
Scientific Committee on Consumer Safety

SCCS

# OPINION ON

# Benzisothiazolinone

## COLIPA n° P96

The SCCS adopted this opinion at its 15th plenary meeting

of 26-27 June 2012

About the Scientific Committees

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.
They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

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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## 1. BACKGROUND

1,2-Benzisothiazol-3(2H)-one (CAS No 2634-33-5, EC No 220-120-9) with the INCI name benzisothiazolinone is currently not listed in Annex VI of the Cosmetics Directive and therefore cannot be used as preservative.

COLIPA has requested the inclusion of benzisothiazolinone in Annex VI in order to allow the use of benzisothiazolinone in cosmetic products.

A first scientific opinion (SCCNFP/0811/04) was adopted 1 July 2004 by the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers with the following 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."*

The present submission II provides the data requested by this opinion.

## 2. TERMS OF REFERENCE

1. *Does SCCS consider benzisothiazolinone safe when used as a preservative up to a maximum authorised concentration of 0.01% in cosmetic products, based on the provided data?*

2. *And/or does the SCCS have any scientific concern with regard to the use of benzisothiazolinone in cosmetic products?*

# 3. OPINION

## 3.1. Chemical and Physical Specifications

Benzisothiazolinone is listed in the EU Cosmetics Inventory, Section 1 with indicated function "antimicrobial". It is currently not regulated in the annexes of the Cosmetics Directive 76/768/EEC.

### 3.1.1. Chemical identity

#### 3.1.1.1. Primary name and/or INCI name

Benzisothiazolinone (INCI)

#### 3.1.1.2. Chemical names

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

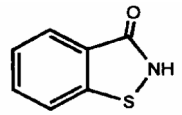
#### 3.1.1.3. Trade names and abbreviations

BIT; Thor BIT; ACTIDE® BIT; Microcare® BIT; Nuosept BIT Technical; Promex BIT COLIPA P96

#### 3.1.1.4. CAS / EC number

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

#### 3.1.1.5. Structural formula




#### 3.1.1.6. Empirical formula

Formula: $C_7H_5NOS$

### 3.1.2. Physical form

Off-white to yellowish solid

### 3.1.3. Molecular weight

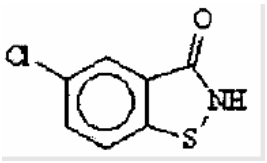
Molecular weight: 151.19 g/mol

## 3.1.4. Purity, composition and substance codes

Substance code: /
Batches used: batch no 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 no 2001 014 spec., 20% max, found 15%)
Ash content: /
Sodium chloride: < 0.1% w/w (for batch no 2001 014 spec., 0.2% max, found 0.02%)
Lead: /
Mercury: /
Arsenic: /
Iron: /

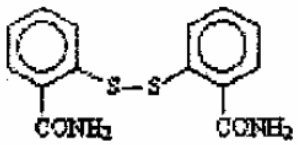
Impurities

- 5-Chloro-1,2-benzisothiazolin-3(2H)-one: 0.15-0.22% w/w
(for batch no 2001 014, specification 4 ppm max, found 3 ppm)




CAS: 4337-43-3
EC: 224-385-1
emp. Formula: $C_7H_4ClNOS$

- 2,2-Dichlorobisbenzamide: < 0.1% w/w
(for batch no 2001 014, specification 0.5% max, found 0.03%)




CAS: 2527-57-3
EC: 219-766-4
emp. Formula: $C_{14}H_{12}N_2O_2S_2$

Residual solvents: /

## 3.1.5. Impurities / accompanying contaminants

See 3.1.4.

## 3.1.6. 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

### 3.1.7. Partition coefficient (Log Pow)

Log Pow: 0.4 at 20 °C (OECD Guideline 117)

Effect of pH and temperature on Log Pow (OECD Guideline 117 (HPLC))

Log Pow: 0.99 at 20 °C and pH 5
Log Pow: 0.63 at 10 °C and at pH 7
Log Pow: 0.70 at 20 °C and pH 7
Log Pow: 0.76 at 30 °C and pH 7
Log Pow: – 0.90 at 20 °C and pH 9

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

### 3.1.8. Additional physical and chemical specifications

| | |
|---|---|
| Melting point: | 156.6 °C (Directive 92/69/EEC, A1) |
| Boiling point: | 327.6 °C (Directive 84/449/EEC, A2) |
| Density: | 1.483 g/cm³ at 20 °C (OECD Guideline 109) |
| Vapour Pressure: | 0.0000037 hPa at 25 °C (Directive 92/69/EEC, A4) |
| pKa: | 7.3 at 25 °C |
| Flash point: | / |
| Viscosity: | / |
| Refractive index: | / |
| UV_Vis spectrum (200-800 nm): | / |

### 3.1.9. Homogeneity and Stability

No data

**General Comments to physico-chemical characterisation**

No data on stability are provided

## 3.2. Function and uses

Benzisothiazolinone is listed in the EU Cosmetics Inventory, Section 1 with indicated function "antimicrobial". It is currently not regulated in the annexes of the Cosmetics Directive 76/768/EEC.

The submitted dossier requests a maximum level of 100 ppm as preservative in cosmetic products. This is greater than the level proposed in the earlier submission, which the applicant justifies with the availability of more recent studies, demonstration of a greater margin of safety.

Aside from its use in cosmetic products, there are also other uses:

Under the Biocide review programme for existing substances (Commission Regulation (EC) No 1451/2007), Benzisothiazolinone is examined for use in the following product types:

Private area and public health area disinfectants and other biocidal products (2), In-can preservatives (6), Film preservatives (7), Fibre, leather, rubber and polymerised materials preservatives (9), Masonry preservatives (10), Preservatives for liquid-cooling and processing systems (11), Slimicides (12), Metalworking-fluid preservatives (13), Embalming and taxidermist fluids (22).

