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

OPINION

CONCERNING

METHYLISOTHIAZOLINONE

COLIPA n° P 94

Adopted by the SCCNFP on 23 April 2004
by means of the written procedure

1. Terms of Reference

1.1 Context of the question

The adaptation to technical progress of the Annexes to Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products. Request for inclusion of Methylisothiazolinone in Annex VI, part 1 – List of preservatives allowed – to Council Directive 76/768/EEC.

In response to the opinion of the SCCNFP concerning Methylisothiazolinone, adopted during the 23rd plenary meeting of 18 March 2003 (doc. n° SCCNFP/0625/02), additional information on the physico-chemical properties of the substance, an in vitro percutaneous absorption study and two studies on mutagenicity/genotoxicity was submitted to the SCCNFP for evaluation.

1.2 Request to the SCCNFP

The SCCNFP is requested to answer the following questions :

* *Is the SCCNFP of the opinion that the information submitted is sufficient to allow an adequate risk assessment of 2-Methyl-4-isothiazolin-3-one to be carried out?*

* *If yes, is 2-Methyl-4-isothiazolin-3-one safe when used as a preservative in cosmetic products?*

* *Does the SCCNFP recommend any further restrictions on the conditions of use and a maximum use concentration for 2-Methyl-4-isothiazolin-3-one when used as a preservative in cosmetic products?*

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

2.1.1. Primary name

Methylisothiazolinone (INCI)

2.1.2. Chemical names

Chemical name : 2-Methyl-4-isothiazolin-3-one
CAS name : 2-Methyl-4(2H)-isothiazolin-3-one

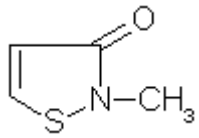
2.1.3. Trade names and abbreviations

Trade name : Kordek® 573T Industrial Microbiocide, RH-24.573, RH-4573, RH-573
COLIPA n° : P94

2.1.4. CAS / EINECS number

CAS : 2682-20-4
EINECS : 220-239-6

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : C_4H_5NOS
Mol. weight : 115.16

2.1.7. Purity, composition and substance codes

Batch tested : All batches used in the present dossier
Identification : by UV, IR, NMR and MS

Purity : 97.50 - 99.99 %
Chemical impurities : ≤ 4 % (including ≤ 0.39% 5-chloro-2-methyl-4-isothiazolin-3-one and ≤ 0.02% 5-chloro-2-methyl-4-isothiazolin-3-one)

2.1.8. Physical properties

Appearance	:	Off-white to light brown solid at room temperature
Melting point	:	46.7 – 48.3°C
Boiling point	:	thermal decomposition at 155°C
Density	:	1.35 at 25°C
Rel. vap. dens.	:	/
Vapour Press.	:	0.73 Pa at 20°C
Log P_{ow}	:	-0.486 at 24°C

2.1.9. Solubility

Water (pH 5)	:	>1000 g/l (20 °C)
Buffer	:	>574.6 g/l (pH 5), >489.0 g/l (pH 9)
Ethyl acetate	:	562 g/l (10°C), 688 g/l (30°C)
Hexane	:	0.93 g/l (10°C), 2.4 g/l (30°C)

2.1.10. Stability

Stable at 54°C for two weeks (study period)

2.2. Function and uses

Methylisothiazolinone is proposed to be used as a preservative in cosmetic products. Main uses are leave-on products, namely hand and body lotions and moisturisers (including facial moisturisers), sun tanning lotions and some rinse-off products like shampoos (mostly zinc pyrithione based anti-dandruff shampoos), surfactants and conditioners.

The requested use concentration is 100 ppm active ingredient in the finished cosmetic products.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

