 102 (preliminary test: only TA98, TA100 and TA 102)
Replicates:	3 plates per dose level
Assay conditions:	with and without metabolic activation (rat liver S9 mix from rats induced with Aroclor 1254); 48 – 72 hr incubation time
Test substance:	R0027603A
Batch:	054 D001
Purity:	98.3%
Concentrations:	10 – 5000 µg/plate (preliminary test) 78- 2500 µg/plate (main test)
Vehicle:	Dimethylsulfoxide (DMSO); mitomycin C (positive control) was dissolved in distilled water
GLP:	in compliance
Study period:	February 2007 – May 2007

The test item was tested in a preliminary test and three mutagenicity experiments. The preliminary test, both experiments without S9 mix, the first and the third experiments with S9 mix were performed according to the direct plate incorporation method, whereas the second experiment with S9 mix was performed according to the preincubation method.

The strains were exposed to the test material dissolved in DMSO on plated agar in the presence and absence of rat liver metabolic activating system. DMSO alone served as negative control. The following positive controls were used in experiments without S9 mix: sodium azide (for TA 1535 and TA 100), 9-aminoacridine (for TA 1537), 2-nitrofluorene (for TA 98) and mitomycin C (for TA 102). For experiments with S9 mix, 2-anthramine (for TA

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1535, TA 1537 and TA 98 and TA 100) and benzo[a]pyrene (for TA 100) were used as positive controls. After 48 to 72 hours of incubation at 37°C, revertants were scored.

Results

In the preliminary mutagenicity assay, the dose levels tested were 10, 100, 500, 1000, 2500 and 5000 µg/plate. Emulsion formed at dose levels ≥ 1000 µg/plate which made the plates unreadable at dose-levels ≥ 2500 µg/plate in the TA 102 strain. No noteworthy toxicity was noted towards the three strains used, either with or without S9 mix.

Based on the results from the preliminary test, selected treatment levels for the main test were: 78.13, 156.3, 312.5, 625, 1250 and 2500 µg/plate, for all tester strains in all experiments with and without S9 mix. A moderate to strong emulsion was observed in the Petri plates when scoring the revertants at dose-levels ≥ 625 µg/plate. The strong emulsion made the plates unreadable at 2500 µg/plate. In the experiments without S9 mix, no toxicity was noted in the five strains used. The test item did not induce any noteworthy increase in the number of revertants, in either experiment, in any of the five strains. In the experiments with S9 mix, no toxicity was noted in the five strains used. In the TA 98 strain, a 1.9-2.0-fold increase in the number of revertants was noted in the first experiment (direct plate incorporation method) at the dose-levels of 625 and 78.13 µg/plate. These very slight increases in revertants were not considered as biologically relevant since they were neither dose-related nor reproducible (between plates within the first experiment and in the third experiment). The test item did not induce any noteworthy increase in the number of revertants, in either experiment, in any of the remaining four tester strains.

Conclusion

Under the experimental conditions of this study, R0027603A did not show mutagenic activity in the bacterial reverse mutation test with *Salmonella typhimurium*.

Ref.: 10

In Vitro Mammalian Cell Gene Mutation Test

Guidelines:	OECD TG 476
Species/strain:	L5178YTK ^{+/-} mouse lymphoma cells
Replicates:	2
Test Substance:	R0027603A
Batch:	Pilote 2
Purity:	98.2%
Vehicle:	Dimethylsulfoxide (DMSO)
Concentrations:	range-finder study, 3 hr (with and without S9): 3.75, 7.5, 15, 30, 120 µg/ml range-finder study 24hr (without S9): 0.47, 0.94, 1.87, 3.57, 7.5, 15, 30, 60, 120 µg/ml experiment 1, 3 hr (with and without S9): 2.5, 5, 10, 20, 30, 40, 50, 60, 80, 100 µg/ml experiment 2, 3 hr and 24 hr (3 hr with and 24 hr without S9): 10, 20, 40, 60, 80, 100, 120, 140 µg/ml
Controls:	Methyl methane sulphonate without S9 (positive control) Benzo[a]pyrene with S9 (positive control) Vehicle (negative control)
GLP:	in compliance
Study period:	November 2008 – January 2009

R0027603A was investigated for the induction of gene mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells with and without metabolic activation. Liver S9 fraction from Aroclor 1254-induced rats was used as the exogenous metabolic activation system. Test concentrations and exposure times were based on the level of toxicity. Methyl

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methane sulphonate and benzo[a]pyrene served as positive controls for the cultures without and with metabolic S9 activation, respectively.

Results

In the preliminary cytotoxicity assays (3 hr and 24 hr), precipitates were observed at the highest dose tested (120 µg/ml). No significant changes in osmolarity or pH were observed in the range-finder experiments.

