THE SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD PRODUCTS INTENDED FOR CONSUMERS

OPINION

CONCERNING

BENZISOTHIAZOLINONE

COLIPA n° P 96

Adopted by the SCCNFP on 1 July 2004
By means of the written procedure

1. Terms of Reference

1.1 Context of the question

According to Article 4, 1. (e) and (f) of Council Directive 76/768/EEC, Member States shall prohibit the marketing of cosmetic products containing preservatives other than those listed in Annex VI, part 1, beyond the limits and outside the conditions laid down, unless other concentrations are used for specific purposes apparent from the presentation of the product.

Benzisothiazolinone is currently not listed in Annex VI and therefore cannot be used as a preservative.

The European Commission received a request for inclusion of Benzisothiazolinone in Annex VI, part 1 – List of preservatives allowed – to Council Directive 76/768/EEC.

1.2 Request to the SCCNFP

The SCCNFP is requested to answer the following questions:

* *Does the data provided in the safety dossier justify that Benzisothiazolinone is safe when used as a preservative up to a maximum authorised concentration of 0.0050% (50 ppm) in cosmetic products?*

* *Does the SCCNFP recommend any limitations and requirements or conditions of use for Benzisothiazolinone in cosmetic products based on the toxicological profile and risk assessment presented in the safety dossier?*

1.3 Statement on the toxicological evaluation

The SCCNFP is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food products intended for consumers.

The Commission's general policy regarding research on animals supports the development of alternative methods to replace or to reduce animal testing when possible. In this context, the SCCNFP has a specific working group on alternatives to animal testing which, in co-operation with other Commission services such as ECVAM (European Centre for Validation of Alternative Methods), evaluates these methods.
The extent to which these validated methods are applicable to cosmetic products and its ingredients is a matter of the SCCNFP.

SCCNFP opinions include evaluations of experiments using laboratory animals; such tests are conducted in accordance with all legal provisions and preferably under chemical law regulations. Only in cases where no alternative method is available will such tests be evaluated and the resulting data accepted, in order to meet the fundamental requirements of the protection of consumer health.

2. Toxicological Evaluation and Characterisation

2.1. General

Benzisothiazolinone is listed in the EU Cosmetics Inventory, Section 1 with indicated function "antimicrobial", without any restrictions.

2.1.1. Primary name

Benzisothiazolinone (INCI)

2.1.2. Chemical names

1,2-Benzisothiazol-3(2H)-one (IUPAC)
1,2-Benzisothiazol-3-one
1,2-Benzisothiazolin-3-one
Benzo[a]isothiazol-3-one

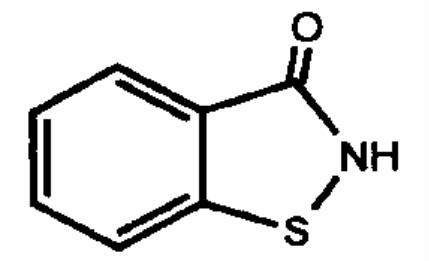
2.1.3. Trade names and abbreviations

COLIPA n°	:	P96
Trade name	:	BIT; Thor BIT; ACTIDE® BIT; Microcare® BIT; Nuosept BIT Technical; Promex BIT

2.1.4. CAS / EINECS number

CAS	:	2634-33-5
EINECS	:	220-120-9

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula	:	C_7H_5NOS
Mol. weight	:	151.19 g/mol

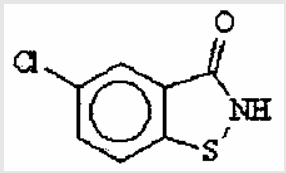
2.1.7. Purity, composition and substance codes

All the tests carried out (with the exception of the BCOP, 3T3 NRU and SIRC NRU tests as well as the human in-use studies) were required in terms of the requirements for registration of products under the European Biocidal Products Directive, and that none were required exclusively for purposes of registration under the European Cosmetics Directive 76/768/EEC.

Substance code	:	/
Batches used	:	batch n° 2001 014
Purity	:	74.02-84.02 % w/w (84 % corresponds to 15 % water content) > 99 % w/w on a dry basis
Loss on drying	:	/
Water content	:	15-29 % w/w (for batch n° 2001 014 spec., 20 % max, found 15 %)
Ash content	:	/
Sodium chloride	:	< 0.1 % w/w (for batch n° 2001 014 spec., 0.2 % max, found 0.02 %)
Lead	:	/
Mercury	:	/
Arsenic	:	/
Iron	:	/

Impurities

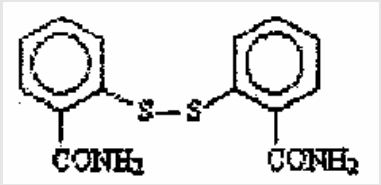
- 5-Chloro-1,2-benzisothiazolin-3(2H)-one : 0.15-0.22 % w/w
  (for batch n° 2001 014, specification 4 ppm max, found 3 ppm)



CAS n° : 4337-43-3
EINECS n° : 224-385-1

emp. formula $C_7H_4Cl\,N_2\,O\,S$

- 2,2-Dichlorobisbenzamide : < 0.1 % w/w
  (for batch n° 2001 014, specification 0.5 % max, found 0.03 %)