Benzisothiazolinone is used as a slimicide in the manufacture of disposable powder-free polyvinyl chloride gloves.

(Add. ref. 41)

Benzisothiazolinone is widely used in industry as a preservative in water-based solutions such as pastes, paints and cutting oils.

(Add. ref. 42, 43)

## 3.3. Toxicological Evaluation

Throughout the studies quoted (unless otherwise specified) the purity of the test material used was 99.02%, with an active content of 70.02 to 84.02% w/w, water content of 15% to 29% w/w and total impurities of 0.26 to 0.33% w/w.

### 3.3.1. Acute toxicity

#### 3.3.1.1. Acute oral toxicity

***Taken from SCCNFP/08441/04***

| | |
|---|---|
| Guideline: | / |
| Method: | EPA OPP 81-1 |
| Species/strain: | Rat, Sprague-Dawley derived, albino |
| Group size: | 30 (3 groups of 5 males and 5 females each) |
| Test item: | Benzisothiazolinone |
| Test substance: | Nuosept BIT Technical |
| Batch: | # 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 LD50 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 bw (upper) and 1.049 mg/kg bw (lower). The LD50 for males was 2.100 mg/kg bw with 95% Confidence Limits of 5.029 mg/kg bw (upper) and 877 mg/kg bw (lower). The data does not permit calculation of the LD50 for females by Probit Analysis. Graphically, the LD50 for females was estimated to be 1.050 mg/kg bw.

Ref.: 10

### 3.3.1.2. Acute dermal toxicity

***Taken from SCCNFP/08441/04***

| | |
|---|---|
| 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: | # 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 LD50 of the test substance is greater than 5000 mg/kg bw 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

### 3.3.1.3. Acute inhalation toxicity

No data submitted

## 3.3.2 Irritation and corrosivity

### 3.3.2.1. Skin irritation

***Taken from SCCNFP/08441/04*** *(with some modifications)*

| | |
|---|---|
| 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: | # 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) |
| Readings: | 1, 24, 48, 72 hours and 7 days |
| GLP: | in compliance |

Date: 1995

Results
The test substance was moistened with water for application (0.5 ml water/0.5 g test substance =41.15% a.i.). 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.

Conclusion
Nuosept BIT Technical (Benzisothiazolinone) is a skin irritant.

Ref.: 15

**Human study**

50 healthy human volunteers (20 males and 30 females aged between 19-60 years) 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 of which 0.15% w/w (75 ppm) 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.
Subjects with known sensitivity to isothiazolinones were excluded. 47 subjects completed the study as planned, and diary 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.: 22

### 3.3.2.2. Mucous membrane irritation

***Taken from SCCNFP/08441/04***

Guideline: /
Method: EPA OPP 81-4
Species/strain: New Zealand albino rabbits
Group size: 9 (4 males and 5 females)
Test substance: Nuosept BIT Technical
Batch: # 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 |
| Date: | 1995 |

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.: 17

**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: | Bovine |
| Number corneas: | 45 |
| Test substance: | 1,2-Benzisothiazolin-3-one |
| Batch: | 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 |
| Date: | 2003 |

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.

Ref.: 18

Comment
The method used was not a validated *in vitro* method in 2003. Meanwhile, it is an OECD test guideline (number) which can detect strong irritants.

**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: /
Species: BALB/c 3T3 Mouse fibroblast cell line
SIRC Rabbit corneal cell line
Test substance: 1,2-Benzisothiazolin-3-one
Batch: LHW 1355
Purity: > 99%
Dose: 0-100 ppm range finding; 0-10 ppm testing
Exposure time: 24 hours
GLP: in compliance
Date: 2002

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 EC50 value was calculated. The EC50 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 | EC50 3T3 | EC50 SIRC |
|---|---|---|
| Chloromethylisothiazolinone/methylisothiazolinone (3:1) | 1.19 | 1.89 |
| Methylisothiazolinone | 5.76 | 5.98 |
| Methyldibromoglutaronitrile 20% (in phenoxyethanol) | 28.4 | 29.36 |
| Methyl/ethyl/propyl/butyl/isobutyl parabens in phenoxyethanol | 439.5 | 489.0 |

Ref.: 19

Comment
The SCCS considers the above cytotoxicity data of little value in its overall evaluation of the safety of benzisothiazolinone.

### 3.3.3. Skin sensitisation

***Taken from SCCNFP/08441/04***

**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: 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
Date: 1996

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.: 20

**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: | #170-138 |
| Purity: | 1,2-Benzisothiazolin-3-one (BIT) 82.3% a.i. |
| Dosage: | Induction: Weekly application of 0.3 g of test substance 95% w/w in corn seed oil for 3 consecutive weeks.<br>Challenge: 14 days after last induction with same dose as induction to naive site |
| Vehicle: | Corn seed oil |
| GLP: | in compliance |
| Date: | 1995 |

Results
It was found that a 6 hour exposure under 25 mm Hilltop chambers to 95% w/w (78.19% active) BIT powder in corn oil was suitable for the test group. 0.04% DNCB (dichloronitro-benzene) in acetone was used as the positive control.
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
Nuosept BIT Technical was not a sensitizer in this test.

Ref.: 21

**Human study**

15 healthy human volunteer patients (2 males and 13 females aged between 25-66 years) 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 chloromethyliso-thiazolinone.
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 diary to record usage of the products, which were weighed at the end of study.

Results
10 subjects completed the study as planned, and diary 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** | **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 |

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.

Ref.: 23

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.

**Local Lymph Node Assay and Human Repeat Insult Patch Test**

The relative sensitising potencies and potential for cross sensitisation/reaction of chloromethylisothiazolinone (CMI or CIT/MIT), methyl trimethylene isothiazolinone (MTI) and benzisothiazolinone (BIT) were considered from newly generated and historical data. However, although CMI was specifically mentioned, it was not stated whether the mixture of chloromethylisothiazolinone with methylisothiazolinone was actually used. Original experimental data were not provided in this review.