In experiment 1 (3 hr exposure with and without S9), concentrations from 2.5 to 100 µg/ml were tested, precipitation occurred at the highest dose tested. In experiment 2, concentrations between 10 and 140 µg/ml were used; in the absence of S9, haziness was observed at 120 and 140 µg/ml, whereas in the presence of S9, precipitation occurred at 100 µg/ml and above, 80 µg/ml was selected as the highest dose for total growth determination. Clear increases in mutant induction were observed by the positive control chemicals methyl methane sulphonate and benzo[a]pyrene. In experiments 1 and 2, there were no notable increases in mutant frequencies in the presence or absence of metabolic activation after 3 hr incubation and in the absence of metabolic activation after 24 hr incubation.

Conclusion

The test item R0027603A did not induce an increase in mutant frequency at the *tk* locus of L5178Y mouse lymphoma cells when tested under the conditions employed in this study.

Ref.: 11

Comments

Elevated relative total growth values were seen at all concentrations in the absence of S9-mix, which were due to relatively reduced viability and cell counts for the vehicle control treatments, although the reason for this effect remained unclear.

The test conditions included treatments up to precipitating concentrations; precipitation prevented testing of higher doses.

***In Vitro* Micronucleus Test**

Guideline:	OECD TG 487, draft
Species/strain:	L5178Y <i>tk</i> ^{+/-} mouse lymphoma cells
Replicates:	2
Assay conditions:	Preliminary test: with and without S9:3 hr treatment; without S9: 24 hr treatment Experiment 1: with and without S9: 3 hr treatment, 24 hr recovery Experiment 2: without S9:24 hr treatment, 0 hr recovery; with S9: 3hr treatment, 24 hr recovery
Test Substance:	R0027603A
Batch:	054 D001
Purity:	98.3%
Solvent:	Dimethylsulfoxide (DMSO)
Concentrations:	Preliminary test (with and without S9): 39.06, 78.12, 156.25, 312.5, 625, 1250, 2500 and 5000 µg/ml Experiment 1: without S9: 4.11, 12.3, 37, 111, 333, 1000, 1500, 1750, 2000 µg/ml with S9: 1.37, 4.11, 12.3, 37, 111, 333 µg/ml Experiment 2: without S9: 4.11, 12.3, 37, 111, 333, 500, 600, 700, 800, 900 µg/ml with S9: 1.37, 4.11, 12.3, 37, 111, 333, 500, 750, 1000 µg/ml
Controls:	without S9: mitomycin C as clastogen, colchicine as aneugen (positive control) with S9: Cyclophosphamide as clastogen (positive control)
GLP:	in compliance
Study period:	March 2007 – April 2007

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A preliminary test was performed in order to determine cytotoxicity of the test item. Cytotoxicity was evaluated by determining the population doubling (PD) of cells (and quality of the cells on the slides).

In the preliminary test, a slight to marked precipitate was observed at the end of the treatment period at dose-levels $\geq 78.12 \mu\text{g/ml}$. Following the 3-hour treatment without S9 mix, a slight to strong toxicity was induced at dose-levels $\geq 1250 \mu\text{g/ml}$. Following the 24-hour treatment without S9 mix, a slight to strong toxicity was induced at dose-levels $\geq 625 \mu\text{g/ml}$ and following the 3-hour treatment with S9 mix, no noteworthy toxicity was induced at treated dose-levels.

Dose levels of the main experiments were adjusted based on the findings (toxicity and/or precipitate) of the preliminary study.

In experiments without S9 mix, precipitate was observed at the end of the treatment period at dose-levels $\geq 111 \mu\text{g/ml}$ (3 hr treatment) or $\geq 37 \mu\text{g/ml}$ (24-hr treatment). Following the 3-hr treatment, slight to moderate toxicity was induced at dose-levels between 4.11 and 333 $\mu\text{g/ml}$, as shown by 30-51% decrease in the PD. At higher dose-levels strong toxicity was induced, also due to higher cell concentration at the end of the treatment period. Following the 24-hour treatment, slight to strong toxicity was induced at dose-levels between 111 and 333 $\mu\text{g/ml}$, as shown by 35-51% decrease in the PD. At higher dose-levels strong toxicity was induced. Therefore, dose-levels selected for micronucleus analysis were 4.11, 12.3 and 37 $\mu\text{g/ml}$ for the 3 hr treatment and 37, 111 and 333 $\mu\text{g/ml}$ for the 24-hr treatment (the highest dose levels caused a 51% decrease in PD, respectively). No significant increase in the frequency of micronucleated cells was noted after the 3- and 24-hr treatment periods in experiments without S9 mix.

In experiments with S9 mix, precipitate was observed at the end of the treatment period at dose-levels $\geq 111 \mu\text{g/ml}$ (first experiment) or $\geq 37 \mu\text{g/ml}$ (second experiment). A slight to moderate toxicity was induced at dose-levels $\geq 333 \mu\text{g/ml}$, as shown by 32-63% decreases in the PDs. Therefore, dose levels of 37, 111 and 333 $\mu\text{g/ml}$ (first experiment) and 333, 500 and 750 $\mu\text{g/ml}$ (second experiment) were selected for micronucleus analysis. The highest dose levels caused 49% and 56% reductions in PDs, respectively. No significant increase in the frequency of micronucleated cells was noted in the experiments with S9 mix. Under the different experimental condition applied, positive controls showed clear unequivocal increases in the frequency of micronucleated cells.