CAS n° : 2527-57-3
EINECS n° : 219-766-4

emp. formula $C_{14}H_{12}\,N_2\,O_2\,S_2$

Residual solvents: /

2.1.8. Physical properties

Appearance	:	Off-white to yellowish solid
Melting point	:	156.6 °C (Directive 92/69/EEC, A1)
Boiling point	:	327.6 °C (Directive 84/449/EEC, A2)
Density	:	1.483 g/cm^3 at 20 °C (OECD Guideline 109)
Vapour Press.	:	0.0000037 hPa at 25 °C (Directive 92/69/EEC, A4)
pK_a	:	7.3 at 25 °C

Log P_{ow} : 0.4 at 20 °C (OECD Guideline 117)

Effect of pH and temperature on Log P_{ow} (OECD Guideline 117 (HPLC))

Log P_{ow} : 0.99 at 20 °C and pH 5
Log P_{ow} : 0.63 10 °C and at pH 7
Log P_{ow} : 0.70 at 20 °C and pH 7
Log P_{ow} : 0.76 at 30 °C and pH 7
Log P_{ow} : – 0.90 at 20 °C and pH 9

Conclusions
With increasing pH from 5 to 9, the Log P_{ow} decreases very strongly. Only a slight increase of Log P_{ow} is observed between 10 °C and 30 °C.

2.1.9. Solubility

Solubility in water : 1.1 g/l (0.11 %) at 20°C
6.0 g/l (0.60 %) at 30°C (Directive 92/69/EEC, A6)

Effect of pH and temperature on solubility in water (OECD Guideline 105)
at 10°C and pH 4.8 : 0.736 g/l
at 20°C and pH 4.8 : 0.938 g/l
at 30°C and pH 4.8 : 1.198 g/l
at 20°C and pH 6.7 : 1.288 g/l
at 20°C and pH 9.1 : 1.651 g/l

2.1.10. Stability

No data

General comments on analytical and physico-chemical characterisation

No data on stability are provided.

2.2. Function and uses

Benzisothiazolinone is proposed for use as a preservative in leave-on and rinse-off cosmetic products, excluding oral care products at a maximum final concentration of 50 ppm.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

Guideline	:	/
Method	:	EPA OPP 81-1
Species/strain	:	Rat, Sprague-Dawley derived, albino
Group size	:	30 (3 groups of 5 ♂ and 5 ♀ each)
Test item	:	Benzisothiazolinone
Test substance	:	Nuosept BIT Technical
Batch no	:	# 170-138 (PSL Code no E50629-1R (powder)
Purity	:	1,2-Benzisothiazolin-3-one 82.3 %; water 17.7 %
Dose	:	1000, 2000 and 5000 mg/kg/bw/day test substance (823, 1646 and 4115 mg/kg active ingredient)
Vehicle	:	Water; the test substance was applied as 40 % w/w suspension in water
Route	:	oral intubation/gavage
GLP	:	in compliance

Results
Based on the findings, the Acute Oral Defined LD_{50} of Nuosept Bit Technical, Lot #170-138 calculated by Probit Analysis was 1450 milligrams of the test substance per kilogram of bodyweight (when administered as a 40 % w/w suspension in distilled water) with 95 % Confidence Limits of 2004 mg/kg (upper) and 1.049 mg/kg (lower). The LD_{50} for males was 2.100 mg/kg with 95 % Confidence Limits of 5.029 mg/kg (upper) and 877 mg/kg (lower). The data does not permit calculation of the LD_{50} for females by Probit Analysis. Graphically, the LD_{50} for females was estimated to be 1.050 mg/kg.

Ref.: 10

2.3.2. Acute dermal toxicity

Guideline	:	/
Method	:	EPA OPP 81-2
Species/strain	:	Rat, Sprague-Dawley derived, albino
Group size	:	10 (5 male/5 female)
Test item	:	Benzisothiazolinone
Test substance	:	Nuosept BIT Technical
Batch no	:	# 170-138 (PSL Code no E50629-1R (powder)
Purity	:	1,2-Benzisothiazolin-3-one 82.3 %; water 17.7 %
Dose	:	5000 mg/kg/bw/day (Limit test) (4115 mg/kg active ingredient)
Vehicle	:	Water; the test substance was moistened with water for application (1 ml water/1 g test substance)
Route:		topical application (24 h)
Exposure period	:	1 x 24 h. observation period 14 d
GLP	:	in compliance

Results

An Acute Dermal Toxicity test was conducted with rats to determine the potential for Nuosept Bit Technical, Lot # 170-138 to produce toxicity after topical application. Based on the results of testing, the single dose Acute Dermal Toxicity LD_{50} of the test substance is greater than 5000 mg/kg of bodyweight when applied as received, moistened with distilled water.
5000 mg of the test substance per kilogram of bodyweight was moistened with distilled water and applied to the skin of ten healthy rats (224-232 g) for 24 hours. The animals were observed for signs of gross toxicity and mortality at least once daily for another 14 days. Bodyweights were recorded just prior to application and again on days 7 and 14 (termination). Necropsies were performed on all animals at terminal sacrifice.
All animals survived, gained weight and appeared active and healthy during the study. There were no signs of gross toxicity, adverse pharmacologic effects or abnormal behaviour. Gross necropsy findings at terminal sacrifice were generally unremarkable.