Using the LLNA, the EC3 for Benzisothiazolinone was established at 10.4%, that for MTI at 2% and CIT/MIT at 0.01%.

Using the HRIPT resulted in no reactions to BIT at 360 ppm and 9% of volunteers reacting at 725 ppm. There were also no reactions to CMIT/MIT at 10 ppm and 4.4% reacted at 20 ppm. For MTI there were no reactions at 100 ppm but 16% reacted at 300 ppm.

Data are summarized:

| **Preservative** | **Test concentration (ppm)** | **Proportion of human panel sensitised (%)** | **LLNA EC3** |
|---|---|---|---|
| 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.: 24

Comment
The HRIPT is not considered ethical.

**Local Lymph Node Assay**
An EC3 of 2.3% for 1,2-benzisothiazolin-3-one (CAS 2634-33-5) and 1.9% for 2-methyl-2H-isothiazol-3-one (CAS 2682-20-4) is tabulated in a review but no experimental details are provided.

(Add. ref.: 49)

**Overall Comment Sensitisation**

Benzisothiazolinone is a contact allergen with the guinea pig maximization (Magnusson Kligman) test indicating BIT as a moderate sensitiser, as does the LLNA with an EC3 of 2.3% for BIT.

Since the human in-use study provided by the applicant included a low number of subjects, only limited conclusions could be drawn. Therefore, the SCCS conducted a literature search on the sensitising potential of benzisothiazolinone in humans. 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.

(Add. ref.: 37, 38, 39, 40)

Benzisothiazolinone is widely used in industry as a preservative in water-based solutions such as pastes, paints and cutting oils. Occupational dermatitis has been reported mainly due to cutting fluids, paint manufacture, pottery mould-makers, acrylic emulsions manufacture, printers, paper makers etc. which contain benzisothiazolinone.

(Add. ref. 42, 44, 45, 46, 47, 48)

Moreover, benzisothiazolinone is used as a slimicide in the manufacture of disposable powder-free polyvinyl chloride (PVC) gloves. Of 31 glove brands studied 9 (30%) contained 3–26 ppm benzisothiazolinone. Individuals wearing such gloves developed allergic contact dermatitis. A concentration of 20 ppm benzisothiazolinone in a glove seems to be enough for sensitization.

(Add. ref. 41)

### 3.3.4. Dermal / percutaneous absorption

**New study**

| | |
|---|---|
| Guideline: | OECD 428 |
| Species/strain: | human skin (5 abdominal, 1 breast); 400 µm dermatomed skin |
| Group size: | 10 membranes |
| Method | flow through diffusion cell |
| Membrane integrity | Tritiated water |
| Test substance: | benzisothiazolinone |
| Batch: | 12409EE |
| Purity: | 97% |
| Radiochemical | $^{14}$C benzisothiazolinone; 99.9% |
| Vehicle: | water |
| Test item: | 0.01% w/v benzisothiazolinone aqueous |
| Dose volume: | 20 µl/cm$^2$ |
| Receptor | Tissue culture medium containing 6% PEG, 0.01% sodium azide, 1% glucose and streptomycin |
| Solubility | (in water) 1 mg/ml |
| Method of Analysis: | liquid scintillation |
| GLP: | in compliance |
| Study period: | 2008 |

The absorption of radiolabelled 0.01% w/v benzisothiazolinone in aqueous solution was determined from the use of 10 chambers with human dermatomed skin.

**1 Distribution of Radioactivity (% Applied Dose) at 24 h Post Dose Following Topical Application of [$^{14}$C]-Benzisothiazolinone in Water (0.01%, w/v) to Human Split-Thickness Skin**

