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

OPINION

CONCERNING

METHYLISOTHIAZOLINONE

COLIPA n° P94

Adopted by the SCCNFP during the 23rd plenary meeting
of 18 March 2003

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.

1.2 Request to the SCCNFP

The SCCNFP is requested to answer the following questions :

* Is the use of 2-Methyl-4-isothiazolin-3-one as a preservative in cosmetic products safe?

* Is there a need for setting a new concentration limit for the use of this substance 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 name)

2.1.2. Synonyms

2-Methyl-4-isothiazolin-3-one

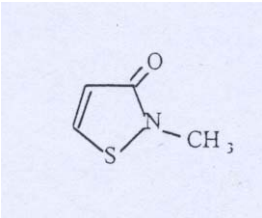
2.1.3. Trade names and abbreviations

Neolone™ 950 (= 9.5 % formulation of a.i. in water for cosmetic applications)
Kordek 573T, Industrial Microbiocide
RH-24,573
RH-573T, Industrial Microbiocide
MI

2.1.4. CAS no.

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

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : C_4H_5NOS
Mol. weight : 115.2

2.1.7. Purity, composition and substance codes

Purity active ingredient

Methyisothiazolinone : 96.8 %weight
Impurity (manufacturing by-products)

5-Chloro-2-Methyl-4-isothiazolin-3-one	:	0.1 %weight
4,5-Dichloro-2-Methyl-4-isothiazolin-3-one	:	
N,N'-Dimethyl-3,3'-dithiodipropionamide	:	0.2 %weight
N,N'-Dimethyl-3,3'-trithiodipropionamide	:	0.5 %weight
N-Methyl-3-chloropropionamide	:	
Ammonium chloride	:	
Water	:	0.2 %weight
Ethylacetate	:	0.1 %weight
Acetic acid	:	0.1 %weight
LC unknowns*	:	1.5 %weight

* Fraction of about 9 minor components, each present at a level generally < 0.1%, which have been tentatively identified by Liquid Chromatography/Mass Spectrometry (LC/MS) to be chlorinated products of monosulfide by-products produced during the amidation of methyl-3-mercaptopropionate.

2.1.8. Physical properties

Appearance	:	dark brown
Melting point	:	48.0 – 49.5 °C
Boiling point	:	/
Density	:	1.35 g/ml at 25 °C
Rel. vap. dens.	:	
Vapour Press.	:	2 x 10^{-2} torr at 25 °C
Log P_{ow}	:	-0.486
pH at 25 °C	:	2.58

2.1.9. Solubility

Solubility in	water	≥ 100 g/100 ml of solvent
	acetonitrile	≥ 78.6 g/100 ml of solvent
	methanol	≥ 79.1 g/100 ml of solvent
	hexane	2371 ppm
	xylene	15.65 g/100 ml of solvent

2.2. Function and uses

Cosmetic preservative. 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.

Requested use concentration : 100 ppm a.i.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

Groups of six male and six female Crl:CD®BR strain rats, were dosed orally by gavage with 2-Methyl-4-isothiazolin-3-one [MI, RH-573 Technical] as a solution of the ingredient [a.i.] in water. The administered doses were 75, 150, 180, and 225 mg MI a.i./kg bw. Two additional groups of six males were gavaged with solutions of MI at 225 or 300 mg/kg in order to determine the LD_{50} for male rats. Following dosing the animals were allowed free access to food and water and were observed for 14 days.

Signs of intoxication were observed at all doses in females and at dose levels of 150 mg/kg and higher in males. The signs began by 1 hr post-dosing and included : passiveness, ataxia, scant or no faeces, diarrhoea or soft faeces, mucus in faeces, yellow or brown stained anogenital area, red-stained muzzle and/or lacrimation. These signs resolved, in surviving rats, by day 6. Necropsy of decedents showed reddened intestines, red-tinged fluid or red/red-tinged material in the intestines; reddened glandular portion of the stomach; red-tinged fluid or mucous in the stomach and stomach distended by air. Necropsy of the survivors revealed no gross changes.