Ref.: 11

2.3.3. Acute inhalation toxicity

No data

2.3.4. Repeated dose oral toxicity

Guideline	:	OECD 407
Species/strain	:	Rat, Wistar Hsd Cpb:WU
Group size	:	12 (6 male/6 female)
Test item	:	Benzisothiazolinone, Code 072/1-PBP
Test substance	:	Promex BIT (paste)
Purity	:	1,2-Benzisothiazolin-3-one, 84.29 %; water, 15 %, purity of active ingredient on dry weight basis, 99.02 %
Batch no	:	2001 014//sample no. KP 070601//cb 181100
Dose levels	:	0, 15, 45 and 135 mg/kg bw/day (12.63, 37.89 and 113.67 mg/kg/a.i.), suspended in 0.5 % CMC
Route	:	daily oral intubation/gavage
Exposure period	:	28 days
GLP	:	in compliance

Results
Oral administration of 1,2-Benzisothiazolin-3-one, by gavage in Wistar rats at the dose of 15 mg/kg/day bw (12.63 mg a.i./kg/day) had no adverse effect on general health, neurological effects, growth, food consumption, haematological and clinical chemistry parameters, organ weights and its ratios, gross and histopathological changes.
Treatment related signs of slight salivation were observed in all the males in the main group at 135 mg/kg/day and its recovery group from treatment day 17 and in two females from the test group and two in the recovery group from treatment day 20 till the end of the treatment period. During the recovery period, treatment related signs of salivation were not observed in the 135 mg/kg/day group indicating the reversibility of the effect.
Body weight was unaffected in the 15 and 45 mg/kg/day groups. At 135mg/kg/day there was a significant decrease in weight gain and cumulative weight gain in the male group with the exception of the first week where it was not statistically significant.
Weekly body weights were significantly lower in the high dose (135 mg/kg/day) recovery period for males and on weeks 4, 5, and 6 for females.

The only treatment related effect seen in the 45-mg/kg/day group was that lesions were seen in the non-glandular stomach, which could be related to the irritant/corrosive effect of the test substance and were not seen in the other groups.
The NOAEL in this study was 15 mg/kg/day bw (12.63 mg a.i./kg/day).

Ref.: 12

2.3.5. Repeated dose dermal toxicity

No data

2.3.6. Repeated dose inhalation toxicity

No data

2.3.7. Sub-chronic oral toxicity

Guideline	:	OECD 408
Species/strain	:	Rat, Wistar
Group size	:	Total 20; 10 male/10 female
Test item	:	Benzisothiazolinone
Batch no	:	G00Z-0600-1907-13//072/1.PBP//2001 014//KP 070601
Purity	:	1,2-Benzisothiazolin-3-one, 84.29 %; water 15 %; purity of active ingredient on dry weight base 99.1 %
Dose levels	:	10, 30 and 75 mg/kg/bw/day (8.42, 25.26 and 63.15 mg/kg/bw/day a.i.)
Vehicle	:	0.5 % Carboxymethyl-Cellulose (CMC)
Route	:	daily oral intubation/gavage
Exposure period	:	90 days
GLP	:	in compliance

Results
The oral administration of 1,2-Benzisothiazolin-3-one by gavage in Wistar rats at the dose of 10 mg/kg/day (8.42 mg a.i./kg/day) body weight had no adverse effect on general health, neurological effects, growth, food consumption, haematological and clinical chemistry parameters, sperm evaluation, organ weights and its ratios and gross and histopathological changes.
At 30 mg/kg/day (25.26 mg a.i./kg/day) there were no treatment related clinical signs. Food consumption was lower in females in weeks 2 and 4. There were some changes primarily in the non glandular stomach region both macroscopically and histologically which were considered treatment related and were reversible. These effects may have been due to the irritant nature to the test substance.
At 75 mg/kg/day (63.15 mg a.i./kg/day) there was a significant reduction in food consumption in males and less so in females, which returned to control levels in the recovery period.
The NOAEL in this study was 10 mg/kg/day/bw (8.42 mg a.i./kg/day).

Ref.: 13

2.3.8. Sub-chronic dermal toxicity

No data

2.3.9. Sub-chronic inhalation toxicity

No data

2.3.10. Chronic toxicity

No data

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

Guideline	:	/
Method	:	EPA OPP 81-5
Species/strain	:	New Zealand albino rabbits
Group size	:	6, 3 males and 3 females
Test substance	:	Nuosept BIT Technical
Batch number	:	# 170-138
Purity	:	1,2-Benzisothiazolin-3-one, 82.3 %; Water, 17.7 %
Dose	:	Slurry of 0.5 g in 0.5 ml water
Exposure	:	Semi occlusive
Exposure time	:	4 hour(s)
GLP	:	in compliance

Results
The test substance was moistened with water for application (0.5 ml water/0.5 g test substance = 41.15% a.i.) All animals appeared active and healthy throughout the duration of the study. One hour after patch removal, well-defined moderate erythema and oedema was noted at all treated sites. This decreased with time. Desquamation occurred at one site and all animals were free of erythema and oedema by day 7. The primary dermal irritation index was 2.8 based on the 1-72 hours evaluation averages.

Conclusion
Benzisothiazolinone is moderately skin irritating.

Ref.: 14

Human study

50 healthy human volunteers (20 males and 30 females) were recruited for a randomised double blind open epicutaneous application study to compare the effects of a cream with and without the preservative Microcare® SI. The protocol was approved by an independent ethics committee and the test was performed in compliance with the principles of the Declaration of Helsinki. The test substance was Microcare® SI - an aqueous blend of 2.5% of methylisothiazolinone (MIT) and 2.5% of benzisothiazolinone (BIT).