| | Cell Number and Donor Number | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cell 1 0221 | Cell 2 0221 | Cell 3 0223 | Cell 4 0223 | Cell 5 0223 | Cell 6 0225 | Cell 7 0225 | Cell 8 0225 | Cell 13 0233 | Cell 14 0233 | Mean | SD |
| Skin Wash | 35.87 | 11.66 | 8.11 | 12.00 | 8.59 | 17.56 | 9.41 | 20.10 | 16.14 | 18.59 | 15.80 | 8.27 |
| Cell Wash | 0.52 | 0.19 | 0.26 | 0.21 | 0.22 | 0.42 | 1.28 | 0.59 | 0.56 | 0.58 | 0.48 | 0.32 |
| Tissue Swab | 24.53 | 31.27 | 18.50 | 19.66 | 22.57 | 22.69 | 24.20 | 22.21 | 29.02 | 42.96 | 25.76 | 7.17 |
| Pipette Tip | 0.01 | 0.01 | 0.01 | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.00 |
| Dislodgeable Dose | 60.92 | 43.14 | 26.87 | 31.87 | 31.38 | 40.68 | 34.90 | 42.91 | 45.73 | 62.14 | 42.05 | 11.91 |
| Stratum Corneum 1-5 | 0.83 | 1.44 | 0.30 | 1.89 | 2.25 | 0.83 | 1.20 | 0.94 | 4.53 | 1.03 | 1.52 | 1.20 |
| Stratum Corneum 6-10 | 0.53 | 0.82 | 0.46 | 2.48 | 1.36 | 1.12 | 1.45 | 1.86 | 4.00 | 0.64 | 1.47 | 1.09 |
| Stratum Corneum 11-15 | 0.25 | 0.67 | 0.59 | 1.58 | 1.45 | 1.33 | 0.76 | 1.41 | 1.71 | 0.84 | 1.06 | 0.49 |
| Stratum Corneum 16-20 | 0.20 | 0.79 | 0.48 | 0.99 | 1.10 | 1.21 | 0.75 | 0.62 | 2.19 | 0.57 | 0.89 | 0.55 |
| Stratum Corneum | 1.81 | 3.71 | 1.82 | 6.94 | 6.16 | 4.48 | 4.15 | 4.82 | 12.43 | 3.08 | 4.94 | 3.11 |
| Unexposed Skin | 0.01 | *0.00 | 0.02 | 0.01 | *0.00 | 0.00 | 0.15 | 0.03 | *0.00 | *0.00 | °0.02 | °0.05 |
| Total Unabsorbed | 62.75 | 46.85 | 28.71 | 38.82 | 37.54 | 45.17 | 39.20 | 47.77 | 58.16 | 65.22 | 47.02 | 11.83 |
| Epidermis | 12.14 | 25.12 | 32.41 | 24.50 | 23.20 | 12.16 | 16.18 | 12.03 | 23.52 | 16.16 | 19.74 | 6.98 |
| Dermis | 0.66 | 6.94 | 7.25 | 4.17 | 2.88 | 4.11 | 8.47 | 3.27 | 6.12 | 0.70 | 4.46 | 2.70 |
| Receptor Fluid | 22.09 | 17.78 | 28.53 | 29.85 | 32.46 | 34.91 | 32.92 | 33.99 | 7.56 | 14.10 | 25.42 | 9.52 |
| Receptor Rinse | 0.06 | 0.11 | 0.32 | 0.28 | 0.34 | 0.18 | 0.28 | 0.26 | 0.16 | 0.14 | 0.21 | 0.09 |
| Total Absorbed | 22.15 | 17.88 | 28.85 | 30.13 | 32.79 | 35.09 | 33.20 | 34.25 | 7.72 | 14.24 | 25.63 | 9.58 |
| Dermal Delivery | 34.95 | 49.94 | 68.52 | 58.80 | 58.87 | 51.36 | 57.85 | 49.55 | 37.36 | 31.10 | 49.83 | 12.05 |
| Mass Balance | 97.69 | 96.80 | 97.23 | 97.62 | 96.41 | 96.53 | 97.05 | 97.32 | 95.52 | 96.31 | 96.85 | 0.67 |

**Distribution of [$^{14}$C]-Benzisothiazolinone (µg equiv./cm$^2$) at 24 h Post Application Following Topical Application of [$^{14}$C]-Benzisothiazolinone in Water (0.01%, w/v) to Human Split-Thickness Skin**

| | Cell Number and Donor Number | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cell 1 0221 | Cell 2 0221 | Cell 3 0223 | Cell 4 0223 | Cell 5 0223 | Cell 6 0225 | Cell 7 0225 | Cell 8 0225 | Cell 13 0233 | Cell 14 0233 | Mean | SD |
| Skin Wash | 0.75 | 0.24 | 0.17 | 0.25 | 0.18 | 0.37 | 0.20 | 0.42 | 0.34 | 0.39 | 0.33 | 0.17 |
| Cell Wash | 0.01 | 0.00 | 0.01 | 0.00 | 0.00 | 0.01 | 0.03 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Tissue Swab | 0.51 | 0.65 | 0.39 | 0.41 | 0.47 | 0.47 | 0.51 | 0.46 | 0.61 | 0.90 | 0.54 | 0.15 |
| Pipette Tip | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Dislodgeable Dose | 1.27 | 0.90 | 0.56 | 0.67 | 0.66 | 0.85 | 0.73 | 0.90 | 0.96 | 1.30 | 0.88 | 0.25 |
| Stratum Corneum 1-5 | 0.02 | 0.03 | 0.01 | 0.04 | 0.05 | 0.02 | 0.03 | 0.02 | 0.09 | 0.02 | 0.03 | 0.02 |
| Stratum Corneum 6-10 | 0.01 | 0.02 | 0.01 | 0.05 | 0.03 | 0.02 | 0.03 | 0.04 | 0.08 | 0.01 | 0.03 | 0.02 |
| Stratum Corneum 11-15 | 0.01 | 0.01 | 0.01 | 0.03 | 0.03 | 0.03 | 0.02 | 0.03 | 0.04 | 0.02 | 0.02 | 0.01 |
| Stratum Corneum 16-20 | 0.00 | 0.02 | 0.01 | 0.02 | 0.02 | 0.03 | 0.02 | 0.01 | 0.05 | 0.01 | 0.02 | 0.01 |
| Stratum Corneum | 0.04 | 0.08 | 0.04 | 0.15 | 0.13 | 0.09 | 0.09 | 0.10 | 0.26 | 0.06 | 0.10 | 0.06 |
| Unexposed Skin | 0.00 | *0.00 | 0.00 | 0.00 | *0.00 | 0.00 | 0.00 | 0.00 | *0.00 | *0.00 | °0.00 | °0.00 |
| Total Unabsorbed | 1.31 | 0.98 | 0.60 | 0.81 | 0.78 | 0.94 | 0.82 | 1.00 | 1.21 | 1.36 | 0.98 | 0.25 |
| Epidermis | 0.25 | 0.52 | 0.68 | 0.51 | 0.48 | 0.25 | 0.34 | 0.25 | 0.49 | 0.34 | 0.41 | 0.15 |
| Dermis | 0.01 | 0.14 | 0.15 | 0.09 | 0.06 | 0.09 | 0.18 | 0.07 | 0.13 | 0.01 | 0.09 | 0.06 |
| Receptor Fluid | *0.46 | *0.37 | *0.60 | *0.62 | *0.68 | *0.73 | *0.69 | *0.71 | *0.16 | *0.29 | °0.53 | °0.20 |
| Receptor Rinse | 0.00 | 0.00 | 0.01 | 0.01 | 0.01 | 0.00 | 0.01 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 |
| Total Absorbed | 0.46 | 0.37 | 0.60 | 0.63 | 0.68 | 0.73 | 0.69 | 0.72 | 0.16 | 0.30 | 0.54 | 0.20 |
| Dermal Delivery | 0.73 | 1.04 | 1.43 | 1.23 | 1.23 | 1.07 | 1.21 | 1.03 | 0.78 | 0.65 | 1.04 | 0.25 |
| Mass Balance | 2.04 | 2.02 | 2.03 | 2.04 | 2.01 | 2.02 | 2.03 | 2.03 | 2.00 | 2.01 | 2.02 | 0.01 |