Conclusion

Under the experimental conditions of this test, R0027603A did not induce an increase in cells with micronuclei in L5178Y *tk*^{+/-} mouse lymphoma cells.

Ref.: 12

Comment

The test conditions included treatments up to precipitating concentrations; precipitation prevented testing of higher doses.

3.3.6.1. Mutagenicity / Genotoxicity <i>in vivo</i>

Mouse bone marrow micronucleus test

Guideline: OECD TG 474, EC B.12
 Species/strain: Rat, Sprague-Dawley, Ico: OFA-SD
 Group size: 3 male and 3 female animals (preliminary study)
 5 animals /sex/dose (main study)
 Test substance: R0027603A
 Batch: Pilote 2
 Purity: 98.2%
 Dose levels: 2000 mg/kg bw/d (preliminary study)
 500, 1000 and 2000 mg/kg bw/d (main study)
 Route: oral (gavage) – single administration

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Vehicle: 0.5% carboxymethylcellulose
 Controls: vehicle (negative controls)
 cyclophosphamide (positive controls)
 Sacrifice times: 24 hr after treatment
 48 hr after treatment (only at the highest dose)
 GLP: in compliance
 Study period: December 2008 – December 2009 (end of in vivo phase: 29 January 2009)

Doses for the main study were based on the results of a preliminary study performed at the limit dose of 2000 mg/kg bw at which no clinical signs and no deaths were observed.

In the main study, groups of rats received a single oral administration (10 ml/kg bw) of R0027603A in vehicle at 500, 1000 and 2000 mg/kg bw. Two groups of male and female animals received the highest dose which was investigated 24 hr and 48 hr after treatment. Animals receiving vehicle only served as negative, animals receiving cyclophosphamide at 15 mg/kg bw served as positive controls. At the time of sacrifice, animals were killed by cervical dislocation and bone marrows were obtained from the femurs. Anucleated erythrocytic cells were separated and slides were prepared. For each animal, the number of 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).

Results

Neither mortality nor clinical signs were observed in the animals of either sex given 500, 1000 or 2000 mg/kg bw/day. The mean values of MPE as well as the PE/NE ratio for the vehicle and positive controls were consistent with the historical data of the performing laboratory. Cyclophosphamide induced a significant increase ($p < 0.05$) in the frequency of MPE, indicating the sensitivity of the test system under the experimental conditions. The mean MPE values and PE/NE ratios in the test item-treated groups were similar to those of the vehicle group.

Conclusion

Under the experimental conditions of this study, the test item R0027603A did not induce damage to the chromosomes or the mitotic apparatus of rat bone marrow cells after a single oral administration at the dose-levels of 500, 1000 and 2000 mg/kg bw/day.

Ref.: 13

Comment

According to OECD TG 474 considerations should be undertaken in order to determine whether the substance would be able to reach the bone marrow. No documentation was provided that this has been done before the conduction of the study. Plasma samples were taken 0.5 and 2 hr after treatment for determination of plasma levels of the substance, but the analysis has not been performed. The SCCS is of the opinion that this information would have been essential to demonstrate the validity of the study. Further this would have been helpful for the evaluation of the substance, because information on systemic bioavailability could have been obtained.

Due to its structural and physicochemical properties of S85 (e.g. very low water solubility) the substance might not have reached the bone marrow in significant amounts by the oral administration pathway. Thus, the study is of limited value for evaluation of the genotoxic potential of the substance and is not in line with animal welfare principles, because its informative value had not been considered before conducting the study.

3.3.7. Carcinogenicity

No data submitted

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3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Guideline:	OECD TG 414
Species/strain:	Rat, Sprague-Dawley, Rj Han:SD
Group size:	24 mated female animals/dose
Test substance:	R0027603A
Batch:	Pilote 2
Purity:	98.2%
Dose levels:	100, 300 and 1000 mg/kg bw/d
Route:	oral (gavage)
Vehicle:	0.5% carboxymethylcellulose, aqueous solution
Dosing schedule:	daily from day 6 to day 20 post-coitum
Dosage volume:	5 ml/kg bw/d
Controls:	24 mated females receiving vehicle
GLP:	in compliance
Study period:	January 2009 – December 2009 (end of in vivo phase: 3 February 2009)

The study was preceded by a preliminary teratology study in Sprague Dawley rats according to OECD TG 414 using dose levels 100, 300 and 1000 mg/kg bw/d of batch 054D001 (end of in vivo phase: 04 May 2007). In this preliminary study, no signs of maternal toxicity were noted at any dose-level, pregnancy parameters were not affected and foetuses did not show external malformations or variations.