The test cream contained Microcare® SI at a level of 0.3% w/w (150ppm) of which 0.15%

w/w (75ppm) was 1,2-benzisothiazolinone. Analysis of the test products showed that the formulation contained slightly lower concentrations of isothiazolinones than expected, equivalent to an addition rate of 0.2-0.25%, such as would be used in cosmetic applications. However, the reduction does not invalidate the study as regards the intended use.

The test subjects were aged between 19-60 years. Subjects with known sensitivity to isothiazolinones were excluded. 47 subjects completed the study as planned, and dairy cards and product weights were available. Three subjects withdraw for reasons not related to the study. The test subjects applied twice daily for 4 weeks 1.5 ml of test cream and vehicle to the inner aspects of both forearms in a randomised and blinded fashion. The test sites were assessed after 2 and 4 weeks and the skin reactions scored according to a 5 point ranking scale.

Results
7 subjects reported redness or itching, tingling or stinging sensation upon application of the test cream. The reaction was reported to disappear after the product had been absorbed into the skin. Determining causation demonstrated that all 7 subjects experienced sensations following application of the base cream whereas only 5 experienced sensations following the test cream.

Conclusion
A skin cream preserved with a mixture of MIT/BIT 1:1 at a concentration of 75 ppm of each active was tolerated as well as the vehicle cream under the conditions of the test.

Ref.: 15

2.4.2. Irritation (mucous membranes)

Guideline	:	/
Method	:	EPA OPP 81-4
Species/strain	:	New Zealand albino rabbits
Group size	:	9, 3 males and 3 females
Test substance	:	Nuosept BIT Technical
Batch number	:	# 170-138
Purity	:	1,2-Benzisothiazolin-3-one, 82.3 %; Water, 17.7 %
Dose	:	0.1 g of the undiluted test substance was instilled into the right eye. The treated eyes of 3 rabbits were rinsed 20-30 seconds after instillation; the eyes of the remaining 6 animals were not rinsed.
Exposure time	:	48 hours
GLP	:	in compliance

Results
From 1 to 48 hours, all treated eyes exhibited severe to maximal irritation including corneal opacity, iritis and conjunctivitis. Overall the severity of irritation increased with time. Due to the irreversible nature of the irritation the test was terminated after 48 hours.

Conclusion
The test substance was severely irritating to the rabbit eye.

Ref.: 16

An assessment of the eye irritancy potential of 1,2-benzisothiazolin-3-one using the Bovine Corneal Opacity and Permeability assay in vitro

Guideline	:	/
Method	:	INVITTOX Protocol 124
Species	:	Cow
Number corneas	:	45
Test substance	:	1,2-Benzisothiazolin-3-one
Batch number	:	LHW 1355
Purity	:	>99%
Dose	:	75, 750 and 7500 ppm aqueous solution of BIT prepared as its sodium salt
Exposure time	:	10 minutes
GLP	:	in compliance

Bovine eyes mounted on holders and incubated with Minimal Essential Medium (MEM) were exposed to test article or positive or negative control. After 10 minutes exposure the corneas were rinsed and again incubated with media.
Corneal opacity was measured with an opacimeter and corneal permeability was determined using sodium fluorescein and measured spectrophotometrically.
The corneal opacity and permeability were combined to give an in-vitro score

Results
The mean in vitro score for BIT at 7500 ppm was 3.012, at 750 ppm 0.666 and at 75 ppm -0.207, compared with 0.490, 0.416 and 0.449 for saline (negative control) and 50.73, 50.23 and 50.25 for ethanol(positive control), respectively.

Conclusion
Benzisothiazolinone was considered to be non-irritant to the eye at all tested concentrations in the BCOP assay under the conditions of the test.

Comment
The method used is not a validated in-vitro method

Ref.: 17

An assessment of the cytotoxicity of 1,2 benzisothiazolin-3-one by in vitro Neutral Red Uptake Assay using BalbC 3T3 & SIRC mammalian cell lines

Guideline	:	ISO 10993-5
Species	:	BALB/c 3T3 Mouse fibroblast cell line SIRC Rabbit corneal cell line
Test substance	:	1,2-Benzisothiazolin-3-one
Batch number	:	LHW 1355
Purity	:	> 99%
Dose	:	0 -100 ppm range finding; 0 -10 ppm testing
Exposure time	:	24 hours
GLP	:	in compliance

Results
The effects of the test substance on cell viability of the two different cell lines was measured by the neutral red uptake. A best-fit dose-response curve for each set of experiments was calculated from the data using non- linear regression and the respective EC_{50} value was calculated. The EC_{50} for 3T3 cells was 3.1414 ppm and that for SIRC cells was 3.6666.
These results may be compared with results previously obtained for other preservative preparations, as illustrated in the following table demonstrating that BIT is less cytotoxic than CIT/MIT, yet more cytotoxic than other commonly used preservatives.