| Test Preparation Vehicle | Water | |
|---|---|---|
| Target Test Item Concentration in Test Preparation (%, w/w) | 0.01 | |
| Actual Test Item Concentration in Test Preparation (%, w/w) | 0.0104 | |
| Target Application Rate of Test Preparation (mg/cm$^2$) | 20 | |
| Actual Application Rate of Test Preparation (mg/cm$^2$) | 20.06 | |
| Target Application Rate of Test Item (µg equiv./cm$^2$) | 2 | |
| Actual Application Rate of Test Item (µg equiv./cm$^2$) | 2.09 | |
| Distribution | Mean | SD |
| Total Dislodgeable Dose (% Applied Dose) | 42.05 | 11.91 |
| Unabsorbed Dose (% Applied Dose) | 47.02 | 11.83 |
| Absorbed Dose (% Applied Dose) | 25.63 | 9.58 |
| Dermal Delivery (% Applied Dose) | 49.83 | 12.05 |
| Mass Balance (% Applied Dose) | 96.85 | 0.67 |
| Dislodgeable Dose (µg equiv./cm$^2$) | 0.88 | 0.25 |
| Unabsorbed Dose (µg equiv./cm$^2$) | 0.98 | 0.25 |
| Absorbed Dose (µg equiv./cm$^2$) | 0.54 | 0.20 |
| Dermal Delivery (µg equiv./cm$^2$) | 1.04 | 0.25 |
| Mass Balance (µg equiv./cm$^2$) | 2.02 | 0.01 |

Total unabsorbed dose = skin wash + tissue swab + pipette tips + stratum corneum + unexposed skin + cell wash
Absorbed dose = cumulative receptor fluid + receptor rinse
Dermal delivery = exposed skin (epidermis + dermis) + absorbed dose
Mass balance = unabsorbed dose + dermal delivery

Conclusion
Following topical application of [$^{14}$C]-Benzisothiazolinone in water (0.01%, w/v) to human skin, the absorbed dose of [$^{14}$C]-Benzisothiazolinone was 25.63% (0.54 µg equiv./cm$^2$). The dermal delivery of [$^{14}$C]-Benzisothiazolinone was 49.83% (1.04 µg equiv./cm$^2$). The total dislodgeable dose was 42.05% of the applied dose.

Ref.: 36

Comment
This was a properly performed study. Therefore, the mean + 1SD may be used for calculating the MOS. This is 61.9% of the applied dose (1.29 µg/cm$^2$) when 0.01% benzisothiazolinone aqueous was applied. The dermal absorption studies had not been performed with representative cosmetic formulations.

## 3.3.5. Repeated dose toxicity

### 3.3.5.1. Repeated Dose (28 days) oral toxicity

***Taken from SCCNFP/08441/04*** *(with modifications)*

| | |
|---|---|
| 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: | 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 |
| Date: | 2001 |

Results

Oral administration of 1,2-Benzisothiazolin-3-one, by gavage in Wistar rats at the dose of 15 mg/kg bw/day (12.63 mg a.i./kg bw/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 bw/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 bw/day group indicating the reversibility of the effect.
Body weight was unaffected in the 15 and 45 mg/kg bw/day groups. At 135 mg/kg bw/day there was a significant decrease in body weight and cumulative net weight gain in the male group throughout the treatment period with the exception of the first week where it was not statistically significant.
Weekly body weights were significantly lower throughout the treatment and recovery period in the high dose (135 mg/kg bw/day) for males and on weeks 4, 5, and 6 for females.
Cumulative net weight gains were also significantly lower throughout the treatment and recovery period in males and females with the exception of the first week where it was not statistically significant.
Increased incidences of histopathological lesions in the non-glandular stomach (hyperkeratosis, epithelial hyperplasia, ulceration) were observed in mid- (45 mg/kg bw/day) and high-dose (135 mg/kg bw/day) males and females. The severity of the lesions was reduced in the high-dose recovery group.

The NOAEL in this study was 15 mg/kg bw/day (12.63 mg a.i./kg bw/day).

Ref.: 12

Comment
The SCCS noted that the NOAEL of 15 mg/kg bw/day (12.63 mg a.i./kg bw/day) was based on the histopathological lesions observed in the non-glandular stomach, which are most likely due to the irritant property of the test substance.

### 3.3.5.2. Sub-chronic (90 days) toxicity (oral, dermal)

***Taken from SCCNFP/08441/04***

| | |
|---|---|
| Guideline: | OECD 408 |
| Species/strain: | Rat, Wistar |
| Group size: | Total 20; 10 male/10 female |
| Test item: | Benzisothiazolinone (Promex-BIT) |
| Batch: | 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% carboxymethylcellulose (CMC) |
| Route: | daily oral intubation/gavage |
| Exposure period: | 90 days |
| GLP: | in compliance |
| Study period: | 2001-2002 |