See doc. n° SCCNFP/0625/02

2.3.2. Acute dermal toxicity

See doc. n° SCCNFP/0625/02

2.3.3. Acute inhalation toxicity

See doc. n° SCCNFP/0625/02

2.3.4. Repeated dose oral toxicity

See doc. n° SCCNFP/0625/02

2.3.5. Repeated dose dermal toxicity

See doc. n° SCCNFP/0625/02

2.3.6. Repeated dose inhalation toxicity

See doc. n° SCCNFP/0625/02

2.3.7. Sub-chronic oral toxicity

See doc. n° SCCNFP/0625/02

2.3.8. Sub-chronic dermal toxicity

See doc. n° SCCNFP/0625/02

2.3.9. Sub-chronic inhalation toxicity

See doc. n° SCCNFP/0625/02

2.3.10. Chronic toxicity

See doc. n° SCCNFP/0625/02

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

See doc. n° SCCNFP/0625/02

2.4.2. Irritation (mucous membranes)

See doc. n° SCCNFP/0625/02

2.5. Sensitisation

See doc. n° SCCNFP/0625/02

2.6. Reproductive toxicity

See doc. n° SCCNFP/0625/02

2.6.1. One-generation reproductive toxicity

See doc. n° SCCNFP/0625/02

2.6.2. Two-generation reproductive toxicity

See doc. n° SCCNFP/0625/02

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Guideline	:	/
Test substance	:	methylisothiazolinone
Batch no	:	724.03
Purity	:	radio-purity 99.88 %
Tissue	:	female hairless rat, dorsal skin dermatomed to 300-350 µm
Skin integrity	:	not documented
Method	:	in vitro flow through diffusion cell 0.95 cm²
Receptor fluid	:	calf serum
Formulation	:	water solution
Spec. conditions	:	application under occlusion
Dose	:	concentration tested : 25 – 75 and 150 ppm (0.66, 1.97 and 3.97 µg/cm²)
Replicate	:	4 diffusion cells per concentration
Duration contact	:	24 hours
Analyt. method	:	liquid scintillation (^{14}C- methylisothiazolinone - radiolabeled material 39.05 µCi/mg)
Stability	:	/
GLP	:	in compliance

The skin penetration of methylisothiazolinone was evaluated in a flow through diffusion cell across female hairless rat dorsal skin. The test substance was prepared as a water solution at 3 concentrations 0.0025 %, 0.0075 % and 0.015 %. The recommended use level will be 100 ppm (i.e. 0.01 %). Approximately 25 µl of the formulation (exactly measured) were applied on the skin for 24 hours. The excess from the skin surface was removed by washing with water and with soap, then the skin was dried with a cotton swab. The substance was measured using liquid scintillation in the receptor fluid after 1, 2, 4, 19, 21 and 24 hours of diffusion. At the end of the test methylisothiazolinone was assayed in the washing liquids, in the horny layer removed by tape stripping, in the epidermis and the dermis. The mass balance of the experiment was calculated. The absorption was evaluated only from the receptor fluids and the dermis methylisothiazolinone contents.

Remark
The protocol is not following the SCCNFP recommendations (i) rat skin was used in place of human or pig, (ii) the methylisothiazolinone epidermal content was not considered for the evaluation of the absorption. The study is considered inadequate.
Results

The cumulative amount of methylisothiazolinone (taking into account : the receptor fluid, the epidermis and the dermis see table) penetrating after a contact of 24 hours through the hairless rat skin is between 67.9 ± 9.1 % for the lowest concentration (25 ppm) and 80.9 ± 15.3 % for the highest concentration (150 ppm).

% of applied dose		Epidermis	Dermis	Receptor fluid	TOTAL
25 ppm	1	30,50	0,20	25,40	56,1
	2	35,50	0,40	32,00	67,9
	3	64,20	0,00	14,10	78,3
	4	55,30	0,00	14,10	69,4
	mean	46,38	0,15	21,40	**67,93**
	STD	16,00	0,19	8,85	**9,12**
75 ppm	1	31,10	0,80	43,60	75,5
	2	25,30	1,10	21,60	48
	3	37,20	0,80	38,40	76,4
	4	43,60	0,40	31,00	75
	mean	34,30	0,78	33,65	**68,73**
	STD	7,88	0,29	9,55	**13,83**
150 ppm	1	32,20	0,60	32,80	65,6
	2	27,50	1,20	44,20	72,9
	3	30,00	0,80	69,80	100,6
	4	25,90	1,00	57,80	84,7
	mean	28,90	0,90	51,15	**80,95**
	STD	2,77	0,26	16,09	**15,28**

Ref.: 5

2.7.2. Tissue distribution *in vivo*

Guideline : /
Species/strain : CD-1 mice
Group size : 3 males and 3 females per observation time (15 animals for each sex)
Test substance : ^{14}C-methylisothiazolinone, radiochemical purity 96.7 %, 13.72 µCi/mg
Batch number : radiolabeled material: 395-0113
non radiolabeled material: 800IJ123
Analyt. method : liquid scintillation
Vehicle : distilled water
Dose level : 100 mg/kg bw, oral gavage, single dose
Observ. Time : 1, 3, 6, 24 and 48 hours
GLP : in compliance

Fifteen male mice (≈ 27 g) and fifteen female mice (≈ 23 g) were used for this assay. The radioactive compound was administered orally as a single dose of approximately 100 mg/kg bw as a water solution. This dose is corresponding to the LD_{10}. At 1, 3, 6, 24 and 48 hours post dose, 3 animals per sex were sacrificed and radioactivity was determined in: blood, plasma, liver (gall bladder was removed), bone marrow (from both femurs) and both femur bones.