Cytotoxicity values for cosmetic preservatives

Cosmetic Preservative	EC_{50} 3T3	EC_{50} SIRC
Chloromethylisothiazolinone/methylisothiazolinone (3:1)	1.19	1.89
Methylisothiazolinone	5.76	5.98
Methyldibromoglutaronitrile 20% (in phenoxyethnol)	28.4	29.36
Methyl/ethyl/propyl/butyl/isobutyl parabens in phenoxyethanol	439.5	489.0

Ref.: 18

2.5. Sensitisation

Guinea Pig Maximization Test (Magnusson and Kligman)

Guideline	:	OECD 406
Species/ strain	:	Albino Dunkin Hartley guinea pigs
Size	:	38 (20 test, 10 control, 8 range-finding)
Test substance	:	1,2-Benzisothiazolin-3-one 79.8%, water 19.2%
Batch number	:	386-3
Purity	:	79.8%1,2-Benzisothiazolin-3-one (BIT), water, 19.2 % Diamide content 0.28%, PCP < 1 ppm.
Dosage	:	1st Induction 0.1 % w/v intracutaneous 2nd Induction 20% w/v occlusive epicutaneous 3rd Challenge 10% w/v occlusive epicutaneous
Vehicle	:	Corn seed oil (1st and 2nd concentration), FCA/water (1st concentration), Ethanol (3rd concentration)
GLP	:	in compliance

Results
Results from 2 animals in range-finding studies indicated that 0.1 % w/v in cottonseed oil should be used for intradermal induction.
In topical range-finding studies in 4 animals, it was indicated that 20% in cottonseed oil was minimally irritant and was suitable for topical induction. In further topical range-finding studies in 2 animals it was found that 10% in ethanol was suitable for challenge.
Following challenge, 9 out of 20 animals in the test group reacted positively to 10% w/v test article in ethanol at 24 or 48-hour examinations, giving a response incidence of 45%.

Conclusion
BIT is a moderate contact sensitizer

Ref.: 19

Dermal Sensitisation Test -Buehler Method

Guideline	:	/
Method	:	EPA OPP 81-6
Species/strain	:	Hartley albino guinea pigs
Size	:	8 for range finding, 30 for test protocol
Test substance	:	Nuosept Bit technical (82.3 %1,2-Benzisothiazolin-3-one, water 17.7 %)
Batch number	:	#170-138
Purity	:	1,2-Benzisothiazolin-3-one (BIT) 82.3% ai.
Dosage	:	Induction: Weekly application of 0.3 g of test substance 95% w/w in corn seed oil for 3 consecutive weeks. Challenge: 14 days after last induction with same dose as induction to naive site
Vehicle	:	Corn seed oil
GLP	:	in compliance

Results
It was found that a 6 hour exposure under 25mm Hilltop chambers to 95% w/w (78.19% active) BIT powder in corn oil was suitable for the test group and 0.04% DNCB in acetone was suitable for testing of the control group. No reaction was seen at any test or naive control site following challenge. 7/10 positive control animals exhibited signs of reaction to challenge at 24 hours. This reaction persisted in 5 animals at 48 hours.

Conclusion
The test substance was not a sensitizer in the Buehler test.

Ref.: 20

Human study

15 healthy human volunteer patients (2 males and 13 females) were recruited for a randomised double blind open epicutaneous application study to compare the effects of a cream with and without the preservative Microcare® SI. The volunteer subjects were previously diagnosed as being sensitized to chlormethylisothiazolinone.
The protocol was approved by an independent ethics committee and the test was performed in compliance with the principles of the Declaration of Helsinki.

The test substance was Microcare® SI - an aqueous blend of 2.5% of methylisothiazolinone (MIT) and 2.5% of benzisothiazolinone (BIT). The test cream Doublebase ™ contained Microcare® SI at a level of 0.3% w/w (150ppm) of which 0.15% w/w (75ppm) was 1,2-benzisothiazolinone.
The test subjects were instructed to apply twice daily for 4 weeks 1-1.5 ml of test cream and vehicle to the inner aspects of both forearms in a randomised and blinded fashion. They were asked to complete a dairy to record usage of the products, which were weighed at the end of study.

Results

The test subjects aged between 25-66 years. 10 subjects completed the study as planned, and dairy cards and product weights were available. Three subjects were withdrawn from the study due to adverse reactions, 2 subjects after less than 7 days and 1 after 21 days. They all noticed flare of eczema on their forearms. Two subjects were lost to follow up.
After four weeks application, the frequency of each assessment grade is summarised as follows.

Assessment grading	Product Code 1	2
Withdrawn	3	3
No Assessment	2	2
No Visible Redness	9	10
Slight Redness	0	0
Distinct Redness	1	0
Total	15	15

Products implicated due to adverse reactions, including those of withdrawn subjects are summarised as follows.

Product(s) Implicated	*Frequency*
Product 1	3
Product 2	3
Product 1> Product 2	1
Product 1> Product 2	0

Investigators conclusions
There were three cases of flare of eczema related to the study preparations.
Two were associated with the application of product 1, and one with product 2. However, both products have been applied to eight subjects who have experienced no reactions.
Without unblinding the study, it would appear from these results that Microcare® SI can be tolerated by some, but not all, subjects who are known to be sensitised to chloromethyl isothiazolinone.
Comments
The study included a low number of test subjects, there was no detailed description of their existing Chloromethylisothiazolinone (CMI) allergy, lack of assessment of skin reactions under the 4 week study period, and a significant variation in product usage, from around 35 grams to 110 grams per test period. Limited conclusions can be drawn. The test products seem to elicit dermatitis in some test subjects.