	Mortality				
Dose (mg/kg)	75	150	180	225	300
Males	0/6	0/6	0/6	4/12	6/6
Females	0/6	0/6	4/6	5/6	--
Combined	0/12	0/12	4/12	9/18	6/6

The acute oral LD_{50} for male rats was determined as 235 mg a.i./kg bw, [95% confidence limits of 216 to 336 mg/kg] and for female rats 183 mg/kg bw [95% confidence limits of 154 to 214 mg/kg].

Ref. : 1

Groups of six male and six female Crl:CD®BR rats were dosed with Neolone™ 950 by gavage at dose levels of 2000, 2500, 3000 and 5000 mg/kg bw corresponding to 193.8, 242.3, 290.7, 484.5 mg of a.i. [2- Methyl-4-isothiazolin-3-one] /kg bw. In additional groups of six female rats were dosed with 1000, 1500, 2000 mg Neolone / kg bw corresponding to 96.9, 145.4, 193.8 mg of a.i. [2- Methyl-4-isothiazolin-3-one] /kg bw.

Dosed-related mortality was observed, details are shown in the table below.

	Mortality					
Dose (mg/kg)	1000	1500	2000	2500	3000	5000

Males	--	--	1/5	3/6	2/6	6/6
Females	1/6	6/6	5/6	--	--	--

Clinical signs of toxicity were observed at all dose levels beginning 1 hr post dosing and continuing through day 4. The signs included; respiratory noises, passiveness, ataxia, lacrimation, salivation, scant and/or soft faeces, diarrhoea, and/or mucus in faeces. There were no effects on body weight among survivors at any dose level when compared to historical control data. Necropsy of the decedents revealed reddened intestines and/or stomach mucosa; clear or red/yellow fluid in the intestines and/or stomach; blackened intestines and distended stomachs.

The acute oral LD_{50} for Neolone™ 950 Preservative in male rats was 2834 mg product/kg bw [95% confidence limits of 2047 and 4377 mg/kg bw] and in females was 1091 mg product/kg bw [95% confidence limits of 891 and 1334 mg/kg bw]. [This corresponds to 105.7, 274.6 mg/kg a.i. MI].

Ref. : 2

2.3.2. Acute dermal toxicity

Groups of six male and six female Crl:CD®BR rats received occluded topical applications of Neolone™ 950 [2-Methyl-4-isothiazolin-3-one]. The material was applied undiluted at dose levels 2000, 3500 and 5000 mg/kg bw, to shaved intact skin for 24 hours. The animals were then observed for a further 14 days. There were no mortalities over the 14-day observation period. The only clinical signs observed were scant faeces in females from the 3500 and 5000 mg/kg dose groups on days 2 and 3 and in a single male from the 5000 mg/kg dose group on day 3.
Skin effects were noted at all dose levels beginning at day 1 and continuing through the 14-day observation period. These effects included pocketing oedema/oedema, erythema, blanching, desiccation, darkened or reddened areas, scabs, eschar and or sloughing. There were no apparent effects on body weight in either sex at any level and there were no gross changes observed at necropsy.

The acute dermal LD50 for Neolone™ 950 preservative was greater than 5000 mg/kg bw in male and female rats. [This corresponds to > 484.5 mg/kg a.i. MI].

Ref. : 3

2.3.3. Acute inhalation toxicity

No data

2.3.4. Repeated dose oral toxicity

Four groups of ten male and ten female Crl:CD®BR rats were exposed to RH-573 Technical (2-Methyl-4-isothiazolin-3-one], via their drinking water at concentrations of 0, 75, 250 or 1000 ppm a.i. for three months. Dose levels were selected based on range finding studies which indicated that concentrations higher than 1000 ppm would have compromised animal health. There were no mortalities in either sex at any dose level.

No treatment-related systemic or neurological effects were seen in the daily clinical observations, the Detailed Clinical Observations or Functional Observational battery parameters, or motor activity in either sex at any time period at any dose level.
There were no treatment-related effects on body weight in males at dose levels up to and including 250 ppm, or in females at any dose level. Treatment-related decreases in body weight and cumulative body weight gain were observed in males exposed to 1000 ppm RH-573. Cumulative body weight gain was also decreased in females exposed to 1000 ppm. These decreases were observed throughout the entire treatment period.
There were no treatment-related effects on feed consumption in males at dose levels up to and including 250 ppm, or in females at any dose level. Treatment-related decreases in feed consumption were observed in the high exposure group males throughout the duration of exposure.