Three groups of 24 mated female Sprague-Dawley rats received daily oral gavage doses of 100, 300 or 1000 mg/kg bw/day of the test item from day 6 to day 20 *post coitum*. Control animals received vehicle only. Dosage form concentrations were checked on the first and last day of treatment. The animals were checked daily for mortality and clinical signs. Body weight of individual animals was recorded on days 2, 6, 9, 12, 15, 18 and 21 post coitum; and food consumption was recorded from day 2-6 and in 3-day intervals thereafter. On day 21 *post-coitum*, the animals were sacrificed and a macroscopic *post-mortem* examination of principal thoracic and abdominal organs was performed. After hysterectomy, the weights of gravid uteri were recorded. Ovaries and uteri were examined to determine the numbers of *corpora lutea*, implantation sites, resorptions, and live and dead foetuses were recorded. All foetuses were weighed and sexed, and the foetuses of the first 20 litters in each group were examined for external and soft tissue or skeletal (bone and cartilage) malformations and variations.

Results

Maternal data

There were no unscheduled deaths during the study. There were no test item treatment-related clinical signs. There were no effects of treatment on mean body weight, body weight change or mean food consumption at any dose-level. No test item treatment-related abnormalities were observed at necropsy. The mean gravid uterus weights were slightly higher in test item-treated groups than in the control group, but were not dose-related and did not reach a statistically significant level. At 300 mg/kg bw/day, one female had coloured deposit on the placenta, at 100 mg/kg bw/day, one female had a fused placenta with only one implantation site. As the incidence of these findings was low, a test item treatment-relationship was excluded. All pregnancy parameters (numbers of *corpora lutea*, implantation sites and foetuses, and the extent of pre- and post-implantation losses) were similar to control values in all groups.

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Foetal data

There were no effects of treatment on the sex ratio and foetal body weight. There were no external malformations and variations. One foetus in each test item-treated group lacked one kidney. A narrowed pulmonary trunk was observed in one high-dose foetus. Dilated renal pelvis was observed with higher foetal and litter incidences in the high-dose group than in controls but still within the historical control range. Dilated ureter was also observed in one of the animals having shown dilated renal pelvis at 1000 mg/kg bw/day. As the incidences of these soft-tissue findings were low, they were not ascribed to substance treatment. Skeletal malformations and variations – if present- were observed at low incidences did not reach statistically significant level and remained within the historical control ranges.

Under the experimental conditions of this study, 1000 mg/kg bw/day was identified as the No Observed Effect Adverse Level (NOAEL) for maternal and foetal developmental toxicity.

Ref.: 14

Comment

Mean foetal body weight was increased in the groups given 100 or 1000 mg/kg bw/day and reached a statistically significant level when compared to the control group. Since the difference from the mean control value was only +3% (6.02 g in the 100 and 1000 mg/kg bw/day groups vs. 5.82 g in the control group) and no dose-relationship was noted, the applicant considered mean foetal body weights to be unaffected by the test item treatment.

3.3.9. Toxicokinetics

Plasma levels of R0027603A were determined in satellite groups of a 13 week repeat-dose oral toxicity study (see section 3.3.5.2. Sub-chronic (90 days) oral toxicity).

Guideline:	OECD TG 408
Species/strain:	Rat, Sprague-Dawley; RjHan:SD
Group size:	6 male and 6 female animals per dose group
Test substance:	R0027603A
Batch:	Pilote 2
Purity:	97.7%
Dose levels:	100, 300 and 1000 mg/kg bw/d
Vehicle:	0.5% carboxymethylcellulose, aqueous solution
Dosing schedule:	daily oral doses (gavage) for 91 days
Dosage volume:	5 ml/kg bw/d
Controls:	2 male and 2 female animals receiving vehicle only
GLP:	in compliance
Study period:	Nov 2008 – December 2009 (end of in vivo phase: 27 February 2009)

Animals were dosed as described in section 3.3.5.2. In control animals, blood samples were taken samples were taken 0.5 hours after dosing on day 1 and on day 88. From test-item treated animals, blood samples were taken on day 1 and day 85 at the following six time points post dosing: 0.5, 1, 2, 4, 8 and 24 hr. Two animals per sex and dose group were sampled at each time point, so that each animal was sampled twice on day 1 and 85, respectively. Approximately 0.5 ml of venous blood was taken from the orbital sinus of each animal, the blood was centrifuged and the plasma was kept in individual tubes at -80°C until analysis. Test item in plasma was quantified by LC-MS/MS after protein extraction of the samples. Each study sample was analyzed in three runs.