Ref.: 22

Local Lymph Node Assay and Human Repeat Insult Patch Test

The relative sensitising potencies and potential for cross sensitisation/reaction of chloromethylisothiazolinone (CMIT/MIT), methyl trimethylene isothiazolinone (MTI) and benzisothiazolinone (BIT) were investigated.

Results are summarized in Table below.

Using the LLNA, the EC3 for Benzisothiazolinone was established at 10.4%, that for MTI at 2% and CMIT/MIT at 0.01 %.
Confirmatory testing using the HRIPT resulted in no reactions to BIT at 360ppm and 9% of volunteers reacting at 725ppm. There were also no reactions to CMIT/MIT at 10ppm and 4.4% reacted at 20 ppm, For MTI there were no reactions at 100ppm but 16% reacted at 300 ppm. From these results it was concluded that the threshold for sensitisation for CMIT/MIT was 10 to 20 ppm. The estimation of a threshold for BIT was complicated by the presence in the BIT formulation of ethylenediamine (a known skin sensitiser) however the conclusion was reached that "a realistic no effect level was in the region of 500ppm"

Human Repeat Insult Patch Test and LLNA results for BIT, MTI and CIT/MIT

Biocide	Test concentration (ppm)	Proportion of panel sensitised (%)	EC_3
BIT	725	5/58 (9%)	10.4%
BIT	360	0/54 (0%)	
MTI	300	3/19 (16%)	2.0%
MTI	100	0/211 (0%)	
CIT/MIT	20	2/45 (4.4%)	0.007%-0.01%
CIT/MIT	10	0/175 (0%)	

Ref.: 23

From the dermatological literature case reports describe allergic contact dermatitis to benzisothiazolinone. It is a well documented contact allergen. However, its potency is lower than other marketed cosmetic preservatives, and the irritancy profile makes it a difficult contact allergen to test with.

Ref.: a, b, c, d

2.6. Reproductive toxicity

No data

2.7. Toxicokinetics (incl. Percutaneous Absorption)

No data.

2.8. Mutagenicity / Genotoxicity

2.8.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial Reverse Mutation Assay

Guideline	:	OECD 471 (1997)
Species/strain	:	*Salmonella typhimurium* TA98, TA1537, TA100, TA1535 *Escherichia coli* WP2uvrA pkM 101
Test substance	:	Promex BIT 1,2–Benzisothiazolin-3-one
Batch number	:	2001 014
Lot number	:	KP 070601
Purity	:	99.02 %
Concentrations	:	0, 20, 35, 60, 100 and 175 µg/plate (1st experiment) 0, 30, 50, 75, 120 and 180 µg/plate (2nd experiment)
Replicate	:	3 plates/concentration
Positive controls	:	according to guideline
Metabolic activation	:	Aroclor induced rat liver homogenate
GLP	:	in compliance

Results
Toxicity: in a preliminary study with a series of concentrations up to 5000 µg/plate, there was a decrease in the mean number of revertants from the concentrations up to 160 µg/plate.

Mutagenicity: only the lowest doses could be evaluated in comparison with the untreated plates (10-20 µg/plate).

The study cannot be used for the evaluation due to the high toxicity of the test item towards the bacterial cells.

Ref.: 24

***In vitro* Mammalian Cell Gene Mutation Test**

Guideline	:	OECD 476 (1997)
Species/strain	:	CHO-K1 (Chinese hamster ovary cells) HPRT locus
Test substance	:	Promex BIT; 1,2-Benzisothiazolin-3-one
Batch number	:	2001 014
Lot number	:	KP 070601
Purity	:	99.02%
Concentrations	:	0, 0.65, 1.30, 2.60, 5.20 µg/ml
Treatment time	:	5 hours, with and without metabolic activation
Replicate	:	2 experiments in the same conditions.
Positive controls	:	B(a)P; EMS
Metabolic activation	:	Aroclor 1254 induced rat liver homogenate.
GLP	:	in compliance

Results
Toxicity: in the presence of metabolic activation a toxic effect produced by the test item between 4 and 6 µg/ml was observed; in the absence of metabolic activation a toxic effect produced by the test item was observed between 2 and 4 µg/ml. The toxic doses reduced the survival to less the 50 % of the untreated cells.

Mutagenicity: there was no increase of mutants in the treatment with the test substance, in the presence and in the absence of metabolic activation after 5 hours of treatment

In the absence of metabolic activation, a treatment of 20 hours was not performed as suggested by the guideline.
The study indicates that the test item is not mutagenic in the condition of the test.

Ref. 25

***In vitro* Mammalian Chromosome Aberration test**

Guideline	:	OECD 473 (1997)
Species/strain	:	CHO-K1 cell line (Chinese hamster ovary cells)
Test substance	:	Promex BIT; 1,2–Benzisothiazolin-3-one
Batch number	:	2001 014
Lot number	:	KP 070601
Purity	:	99.02%
Concentrations	:	0, 1.6, 3.2, 6.4 µg/ml in the presence of S9 0, 1.25, 2.50, 5.0 µg/ml in the absence of S9
Replicate	:	2 experiments (200 metaphases analysed)
Treatment time	:	1st experiment: 3 hours 2nd experiment: 3 hours, in the presence of S9; 19 hours, in the absence of S9
Positive controls	:	CPA (55 µg/ml); EMS (600 µg/ml)
Metabolic activation	:	Aroclor 1254 induced rat live homogenate
GLP	:	in compliance.