Results
The oral administration of 1,2-Benzisothiazolin-3-one by gavage in Wistar rats at the dose of 10 mg/kg bw/day (8.42 mg a.i./kg bw/day) 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 bw/day (25.26 mg a.i./kg bw/day) there were no treatment related clinical signs or neurological effects and no adverse effects on growth, haematological and clinical chemistry parameters, sperm evaluation, organ weights and its ratios. 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. Increased incidences of histopathological lesions in the non-glandular stomach (hyperkeratosis, epithelial hyperplasia, ulceration) were observed in males and females.
At 75 mg/kg bw/day (63.15 mg a.i./kg bw/day) there were no treatment related neurological effects and no adverse effects on haematological and clinical chemistry parameters, sperm evaluation, organ weights and its ratios. Treatment related signs of slight salivation were observed in four males and three females in the main group and in three males and five females in the recovery group during the treatment period. During the recovery period, treatment related signs of salivation were not observed indicating the reversibility of the effect. Weekly body weights and cumulative net weight gains were significantly lower in males throughout the treatment and recovery period except for the body weight at week 3 which were lower but not statistically significant. The body weight gains were significantly higher in males and in females (weeks 15, 16 and 17) during the recovery period. There was a significant reduction in food consumption in males (weeks 1, 2, 7, 8, 12 and 13) and in females (weeks 1, 2 and 4), which returned to control levels in the recovery period.
Increased incidences of histopathological lesions in the non-glandular stomach (hyperkeratosis, epithelial hyperplasia, ulceration, keratin cysts) were observed in males and females. The severity of the lesions was reduced in the recovery group.

The NOAEL in this study was 10 mg/kg bw/day (8.42 mg a.i./kg bw/day).

Ref.: 13

Comment
The SCCS noted that the NOAEL of 10 mg/kg bw/day (8.42 mg a.i./kg bw/day) was based on the histopathological lesions observed in the non-glandular stomach, which are most likely due to the irritant property of the test substance.
Therefore, the NOAEL is 25.26 mg a.i./kg bw/day based on systemic effects.

#### 3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

### 3.3.6. Mutagenicity / Genotoxicity

#### 3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

***Taken from SCCNFP/08441/04***

**Bacterial Reverse Mutation Assay**

| | |
|---|---|
| Guideline: | OECD 471 (1997) |
| Species/strain: | *Salmonella typhimurium* TA98, TA1537, TA100, TA1535<br>*Escherichia coli* WP2uvrA pkM 101 |
| Test substance: | Promex BIT 1,2–Benzisothiazolin-3-one |
| Batch: | 2001 014 |
| Lot number: | KP 070601 |
| Purity: | 99.02% |
| Concentrations: | 0, 20, 35, 60, 100 and 175 µg/plate (1st experiment)<br>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 |
| Date: | 2001 |

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.: 25

### *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: | 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 |
| Date: | 2002 |

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 than 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. 26

### *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: | 2001 014 |
| Lot number: | KP 070601 |
| Purity: | 99.02% |
| Concentrations: | 0, 1.6, 3.2, 6.4 µg/ml in the presence of S9-mix<br>0, 1.25, 2.50, 5.0 µg/ml in the absence of S9-mix |
| Replicate: | 2 experiments (200 metaphases analysed) |

| | |
|---|---|
| Treatment time: | 1st experiment: 3 hours<br>2nd experiment: 3 hours, in the presence of S9-mix; 19 hours, in the absence of S9-mix |
| Positive controls: | CPA (55 µg/ml); EMS (600 µg/ml) |
| Metabolic activation: | Aroclor 1254 induced rat live homogenate |
| GLP: | in compliance |
| Date: | 2001 |

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 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 in CHO mammalian cells.

Ref.: 27

### 3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

***Taken from SCCNFP/08441/04***

**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: | 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 bw, oral treatment |
| GLP: | in compliance |
| Date: | 2001 |

Results
Toxicity: in a preliminary test, a dose of 250 mg/kg bw was found not toxic (no clinical signs), whereas 450 and 900 mg/kg bw 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.: 28

**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: | 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 bw; 2 hours 2-acetylaminofluorene (2-AAF): 100 mg/kg bw; 16 hours. |
| GLP: | in compliance |
| Date: | 2002 |

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.: 29

### 3.3.7. Carcinogenicity

No data submitted

### 3.3.8. Reproductive toxicity

#### 3.3.8.1. Two generation reproduction toxicity study

| | |
|---|---|
| Guideline: | EPA Health Effects Test Guidelines OPPTS 870.3800, of aug 1998 |
| Species/strain: | Crl:W1 (Glx/BRL/Han)BR |
| Group size: | 24 males and 24 females (P generation) |
| Test substance: | Proxel Press Paste (1,2-Benzisothiazolin-3-one) |
| Batch: | No. 103 and No. 344 |
| Purity: | Purity of active ingredient: 92.7% +/- 1.1% |
| Dose levels: | P generation: 250, 500 and 1000 ppm<br>F1 generation: 250, 500 1000 ppm |
| Route: | oral |
| Administration: | dietary |
| Control: | control diet only |
| GLP statement: | Yes |
| Study period: | 2000-2001 |

Study design
Groups of 24 male and 24 female rats were given Proxel Press Paste by admixture with the diet, at dose levels of 250, 500 and 1000 ppm. A similar group received the control diet only. The animals received the test diet for 10 weeks before being paired for up to 2 weeks. Dosing continued during this pairing period, and throughout the resulting pregnancies. The P generation females were allowed to litter and rear their offspring (F1a) until weaning.
Administration of the test article continued throughout the weaning of the F1 offspring up until necropsy.
24 animals of each sex were randomly selected from each group to form the filial (F1) generation. Direct treatment of the F1 generation continued during their maturation period (10 weeks), the mating period (up to two weeks) and throughout the resulting pregnancies and weaning of the F2 offspring until necropsy. All F1 females were allowed to litter and rear their F2a offspring to weaning.

Result
Analysis of samples from the diets prepared for administration in weeks 1, 17 and 19 of the study showed that the achieved concentrations were within the target range (all values within 89 to 110% of nominal). Analysis of samples from the diets prepared for

administration in weeks 34, 36, 37 and 38 of the study showed that the achieved concentrations were below the target range.