Results

Analysis of plasmas from animals of treated groups showed that most of the plasma concentration values were in the range of the lower limit of quantification (1 ng/mL): from 1.11 to 2.50 in the 100 mg/kg bw/d group, from 1.05 to 2.73 in animals receiving 300 mg/kg bw/d, and from 1.51 to 3.37 in animals receiving 1000 mg/kg bw/d. As the obtained

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data for plasma concentrations were just above the lower limit of quantification and in the absence of long term stability data of the test item in blood samples (analysis were performed up to 10 months after sampling), the toxicokinetic parameters were not determined. The overall conclusion by the study authors from these plasma profiles was that the test item could be detected in plasma of animals, thus showing systemic exposure.

Ref.: 9

Comment

As the physico-chemical properties of bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane point towards a limited bioavailability, reliable data on toxicokinetics are considered essential for a risk assessment based on an oral NOAEL. The submitted study is of limited value for the assessment of oral bioavailability. It indicates that (at least) a small fraction of the administered dose of R0027603A becomes systemically bioavailable after oral administration, however, a quantification of bioavailability is not possible. Only the parent compound has been analyzed and no conclusions can be drawn with respect to probable breakdown products/metabolites. Further, the reliability of the data obtained is questionable: analyses were performed 10 months after sampling and stability of the substance over this period has not been proven. More proper investigation of some toxicokinetic parameters in the satellite group from the 90 day oral study might have enabled better estimation on oral systemic bioavailability (i.e. analysis of test item and probable metabolites in urines; immediate quantification of test item in plasma).

Plasma samples for substance analysis have also been taken from the in vivo bone marrow micronucleus test (Ref. 13), but these samples were not analysed.

3.3.10. Photo-induced toxicity

Phototoxicity

Guideline:	OECD 432 (2004); EU B.41
Species/strain:	Balb/c 3T3cells, clone 31
Test substance:	R0027603A
Batch:	Pilote 2
Purity:	98.2%
Concentrations:	6.313 – 1111.11 µg/ml
Irradiation:	5 J/cm ² UVA
Positive Control:	Chlorpromazine
Negative control:	PBS (phosphate-buffered saline) + 1% DMSO
Study period:	October 2008 – February 2009
GLP:	in compliance

Phototoxicity of R0027603A was determined by the in vitro 3T3 NRU phototoxicity test by comparing neutral red uptake into cells with and without irradiation. Target concentrations of the substance were obtained by first preparing solutions in DMSO which were further diluted with PBS. DMSO content in final solutions was 1%. Experiments were performed in duplicates. Cells were plated in 96-well microplates and cultivated for 24 hrs. On the second day, plates were incubated with different amounts of test substance and positive control. Negative controls were included in the respective plates. Plates selected for irradiation were exposed to UVA for 50 minutes to obtain 5 J/cm². On the third day Neutral Red uptake into the cells was measured using a spectrophotometer at an optical density of 550 nm. Determination of photo-irritation factors (PIFs) and mean photo effects (MPEs) was performed by using the phototox 2.0 software.

Results

The positive control chlorpromazine yielded a PIF of 55.358 ± 0.231 . From the irradiated and non-irradiated plates where R0027603A has been investigated, IC₅₀ values could not be derived; thus, MPE values were calculated. Due to non-homogeneity (precipitates and

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sediments) of R0027603A at the highest concentrations (1111.11 µg/ml, 526.32 µg/ml and 256.64 µg/ml) only concentrations without precipitation (101.01 µg/ml, 50.505 µg/ml, 25.253 µg/ml, 12.626 µg/ml and 6.313 µg/ml) were used for MPE calculation. MPE values of 0.114 (probable phototoxicity) and 0.045 (no phototoxicity) were obtained from the two replicate tests.

Ref.: 6

Comment

No firm conclusions on phototoxicity can be drawn from the results of this study. Due to precipitation and sedimentation, only five different concentrations equal or lower than 101.1 µg/ml) could be used for calculation of MPE values which yielded contradictory results in the two replicate experiments. In EU B.41 it is stated that "In contrast, if no toxicity was demonstrated (+UV and -UV) and if aqueous solubility limited concentrations to values less than 100 µg/ml, then compatibility of the test substance with the assay may be questioned and confirmatory testing should be considered (e.g. using an in vitro skin model or an ex vivo skin model or an in vivo test)". As R0027603A was not toxic and as there were problems in solubility, allowing only five doses ≤ 101.1 µg/ml to be tested, the substance can be regarded as non-compatible with the 3T3NRU test assay.