Results
Toxicity: 2 preliminary experiments showed that the test item was toxic at concentrations between 75 and 5000 µg/ml and between 14 and 58.94 µg/ml.

Clastogenicity: the study indicates that the test item induced chromosome aberrations at the maximum tested dose in the presence of a metabolic activation, and at all concentrations, in the absence of a metabolic activation system.

The test item is clastogenic on CHO mammalian cells.

Ref.: 26

2.8.2. Mutagenicity / Genotoxicity *in vivo*

Mammalian Erythrocyte Micronucleus Test

Guideline	:	OECD 474 (1997)
Species/strain	:	Swiss albino mice-HsdOla: MF1 strain
Test substance	:	Promex BIT; 1,2–Benzisothiazolin-3-one
Batch number	:	2001 014
Lot number	:	KP 070601
Purity	:	99.02 %
Doses	:	63.15; 126.3; 210.5 mg/kg a.i.
Treatment	:	oral (gavage) twice, at 24 hours of interval. The animals were sacrificed 24 hours after the second treatment.
Positive control	:	CPA, 40 mg/kg, oral treatment

GLP : in compliance

Results
Toxicity: in a preliminary test, a dose of 250 mg/kg was found not toxic (no clinical signs), whereas 450 and 900 mg/kg were toxic.

Clastogenicity: in all treated mice, there was a reduction of the ratio PCE/NCE, thus indicating that the test item has reached the target cells.
The positive control, CPA, induced a number of MN significantly higher than the untreated animals. The test item did not induce a number of MN higher than the untreated animals.

The test item is not clastogenic in mice, treated *in vivo*.

Ref.: 27

Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *In Vivo*

Guideline	:	OECD 486 (1997)
Species/strain	:	Wistar Hanlbm: WIST (SPF) rats
Test substance	:	Promex BIT
Batch number	:	2001014
Purity	:	99.02 %
Doses	:	0 (corn oil), 375, 750 mg a.i./kg bw
Treatment times	:	2 hours, 16 hours, orally, once
Positive controls	:	N,N'-dimethylhydrazine dihidrochloride (DMH): 40 mg/kg; 2 hours 2-acetylaminofluorene (2-AAF): 100 mg/kg; 16 hours.
GLP	:	in compliance

Results
Toxicity: in preliminary experiments, doses of 1200 and 1000 mg a.i./kg bw were found toxic to the animals.

DNA repair: autoradiography was done on, at least three cultures of hepatocytes per animals. There was no indication of induction of UDS by the test item. The two positive controls induced a significant increase of UDS.
The test item does not induce UDS in rat hepatocytes in *in vivo* treatment.

Ref.: 28

2.9. Carcinogenicity

No data

2.10. Special investigations

No data

2.11. Safety evaluation

CALCULATION OF THE MARGIN OF SAFETY

NOT APPLICABLE

2.12. Conclusions

The NOAEL was set at 10 mg/kg/day/bw (90 day study in the rat).

No data on embryotoxicity and developmental toxicity were provided.

Benzisothiazolinone is moderately irritating to the skin and severely irritating to the rabbit eye. It is a moderate contact sensitizer.

No data on percutaneous absorption were provided.

Benzisothiazolinone has been tested for the induction of gene mutation on bacterial and mammalian cells treated *in vitro*, for clastogenicity on mammalian cells treated *in vitro*, for the induction of Micronuclei in mice and for the induction of UDS in rats treated *in vivo*. The study on the induction of gene mutations on bacterial cells is inadequate due to the toxicity of the test item. The compound has been found to be clastogenic in mammalian cells treated *in vitro.* The compound has been found non mutagenic *in vitro*, non clastogenic and DNA damaging *in vivo*.

2.13. References

1. Material Safety Data Sheet, Acticide BIT, Thor GmbH 22-11-2001
2. RCC Ltd, Environmental Chemistry & Pharmanalytics Div., Project No. 702731 "Determination of the Melting Point/Melting Range of ACTICIDE BIT", 22-10-1998
3. RCC Ltd, Environmental Chemistry & Phamanalytics Div., Project No. 840980 "Determination of the Boiling Point/Boiling Range of 1,2-Benzisothiazol-3(2H)-one",2002
4. Thor Specialities (UK) Limited, Study No: RS/01/025 "Determination of the Density of 1,2-benzisothiazol-3(2H)-one (BIT)", 18-01-2002
5. RCC Ltd, Environmental Chemistry & Pharmanalytics Div., Project No. 702696 "Determination of the Vapour Pressure of ACTICIDE BIT", 19-11-1998
6. RCC Ltd, Environmental Chemistry & Pharmanalytics Div., Project No. 702718 "Determination of the Partition Coefficient (n-Octanol/Water) of ACTICIDE BIT", 1998
7. Thor Specialities (UK) Limited, Study No: RS/01/025 "Determination of the Partition Coefficient (n-octanol/water) of 1,2-benzisothiazol-3(2H)-one (BIT) at a range of temperatures and pHs", March 2002
8. RCC Ltd, Environmental Chemistry & Pharmanalytics Div., Project No. 702431 "Determination of the Water Solubility of ACTICIDE BIT", 19-11-1998
9. Thor Specialities (UK) Limited, Study No: RS/01/029 "Determination of the water solubility of 1,2-benzisothiazol-3(2H)-one (BIT) at a range of temperatures and pHs", 2002
10. psl - product safety labs, Study No. 3859 "Acute Oral Toxicity Defined LD50", 24-01-96
11. psl - product safety labs, Study No. 3862 "Acute Dermal Toxicity Limit Test", 24-01-96
12. Toxicology Department Rallis Research Centre, Study No. 3286/01 "Repeated Dose (28-Day) Oral Toxicity Study by Gavage with Promex BIT in Wistar Rats", 27-02-02
13. Toxicology Department Rallis Research Centre, Study No. 3287/01 "Repeated Dose (90-Day) Oral Toxicity Study by Gavage with Promex BIT in Wistar Rats", 2002