There were treatment-related decreases in water consumption in female animals exposed to 250 and 1000 ppm throughout most of the dosing phase and in males at all exposure doses throughout dosing.
Haematology and clinical chemistry revealed no treatment-related changes in either sex at any dose level. There were no treatment-related gross or microscopic pathological findings at any dose level in either sex.

Based on effects on body weight and feed consumption at 1000 ppm (65.7 and 93.5 mg a.i./kg bw/day in males and females respectively) the NOAEL in this study was 250 ppm (equivalent to 19.0 and 24.6 mg a.i./kg bw/day in males and females, respectively).

Ref. : 4

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

No data

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)

2-Methyl-4-isothiazolin-3-one was applied undiluted by a single application of 0.5 ml to the shaved intact skin of 7 New Zealand White rabbits. Contact time was 3 minutes for 5 animals, 1-hr (and 3 min.) for one animal, 4-hrs for one animal. The application sites were semi-occluded. After removal of the patch, the animals were observed for 14 days for signs of irritation.
No mortality of clinical sign of systemic toxicity were observed during the study.
On the 4 and 1 hr sites, skin irritation indicative of corrosivity was observed on day 14 and 7, respectively.
On the 3 min. site, very slight to well defined erythema was noted through day 7 and slight oedema was noted at 1 hr.
The 5 animals exposed during 3 min., very slight to well defined erythema was noted through 48 hrs in most rabbits. Very slight to moderate oedema was noted at 1 and 24 hrs. Very slight to slight oedema was noted in one rabbit at 48 and 72 hrs.

2-Methyl-4-isothiazolin-3-one is corrosive to the skin when applied undiluted.

Ref. : 5

0.5 ml of an aqueous solution of Neolone^{TM} 950 (100 ppm a.i. [MI]) was applied to the skin of a group of six New Zealand White rabbits. Contact time was 4 hours and the application was semi-occluded. After removal of the patch, the animals were observed for 72 hours for signs of irritation.
No mortality or clinical signs of systemic toxicity were observed during the study. There was no erythema or oedema present at any observation period. The Primary Irritation Index (PII) was 0.0.

An aqueous solution of MI is non-irritating to rabbit skin at proposed recommended use concentrations of 100 ppm active ingredient.

Ref. : 6

Human study (modified HRIPT)

The cumulative irritation potential of 2-Methyl-4-isothiazolin-3-one (RH-573; MI) was - investigated in 21 day test with human volunteers. Aqueous dilutions of MI (0.1 ml) were applied to the back under occlusive patches, for a contact period of 23 hours, on 21 consecutive days. On completion of the dosing phase, the subjects were rested without further dosing for 10 - 14 days. Following the rest period, 24 hour patch(es) of the appropriate test material(s) were applied to a naïve site. Subject induced with 50, 100 and 250 ppm MI were challenged with the same respective concentrations of test material as well as distilled water and sodium lauryl sulphate. Subjects induced with 500 ppm MI were challenged with 100, 250 and 500 ppm MI as well as with distilled water and SLS. Four of the subjects induced with 1000 ppm were not only challenged with 1000 ppm but also with 250 and 500 ppm.

Group	Number of subjects	Introduction concentration of MI (ppm)	Total Reactions during dosing	Cumulative irritation	Challenge concentration of MI (ppm)	Reactions on challenge
I	16	50	11/16	0/16	50	0/16
II	15	100	4/15	0/15	100	0/15
III	17	250	6/17	0/17	250	0/17
IV	15	500	7/15	0/15	500	1/15
					250	1/15
					100	0/15
V	16	1000	15/16	1/16	1000	2/16
					500	1/4
					250	1/4

During the introduction phase, a number of irritant reactions (to both MI and vehicle control - distilled water) were observed, these were mainly graded as 1 and were transient in nature. The total reactions for vehicle controls were 7/16, 4/15, 4/17, 4/15 and 12/16 for the groups I, II, III, IV and V, respectively. No reactions were noted on challenge for the vehicle controls in any group.
Cumulative irritation was only observed in one individual from the 1000 ppm induction group.