Photoirritation and photosensitization

Guideline: no corresponding OECD or EU Test guideline
 Species/strain: Guinea Pig (Hartley CrI)
 Group size: two groups of 10 animals (5 male, 5 female)
 one group of 5 animals (3 males, 2 females)
 Test substance: R0027603A
 Batch: Pilote 2
 Purity: 98.2%
 Concentration: 5% [w/w]
 Vehicle: acetone
 Positive control: 8-methoxypsoralen (0.1% [w/w] in corn oil) for photoirritation
 3,3',4',5-tetrachloro-salicylanilide (1% (induction) and 5% (challenge)) in
 purified water for photosensitization
 GLP: in compliance
 Study period: December 2008 – June 2009 (end of in vivo phase: 23 January 2009)

The test concentration of 5% was based on the outcome of a preliminary study performed in two male and two female animals applying concentrations between 2.5 and 25%. In the main test, 25 animals were allocated to three different treatment groups. For each treatment, a dose-volume of 0.1 mL of the test item in vehicle was applied by the cutaneous route. The irradiation doses of UV A and UV B were infra-erythematogenic. The photoirritant potential of R0027603A was investigated after the first treatment and/or irradiation performed on day 1 in all animals according to the subsequent scheme, cutaneous reactions at the treatment sites were scored on day 1 (1, 4 and 24 hr after single treatment)

Group	Number of animals	Anterior left flank	Anterior right flank
1	10	Test item	None
2	10	Test item and UV	Vehicle and UV
3	5	Vehicle and UV	UV

The photosensitizing potential of the test item was assessed by six topical applications and/or UV A + UV B irradiation during an induction period of 8 days (which included that of photoirritant assessment) on the anterior area of the right and left flanks of the animals of all groups. During a rest period of 13 days the animals received no treatment and no

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irradiation. On day 22, a challenge phase was initiated by topical application and/or UV A + UV B irradiation to the posterior area of the right and left flanks of the animals. Cutaneous reactions were recorded during the induction phase (24 hrs after each application) and on day 22 (1, 4, 24 and 4h hr after challenge application). The scheme for assessment of photosensitization is illustrated in the subsequent table:

Group	Number of animals	Induction phase (6 applications, days 1 – 8)		Challenge application (day22)	
		Anterior left flank	Anterior right flank	Posterior left flank	Posterior right flank
1	10	Test item	None	Test item	None
2	10	Test item + UV	Vehicle + UV	Test item + UV	Vehicle + UV
3	5	Vehicle + UV	UV	Test item + UV	UV

Results

Neither mortality nor clinical signs were observed during the study and the body weights were similar to those of controls. Questionable or discrete erythema (grade 0.5 or 1) was observed in some animals of group 2 and 3 at the 1 hr and 4 hr scoring times. During the induction phase, discrete erythema was observed in some animals of group 1. Dryness of the skin was observed in some animals of group 1 and 2. No cutaneous reactions were observed in the challenge phase.

Conclusion

Under the experimental conditions of this study, topical applications of R0027603A followed by UV irradiation did not induce photoirritant or photosensitizing effects in guinea pigs.

Ref.: 7

Photomutagenicity

Guideline: no corresponding OECD or EU Test Guideline
 Species/strain: *Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537, TA 102
 Test substance: R0027603A
 Batch: Pilote 2
 Purity: 98.2%
 Vehicle: DMSO
 Concentrations: 50, 158, 500, 1580 and 5000 µg/plate
 GLP: In compliance
 Study period: November – December 2008

The study was performed according to SCCNFP/0119/99

The concentrations selected for the study were based on the results of a range-finder experiment. Negative controls comprised treatments with vehicle either unirradiated or exposed to the chosen doses of UV light. Further negative controls comprised the photoactivating positive controls, 8-methoxypsoralen (8-MOP) and 9, 10-dimethyl-1,2-benzanthracene (DMBA), but in the absence of UV irradiation (for the photomutation experiment only). Positive controls in the presence of UV irradiation were 8-MOP (TA 1537) and DMBA (TA 102); positive controls in the absence of irradiation were 2-Nitrofluorene (TA 98), Sodium azide (TA 100, TA 1535), 9-Aminoacridine (TA 1537) and Mitomycin C (TA 102).

Once bacteria were plated, plates determined for irradiation were exposed to UVA light of 5 and 10 mJ/cm² (TA 98), 2 and 4 mJ/cm² (TA 100), 6 and 12 mJ/cm² (TA 1535), 8 and 16 mJ/cm² (TA 1537) and 60 and 120 mJ/cm² (TA 102), then incubated at 37°C in the dark for three days. Unirradiated plates were incubated by the same manner after treatment. Revertant colonies were then scored.

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Results

Results from positive and negative controls demonstrated the ability of the test system to detect mutagenic and photomutagenic effects. After the incubation period, precipitation of test article was observed at concentrations ≥ 1580 $\mu\text{g}/\text{plate}$. A marked reduction in revertant numbers was observed in TA102 (at the higher concentrations, without irradiation). All the tester strains showed at least some reduction in revertant numbers at the higher concentration(s) with low UV exposure treatments and the effect was more marked with high UV exposure treatments and also included a diminution of the background bacterial lawn at the highest treatment concentration in strain TA102. Following treatments of all the tester strains, both in the absence and presence of appropriate UV irradiation levels, there were no increases in revertant numbers that were statistically significant when the data were analysed at the 1% level using Dunnett's test.

Conclusion

From the results of the study it can be concluded that the test substance is not phototoxic or photo-mutagenic in this assay system.

Ref.: 8

Comment

The range-finder experiment was performed without UV irradiation.