14. psl - product safety labs, Study No. 3861 "Primary Skin Irritation", 24-01-1996
15. Sequani Consumer Limited. Study No TWP0001. " A four week home-use study to evaluate the tolerance to a topical cream containing a new preservative", June 2002
16. psl - product safety labs, Study No. 3860 "Primary Eye Irritation", 24-01-1996
17. Thor Specialities (UK)Limited, Study RS/02/036. "An assessment of the eye irritancy potentiial of 1,2-benzisothiazolin-3-one using the In-vitro BCOP assay", March 2003
18. Thor Specialities (UK)Limited, Study RS/02/039. "An assessment of the cytotoxicity of 1,2-benzisothiazolin-3-one by the In-vitro Neutral Red Uptake Assay, using BalbC 3T3 and SIRC mammalian cell lines.", January 2003
19. Quintiles England limited, Project Code A/K/42557 "Skin Sensitisation Study In The Guinea Pig Magnusson and Kligman Maximisation Test", 02-1997
20. psl - product safety labs, Study No. 3863 "Dermal sensitization test–(Buehler)", 1996
21. Sequani Consumer Limited. Study No TWP0001. "A four week home-use study to evaluate the tolerance to a topical cream containing a new preservative", June 2002.
22. Sequani Consumer Limited. Study No TWP0002 June 2002. " A four week home-use study to evaluate the tolerance to a topical cream containing a new preservative in subjects sensitized to a mixture of isothiazolinones". Start of experimental work December 2002
23. D.A.Basketter, R.Rodford, I.Kimber, I.Smith, J.Wahlberg. Skin sensitisation risk assessment: a comparative evaluation of 3 Isothiazolinone biocides. *Contact Dermatitis* 1999; 40:150-4.
24. Toxicology Department Rallis Research Centre, Study No. 3288/01 "Bacterial Reverse Mutation Test with Promex BIT", 01-03-02
25. Toxicology Department Rallis Research Centre, Study No. 3291/01 "In vitro Mammalian Cell Gene Mutation test with Promex BIT", 03-02
26. Toxicology Department Rallis Research Centre, Study No. 3289/01 "In vitro Mammalian Chromosome Aberration test with Promex BIT", 03-02
27. Toxicology Department Rallis Research Centre, Study No. 3290/01 "Mutagenicity Study - Micronucleus Test in Swiss Albino Mice with Promex BIT", 2002
28. RCC Cytotest Cell Research. Study Number: 736501. "Unscheduled DNA synthesis with 1,2-Benzisothiazolin-3-one" Study Commisioned June 2002
29. B.R Alexander (2002) An assessment of the comparative sensitisation potential of some common isothiazolinones. Contact Dermatitis 46, 191-196
30. Steinberg, D.C. (2000) Measuring synergy. Cosmetics & Toiletries 115(11), 59-62
31. Küll, F.C., Eisman, P.C., Sylwestrowicz, J.D. & Mayer, R.L. (1961) Mixtures of quaternary ammonium compounds and long chain fatty acids as antifungal agents. Applied Microbiology 9, 538-541.
32. Seal K.J., Alexander B.R, (2001) Special Agents. Global Cosmetic Industry, 169(6), 24-30
33. Chapman, J.S., Diehl, M.A., Fearnside, K.B. (1998) Preservative tolerance & resistance. International Journal of Cosmetic Science 20, 31-39
34. Kroes R, Galli C, Munro I, Schilter B, Tran L-A, Walker R, Wurtzen G. (2000) Threshold of Toxicological Concern for Chemicals Substances Present in the Diet: A Practical Tool for Assessing the Need for Toxicity Testing. Food & Chemical Toxicol. 38, 2-3, 255-312

a Ayadi M, Martin P. Pulpitis of the fingers from a shoe glue containing 1,2 benzisothiazolin-3-one. Contact Dermatitis 1999; 40: 115-116
b Fischer T, Bohlin S, Edling C, Rystedt I, Wieslander G.Skin disease and contact sensitivity in house painters using water-based paints, glues and putties. Contact Dermatitis 1995; 32: 39-45
c Taran JM, Delaney TA. Allergic contact dermatitis to 1,2-benzisothiazolin-3-one in the carpet industry. Australas J Dermatol 1997; 38: 42-43

d Chew AL, Maibach HI. 1,2-Benzisothiazolin-3-one (Proxel): irritant or allergen? A clinical study and literature review. Contact Dermatitis 1997; 36: 131-136

3. Opinion

The SCCNFP is of the opinion that the information submitted is insufficient to assess the safe use of benzisothiazolinone.

Before any further consideration, the following information is required:

* percutaneous absorption study in accordance with the SCCNFP Notes of Guidance;

* reproduction toxicity data.

4. Other considerations

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5. Minority opinions

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