The group mean achieved intakes of PROXEL Press Paste were:

| Generation | Dose level (ppm) | Intake (mg/kg/day) Males | Females |
|---|---|---|---|
| P | 250 | 18.5 | 27.0 |
| | 500 | 37.2 | 54.2 |
| | 1000 | 75.1 | 112.0 |
| $F_1$ | 250 | 24.0 | 28.2 |
| | 500 | 48.0 | 56.6 |
| | 1000 | 97.8 | 114.8 |
| Combined | 250 | 21.3 | 27.6 |
| | 500 | 42.6 | 55.4 |
| | 1000 | 86.5 | 113.4 |

P Generation: Clinical observations, body weights and food intakes were unaffected by treatment. Mating data, duration of gestation, number of implantations, numbers of pups born and pup survival were similar in all groups.
Mean pup weight of the high dose pups was slightly lower than the control over the first week *post-partum*. But, over the whole lactation period, mean pup weight was similar in all groups.
There were no adverse effects of treatment on seminology data.
In the high dose group, mean liver weight of the males was slightly higher, and mean testes weight slightly lower than control.
Minor limiting ridge hyperplasia in the stomach was noted in some intermediate and many high dose animals. Squamous cell hyperplasia and forestomach gastritis was also seen in a few animals.

F1 Generation: Males in the high dose group gained slightly less weight than the controls during the study and the high dose females gained slightly less weight during the pre-pairing period only. Clinical observations and food intakes were unaffected by treatment.
Physical development of the F1 generation, mating data, duration of gestation and F2a pup sex ratio were unaffected by treatment. Pup survival to day 4 *post-partum* and mean pup weight gain were slightly lower in the high dose group compared to controls. Seminology investigations, organ weights and ovarian follicle counts were unaffected by treatment.
In the intermediate and high dose groups, limiting ridge hyperplasia in the stomach was noted. This was most prominent in the high dose females where there was also squamous cell hyperplasia, fore stomach gastritis, hyperkeratosis and erosion/ulcer.

Conclusion
Dietary administration of 1000 ppm PROXEL Press Paste to rats for two generations produced slight adult toxicity, in the F1 generation in terms of lower body weight gain, and in both generations limiting ridge hyperplasia of the stomach together with incidences of squamous cell hyperplasia, forestomach gastritis, hyperkeratosis and erosion/ulcer. At this concentration, the growth of the offspring was slightly impaired and in the F2a offspring, there was a slight reduction in pup survival.
At 500 ppm, there were incidences of limiting ridge hyperplasia in the stomach only.
There were no adverse effects of treatment at 250 ppm, equivalent to an approximate overall mean intake of 24 mg/kg bw/day.
Note: The dose levels refer to the concentration of the active ingredient (BIT) and a correction factor of 1.074 was made for the stated purity of the PROXEL.

Ref.: 14

Comments
No significant effects were reported for the reproductive parameters at any dose level and only slight effects were noted in the offspring at the highest dose level (slightly lower pup survival to day 4 *post-partum* and slightly lower mean pup weight gain).
Based on this study, a NOAEL of approximately 50 mg a.i./kg bw/day is used as a conservative estimate for the MOS calculation. This NOAEL is lower than the lowest LOAEL for systemic effects (63 mg a.i./kg bw/day) in the 90-day study.

#### 3.3.8.2. Teratogenicity

No evidence for teratogenicity in the two-generation oral reprotoxicity study

Ref.: 14

### 3.3.9. Toxicokinetics

No data provided.

### 3.3.10. Photo-induced toxicity

#### 3.3.10.1. Phototoxicity / photoirritation and photosensitisation

| | |
|---|---|
| Guideline: | OECD Test guideline 432 |
| Test: | Neutral Red uptake phototoxicity test with Balb/c 3T3 cells |
| Test substance: | 1,2–Benzisothiazolin-3-one |
| Batch: | LMS 414, CH-0405-ST-7 |
| Purity: | > 99% |
| UV-A Irradiation: | SOL-500 lamp fitted with a H1-filter mounted at a distance of 60 cm to achieve an UV-irradiance of 5 J/cm$^2$ |
| Concentrations: | 0, eight concentrations between 0.2 to 50 µg/mL in the presence and absence of UV-A exposure |
| Replicate: | 3 main experiments |
| Positive control: | Chlorpromazine |
| GLP: | in compliance |
| Date: | 2004 |

Results
The 3T3 NRU phototoxicity assay was performed on four occasions for BIT, once as a range finding experiment and three times as main experiment. The PIF values calculated from the results for BIT range between 1.197 and 2.166 with a mean value of 1.54. The positive control chlorpromazine yielded PIF values of about 100.

Conclusion
1,2 Benzisothiazol-3(2H)-one is predicted to be non-phototoxic by the 3T3 NRU PT assay OECD Test Guideline 432

Ref.: 16

#### 3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

### 3.3.11. Human data

See section 3.3.3. Sensitisation

### 3.3.12. Special investigations

No data submitted

### 3.3.13. Safety evaluation (including calculation of the MoS)

**CALCULATION OF THE MARGIN OF SAFETY**

**Benzisothiazolinone**

| | | |
|---|---|---|
| **Daily exposure to all cosmetic products (excl. sunscreens)** | **=** | **17.4 g/d** |
| **Concentration Benzisothiazolinone (BIT)** | **=** | **0.01%** |
| **Daily exposure BIT** | **=** | **1.74 mg** |
| **Dermal absorption** | **=** | **61.9%** |
| **Typical body weight of human** | **=** | **60 kg** |
| **Systemic exposure dose** | **=** | **0.018 mg/kg bw/d** |
| **No Observed Adverse Effect Level (2-generation-study, oral, rat)** | **=** | **50 mg/kg bw/d** |
| **NOAEL corrected for 50% oral bioavailability** | **=** | **25 mg/kg bw/d** |

| | | | |
|---|---|---|---|
| **Margin of Safety** | **NOAEL/SED** | **=** | **1392** |

### 3.3.14. Discussion

*Physico-chemical properties*
Benzisothiazolinone is an off-white to yellowish solid that is soluble in water (1.1 g/L at 20°C); its log Pow shows a significant dependence on the pH. No data on stability are provided.