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation

For the calculation of a MoS, the SED (systemic exposure dose) is compared to the No-observed-adverse effect-level (NOAEL). This means that an internal dose (the SED) is compared to an external dose (the NOAEL). The assessment approach taken for most cosmetic ingredients involves a route-to-route extrapolation for the MoS calculation, comparing the SED calculated for the dermal route to the NOAEL from an oral study.

The direct comparison of the dermal SED to an oral NOAEL implicitly assumes full oral bioavailability. In cases where there are indications that oral bioavailability is low, the NOAEL from the oral toxicity study needs to be corrected by the percentage of oral bioavailability in order to perform an adequate route-to-route extrapolation. This requires information on the extent of oral bioavailability.

In the case of bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane, an oral NOAEL of 1000 mg/kg bw/d is available for MoS calculation.

To a limited extent, conclusions on bioavailability and oral absorption of bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane can be drawn from its physico-chemical properties and from the 90 day oral toxicity study, where plasma levels of the substance have been determined in a satellite group. The very low water solubility of 0.0001 mg/l, the high molecular weight of 741.12 g/mol and the high $\log P_{ow}$ value of 9.4 indicate that the substance has low bioavailability through all relevant uptake routes (oral, dermal, inhalation). The results from the satellite group of the 90 day oral repeat dose study show that at least a small fraction of the administered substance becomes systemically bioavailable and that accumulation does not occur, but the measured plasma concentrations were very low (just above the lower limit of quantification).

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The available data point towards a very low oral bioavailability of S85, albeit they are insufficient to quantify the extent of oral absorption. While low systemic availability might be a favourable property of substances being intended to be used as UV filters, the SCCS is of the opinion that the submitted data are not appropriate for the calculation of a MoS based on an oral NOAEL, as an appropriate correction factor for the limited oral bioavailability cannot be derived.

To determine the percentage of oral absorption which would enable the safe use of the substance, a calculation has been done based on the available information.

Absorption through the skin	DA_p	= 4.46%
Amount of cosmetic product applied daily	A	= 18 g/d
Concentration of ingredient in finished product	C	= 10%
Typical body weight of human		= 60 kg
Systemic exposure dose (SED)		
(A (g/d) x 1000 mg/g x C (%) / 100 x DA_p (%) / 100 / 60) = 1.34 mg/kg bw/d		
No adverse observed effect level	NOAEL	= 1000 mg/kg bw/d
(13 week oral rat study)		

MOS = NOAEL/SED

The uncorrected NOAEL (assuming 100 % absorption) is 1000 mg/kg bw/d. The expected SED from the use of bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane in sun protection products is 1.34 mg/kg bw/day. To achieve a MoS of 100, the NOAEL corrected for oral availability therefore needs to be at least 134 mg/kg bw/day. Using an assessment approach based on route-to-route extrapolation, a sufficient high MoS would only be obtained if oral bioavailability of $\geq 13.4\%$ could be demonstrated.

A calculation of the MoS based on route-to-route extrapolation requires quantification of the oral availability of bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane. Ideally this could have been investigated more thoroughly in the oral *in vivo* studies performed.

Alternatively, a safety assessment avoiding the need for route-to-route extrapolation could be done if data from a valid 90-d dermal repeat dose study were available.

3.3.14. Discussion

Physico-chemical specifications

Bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane is a triazine derivative with a molecular weight of 741.12 g/mol. At ambient temperature it exists as a white powder, however, there is no information on particle size distribution of that powder. Water solubility is extremely low, but it is soluble in DMSO, Ethanol and Methanol. The UV/VIS spectrum yields a maximum at 310 nm. Log P_{ow} is stated to be 9.4. Four different batches of the substance have been used for toxicological investigations. All these batches have a high degree of purity ($\geq 97.3\%$ (HPLC)) which is further supported by sharp melting points of the respective batches. Suspensions in 0.5% aqueous carboxymethylcellulose (the vehicle used for most toxicological investigations) were stable for at least 11 days.

From these physico-chemical parameters, low systemic bioavailability by all uptake routes (oral, dermal, inhalation) can be anticipated.

Bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane is a secondary amine and thus prone to nitrosation. It should not be used in combination with nitrosating substances. The nitrosamine content should be < 50 ppb.

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Toxicity

The acute oral and dermal toxicity of bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane was low: the oral as well as the dermal LD₅₀ values in rats were higher than 2000 mg/kg bw. No data were provided on acute inhalation toxicity.

Irritation

Bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane was not irritating to the skin or to the eyes of rabbits.

Sensitisation

Bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane did not induce contact sensitization in the murine Local Lymph Node Assay.