Results
High levels of radioactivity were measured in the earlier time point samples (1 and 3 hours) and then declined. All the tissues contained significant amount of radioactivity. After 1 hour, the highest content was detected in the liver (107 ppm in male, 56 ppm in female) and the lowest in the bone (27 ppm in male, 18 ppm in female). In the bone marrow concentrations decreased progressively from the 1st hour to the end of the test (39 ppm to 1.1 ppm in male, and 29 ppm to 1 ppm in female). The tissue-to-plasma ratio showed that radioactivity partitioned preferentially from plasma into tissues after 24 hours. The study shows that ^{14}C-methylisothiazolinone reaches the bone marrow and remains there for up to 48 hours.

Ref.: 6

2.8. Mutagenicity / Genotoxicity

See doc. n° SCCNFP/0625/02

Additional i*n vitro / in vivo* UDS Assay

Methylisothiazolinone has been tested for the induction of UDS on rat primary hepatocytes, after *in vivo* treatment of Crl:CD rats by gavage with 100, 200 and 300 mg/kg (4 males/dose).
The evaluation has been made after 2 to 4 hours and after 14 to 16 hours. In both cases the positive control was represented by DMA.
The OECD guideline 484 was followed under GLP.
The compound was found unable to induce *in vivo* UDS; the positive control (DMA) was positive in both treatments.

Although OECD 484 guideline indicates the use of DMA only for the short treatment and AAF for the long treatment, the historical data provided for the positive controls indicates that the test can be accepted.

Conclusion
Methylisothiazolinone is non-genotoxic in an *in vivo* UDS assay on rats.

Ref.: 7

2.9. Carcinogenicity

See doc. n° SCCNFP/0625/02

2.10. Special investigations

See doc. n° SCCNFP/0625/02

2.11. Safety evaluation

CALCULATION OF THE MARGIN OF SAFETY

(**Methylisothiazolinone**)
(Preservatives)

Based on an exposure of 18 g of cosmetic products applied daily, containing at maximum 0.01 % (100 ppm) of Methylisothiazolinone :

Maximum amount of ingredient applied	**I (mg)**	**=**	**1.8 mg**
Typical human body weight		**=**	**60 kg**
Maximum absorption through the skin	**A (%)**	**=**	**100 %**
Dermal absorption per treatment	**I x A**	**=**	**1.8 mg**
Systemic exposure dose (SED)	**I x A / 60 kg**	**=**	**0.03 mg/kg bw**
No observed adverse effect level (rat, 90 day, drinking water)	**NOAEL**	**=**	**19 mg/kg bw**
Margin of Safety	**NOAEL / SED**	**=**	**633**

2.12. Conclusions

The requested data provided on physico-chemical properties on methylisothiazolinone are complete.

The percutaneous absorption study is inadequate. A 100% absorption is assumed.

The in vivo UDS assay is adequate. Methylisothiazolinone is considered non genotoxic/mutagenic.

See also doc. n° SCCNFP/0625/02.

2.13. Opinion

The SCCNFP is of the opinion that the proposed use of Methylisothiazolinone as a preservative at a maximum concentration of 0.01% (100 ppm) in the finished cosmetic product does not pose a risk to the health of the consumer.

2.14. References

Ref. 1 to 39 : see doc. SCCNFP/0625/02

40. Summary document
41. TR-01-059: Physico-chemical properties of Methylisothiazolinone Technical
42. TR-01-018: Physico-chemical properties of biocide formulation XB10-R2
43. TR-03-006: Storage stability study of biocide formulation XB10-R2
44. 00R-066: Methylisothiazolinone: in vitro percutaneous absorption
45. 03RC-042: Methylisothiazolinone: tissue distribution
46. 03RC-044: Methylisothiazolinone: in vivo/in vitro UDS study

47. Confidential attachment to TR-01-059: Physico-chemical properties of Methylisothiazolinone Technical, manufacturing process and impurities
48. TM94-144-01: analytical method for organic impurities of Methylisothiazolinone Technical
49. TR13-95-077: LC/MS analysis of low level impurities of Methylisothiazolinone Technical
50. Confidential attachment to TR-01-018: biocide formulation XB10-R2, product composition and impurities
51. 03R-1019: nature and purity of the material used in the toxicological studies