*Irritation, sensitisation*
According to a study conducted in rabbits benzisothiazolinone (BIT) can be classified as a moderate irritant to skin. A study in rabbits classified the compound as a severe eye irritant.

A guinea pig maximization test classified BIT as a moderate contact sensitizer whilst the Buehler test classifies BIT as non-sensitising. Literature data for the local lymph node assay support a classification of BIT as a moderate dermal sensitizer (EC3 2.3%).

From a four week in-use study with humans only limited conclusions can be drawn due to the low number of subjects, no detailed description of pre-existing chloromethyl-isothiazolinone allergy and a significant variation in product use.

Several case reports in the dermatological literature describe allergic contact dermatitis to benzisothiazolinone, and some individuals wearing disposable powder-free polyvinyl chloride gloves containing 3 - 36 ppm benzisothiazolinone have developed allergic contact dermatitis (Aalto-Korte et al. 2007). According to the latter study, a concentration of 20 ppm benzisothiazolinone in a glove seems to be enough for sensitization. Moreover, in the context of occupational uses, benzisothiazolinone (BIT) is a well-documented contact allergen.

As has been seen with MCI/MI and now with MI itself, these isothiazolinones are important contact allergens for the consumer in Europe. Within the mixture, MCI is known to be the more potent allergen (EC3 0.009%). MI is less potent (EC3 1.9%) and is now permitted at up to 100 ppm in leave on and rinse off cosmetic products; contact allergy to MI itself is

now a considerable problem in Europe and this is of concern (Add. ref: 50-61). If BIT is to be used as a preservative in cosmetic products, it is essential that the level be sufficiently low to prevent a repeat of history.

It is recommended that the incidence of contact allergy to BIT and other isothiazolinones be monitored at regular intervals (e.g. annually), by reference to dermatology clinic data in Europe. Necessary early interventions can then be introduced to reduce exposures and hence contact allergy and allergic contact dermatitis as required.

*Dermal absorption*
An in vitro study with human skin has been provided. The mean value + 1SD can be used for calculating the MOS. This was 61.9% of the applied dose (1.29 µg/cm$^2$) when 0.01% benzisothiazolinone aqueous was applied.
The dermal absorption studies had not been performed with representative cosmetic formulations.

*General toxicity*
The acute toxicity of benzisothiazolinone upon oral or dermal administration to rats is low (LD50 of 1193 and 4115 mg/kg bw, respectively). The NOAEL derived from a subacute 28 day oral (gavage) toxicity study in rats was 12.63 mg a.i./kg bw/day. Subchronic toxicity was evaluated in a 90 day oral (gavage) study (according to OECD guideline 408) and provided a NOAEL of 8.42 mg/kg bw/day for the active ingredient. The NOAELs in the 28-day and 90-day studies were based on histopathological lesions observed in the non-glandular stomach, which are most likely due to the irritant property of the test substance and are therefore, not relevant for the safety assessment of benzisothiazolinone as a cosmetic ingredient.

*Reproductive Toxicity*
In a two generation study in rats with dietary administration benzisothiazolinone produced at 1000 ppm slight adult toxicity in the F1 generation in terms of lower body weight gain, and in both generations limiting ridge hyperplasia of the stomach together with incidences of squamous cell hyperplasia, forestomach gastritis, keratosis and erosion/ulcer. At this concentration, the growth of the offspring was slightly impaired and in the F2a offspring, there was a slight reduction in pup survival. At 500 ppm, there were incidences of limiting ridge hyperplasia in the stomach only. There were no adverse effects of treatment at 250 ppm, equivalent to an approximate overall mean intake of 24 mg/kg bw/day (active ingredient). The NOAEL was based on histopathological lesions observed in the non-glandular stomach, which are most likely due to the irritant property of the test substance and therefore, not relevant for the safety assessment of benzisothiazolinone as a cosmetic ingredient. Therefore, the NOAEL of 50 mg a.i./kg bw/day for the systemic effects of benzisothiazolinone will be used for the safety assessment.

*Mutagenicity*
Benzisothiazolinone has been tested for the induction of gene mutation in 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*.

*Carcinogenicity*
No data provided

## 4. CONCLUSION

1. *Does SCCS consider benzisothiazolinone safe when used as a preservative up to a maximum authorised concentration of 0.01% in cosmetic products, based on the provided data?*

The SCCS considers benzisothiazolinone safe for use as a preservative in cosmetics products up to 0.01% with respect to systemic toxicity.

However, its sensitising potential is of concern.

2. *And/or does the SCCS have any scientific concern with regard to the use of benzisothiazolinone in cosmetic products?*

Sensitisation from related isothiazolinones is an important problem in consumers. This has occurred because there has been consumer exposure before safe levels of exposure relevant to sensitisation have been established. Benzisothiazolinone is a skin sensitiser in animal models with potency similar to methylisothiazolinone. Methylisothiazolinone, at 100 ppm (0.01%) in cosmetic products is causing contact allergy and allergic contact dermatitis in the consumer. Benzisothiazolinone is known to be a sensitiser in man and has induced sensitisation at *circa* 20 ppm in gloves.

There is no information on what may be safe levels of exposure to benzisothiazolinone in cosmetic products from the point of view of sensitisation.

Until safe levels of exposure have been established, the use of benzisothiazolinone in cosmetic products as a preservative or for other functions cannot be considered safe in relation to sensitisation.

## 5. MINORITY OPINION

Not applicable

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