Percutaneous absorption

A generally well conducted study on *in vitro* dermal absorption of a 10% sunscreen formulation of bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane is available. However, the study results exhibited a high degree of variability between individual samples. Thus, high standard deviations were obtained for amounts recovered from skin layers and receptor fluids which resulted in an overall high variability in absorbed dose. This might be due to physico-chemical properties of the test substance in combination with methodological issues (high molecular weight of the test item, high log P_{ow}, low solubility in receptor fluid). At 24 h post applications, the absorbed fraction was 1.5% ± 1.48 of the applied dose (corresponding to 8.25 ± 8.16 µg equiv/cm²). Due to high variability between samples, the mean plus two standard deviations (4.46%; 24.57 µg equiv/cm²) were used for MoS calculation.

Repeated dose toxicity

A 4-week and a 13-week oral toxicity study have been performed in rats. In the 4 week study, bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane caused increases in T3 and TSH levels in males and T4 levels in females at all dose-levels, as well as increased T4 levels in males at 1000 mg/kg bw/day. It also caused an increased number of lipophilic vacuoles in the glandular epithelium of the mammary gland in males treated at 1000 mg/kg bw/day. These effects were all considered non-adverse by the study authors. The effects seen in the 4 week study were not confirmed by the 13-week study, where no adverse effects up to a dose level of 1000 mg/kg bw/d were observed. In particular, substance-related effects on thyroid parameters and on male mammary gland were not seen after 13 week treatment. Thus, 1000 mg/kg bw/d was selected as NOAEL from the oral 13 week study.

Mutagenicity/Genotoxicity

Four *in vitro* assays (a bacterial gene mutation assay using different strains of *Salmonella typhimurium*, a photomutagenicity assay using different strains of salmonella typhimurium in the presence and absence of irradiation, an *in vitro* mammalian cell gene mutation test using L5178Y *tk*^{+/-} mouse lymphoma cells and an *in vitro* micronucleus test using L5178Y *tk*^{+/-} mouse lymphoma cells) are available. All these *in vitro* test were negative, however, the use of higher concentrations of test compound in the respective assays were hampered by formation of precipitates.

An *in vivo* mouse bone marrow micronucleus (using gavage administration) test was also negative. However, as it has not been demonstrated that the substance has reached the bone marrow, the results of this study are not of informative value for the safety assessment of the substance.

Under the respective conditions of the tests performed, bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane has no genotoxic potential.

Reproductive Toxicity

Bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane has been investigated in a developmental toxicity study according to OECD TG 414 up to a limit dose level of 1000

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mg/kg bw/d. No test-item related adverse maternal and foetal effects were observed and a NOAEL of 1000 mg/kg bw/d was derived for maternal and foetal effects.

It should be noted, however, that this study does not allow drawing firm conclusions on male and female fertility. Thus, no conclusions on probable adverse effects of R0027603A on fertility can be drawn when discussing its reprotoxic effects.

Toxicokinetics

To a limited extent, conclusions on toxicokinetics of bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane can be drawn from its physico-chemical properties and from the 90 day oral toxicity study, where plasma levels of the substance were determined in a satellite group. The very low water solubility of 0.0001 mg/l, the high molecular weight of 741.12 g/mol and the high log P_{ow} value of 9.4 indicate that the substance has low bioavailability through the relevant uptake routes (oral, dermal, inhalation). This is supported by the results from the satellite group of the 90 day oral repeat dose study. From this study it can be concluded that at least a small fraction of the administered substance can be systemically bioavailable and that accumulation does not occur, but the measured plasma concentrations were very low (just above the lower limit of quantification). The presence of potential metabolites has not been investigated. The extent of oral absorption cannot be determined from this study.

Phototoxicity

The phototoxicity of bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane has been investigated using the *in vitro* Neutral Red phototoxicity test, by an *in vivo* photosensitizing and photoirritation assay and by an *in vitro* photomutagenicity assay. While no conclusions can be drawn from the results of the *in vitro* phototoxicity test (due to contradictory results and methodological deficiencies), the test item was neither photoirritating nor photosensitizing in the *in vivo* assay performed. Further, the substance was not photomutagenic.

Thus, under the assay conditions used in the respective test, bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane was neither phototoxic nor photomutagenic.

4. CONCLUSION

The SCCS considers that the safe use of Bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane as an UV-filter in cosmetic products in a concentration up to maximum 10.0% cannot be assessed based on the available data. As low oral bioavailability of the substance is anticipated, a quantitative risk assessment based on the oral NOAEL cannot be performed in the absence of adequate information on the extent of internal exposure.

In order to properly assess the safety of Bis(butylbenzoate) diaminotriazine aminopropyltrisiloxane additional data such as a 90-day dermal toxicity study or sound data on oral bioavailability are required. However, the SCCS is aware that such studies are no longer permitted in the European Union.

Further recommendation

The SCCS already addresses the issue of limited bioavailability in the revisions of its Notes of Guidance¹. It is of the opinion that further efforts should be undertaken to develop a general strategy for the assessment of substances with low solubility and low systemic bioavailability.

¹ SCCS/1416/11, (http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_s_004.pdf)

5. MINORITY OPINION

Not applicable

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