Ref. : 8

2.4.2. Irritation (mucous membranes)

Neolone™ 950 [9.5% active ingredient in water] was applied to the conjunctival sac of a group of six New Zealand White rabbits, as a 100 ppm aqueous solution (0.1 ml). Eye irritation was evaluated according to Draize criteria at approximately 1, 24, 48, and 72 hours. After the 24 hour observation, each eye (treated and control) was irrigated with 0.9% saline for approximately 60 seconds. No mortality or clinical signs of systemic toxicity were observed. No corneal, conjunctival or iridial effects were observed during the study.

Neolone™ 950 Preservative is non-irritating to rabbit eyes at proposed recommended use concentrations of 100ppm active ingredient.

Ref. : 7

2.5. Sensitisation

The skin sensitisation potential of 2-Methyl-4-isothiazolin-3-one [MI; RH-24,573] was determined using the closed patch method of Buehler [OECD 406]. Four groups of outbred Hartley guinea pigs [5/sex/group] received ten 6-hr induction doses [3 doses/week for 3.5 weeks] of 0.4 ml at concentrations of 1000, 5000, 15,000 and 30,000 ppm a.i. in distilled water. Two weeks after the last induction dose these animals, together with a group of uninduced control animals, were challenged with 1000, 5000, or 15,000 ppm MI in distilled water.
No erythema reactions were observed in the non-induced control animals at any challenge concentration of MI.

	Challenge dose (ppm a.i.)

Induction dose [ppm a.i.]	1000	5000	15000
0			
1000	0/10	0/10	1/10
5000	0/10	2/10	6/10
15000	1/10	1/10	3/10
30000	0/10	2/10	5/10

The concentration of MI required to induce and elicit a response in 50 % of the animals (EC50) were estimated to be ≥ 5000 ppm a.i. for induction at a challenge concentration 15,000 ppm a.i. and ≥ 15,000 ppm a.i. for challenge at an induction concentration of 30,000 ppm a.i.

Ref. : 9

Human Repeated Insult Patch test

One subject, from the 500 ppm induction group (see table Human study, modified HRIPT, point 2.4.1), was found to react on challenge. This individual was found to react to the marker pen and also to a number of consumer products; this reaction was therefore considered to equivocal. Two subjects from the 1000 ppm induction group showed a mild reaction upon challenge and were considered to be sensitised.

Based on this data the threshold for sensitisation appears to be at or around 1000 ppm 2-Methyl-4-isothiazolin-3-one.

Ref. : 8

In an intensified Shelanski and Shelanski Repeated Insult Patch Test, ninety-eight [98] adult volunteers, patch test negative to 100 ppm Kathon® CG, were enrolled into the study. Kordek™ 50C (Methyl-4-isothiazolin-3-one; MI), 0.15 ml of a 100 ppm aqueous solution, was applied to a webril pad and the pad applied to the back of the volunteers under occlusion. Patches were applied four times a week for three weeks [induction phase]. After a week of dosing, the subjects were challenged on a fresh site with MI, 0.15 ml of a 100 ppm aqueous solution applied to a webril pad. One of the 98 subjects showed a positive response [grade 4] on the fifth day of the induction phase. This subject was judged to be pre-sensitised. Of the remaining 97 subjects none reacted to challenge [elicitation] with 100 ppm aqueous MI.
Under the conditions of this test, 100 ppm Methyl-4-isothiazolin-3-one did not induce skin sensitisation in human volunteers.

Ref. : 10

In a Repeat Insult Patch Test, 113 adult volunteers (12 males and 101 females), were enrolled into the study. 0.2 ml of an aqueous solution of 200 ppm 2-Methyl-4-isothiazolin-3-one, was applied by occlusive patches for a contact period of 24-hr per day. Patches were applied three times a week for three weeks [induction phase]. After a week free of dosing, the subjects were challenged on a fresh site with MI, 0.2 ml of a 200 ppm aqueous solution applied to a webril pad.

There was no adverse effect reported in the 100 subjects who completed the study. 13 out of 113 enrolled in the study either violated the protocol or withdrew from the study.

Under the conditions of this test, 200 ppm Methyl-4-isothiazolin-3-one did not induce skin sensitisation in human volunteers.

Ref. : 11

In a Repeat Insult Patch Test, 107 adult volunteers (19 males and 88 females), were enrolled into the study. 0.2ml of an aqueous solution of 300 ppm 2-Methyl-4-isothiazolin-3-one, was applied by occlusive patches for a contact period of 24-hr per day. Patches were applied three times a week for three weeks [induction phase]. After a week free of dosing, the subjects were challenged on a fresh site with MI, 0.2 ml of a 300 ppm aqueous solution applied to a webril pad. There was no adverse effect reported in the 98 subjects who completed the study. 9 out of 107 enrolled in the study either violated the protocol or withdrew from the study.

Under the conditions of this test, 300 ppm Methyl-4-isothiazolin-3-one did not induce skin sensitisation in human volunteers.

Ref. : 12

Sensitisation Potency of MI in relation to MCI/MI.

Animal data

In a study using the Buehler method (Ref. 9), the concentrations of RH-24,573 [MI] required to induce and elicit a response in 50% of guinea pigs [EC_{50}][a] were estimated. The EC_{50} for induction was determined to be > 5000 ppm a.i. at a challenge concentration of 15,000 ppm a.i. For elicitation the EC_{50} was 15,000 ppm a.i. at an induction concentration of 30,000 ppm a.i. A similar study (Ref. 28) performed with MCI/MI [3/1] determined the EC_{50} for induction to be 88 ppm a.i. at a challenge concentration of 2,000 ppm a.i., and the EC_{50} for elicitation to be 429 ppm a.i. at an induction concentration of 1,000 ppm a.i.

In a version of the Local Lymph Node Assay (Ref. 29), the PC_{200} [the concentration giving 2 fold a proliferate response over controls] for MI was 1506 µg and for MCI it was 11 µg.

The sensitisation potential of methylchloroisothiazolinone/methylisothiazolinone [3:1] was compared to that of Methylisothiazolinone in the Open Epicutaneous test. The threshold for induction for MCI/MI was 58 ppm (Ref. 30). For MI the induction threshold was in the range of 3000 ppm. (Ref. 31)

a : EC_{50} is the effective concentration producing the effect under study in 50% of the test population

Human Data

Although no comparative Human Repeat Insult Patch tests have been performed on MCI/MI and MI, it is possible to compare the sensitisation potential based on existing studies of the two substances. The available data, taken partly from Company reports and partly from the open literature, is summarised in the table below.

Table 1: Comparison of Human Repeat Insult Patch Test data on MCI/MI and MI

	MCI/MI [3:1]			MI		
Concentration [ppm]	**Dose [µg/cm^2]**	**Incidence**	**% Response**	**Dose [µg/cm^2]**	**Incidence**	**Response**
	0.42	0/416	0.0	-	-	-
	0.50	0/103	0.0	-	-	-
7.5	0.75	0/184	0.0	-	-	-
10	0.83	0/602	0.0	-	-	-
12.5	1.04	1/84	1.2	-	-	-
15	1.25	0/200	0.0	-	-	-
15	1.34	2/189	1.1	-	-	-
20	1.67	2/45	4.4	-	-	-
50[b]	2.50	0/109	0.0	-	-	-
100[b]	5.00	5/ 1 16	4.3	5	0/97	0.0
150[c]	7.50	7/196	3.6	-	-	-
200	-	-	-	10	0/100	0.0
300	-	-	-	15	0/98	0.0
400	-	-	-	20	1/116	0.9
500	-	-	-	45	1/210	0.5
600[d]	-	-	-	30	0/75	0.0

b : Based on the summation of results of Draize tests conducted by Maibach cited in ref. 6 p.105

c : Subjects received six induction exposures at 150 ppm in petrolatum followed by four induction exposures at 300 ppm in water - Maibach cited in ref. 6 p 104

d : Study in progress the results to date are reported.

Ref. : 32, 33, 34

Based on the results of the HRIPT data there is at least a factor of 30 difference in the sensitisation [induction] potential of the two isothiazolinone products. This compares favourably with the Open Epicutaneous Test [OET] which shows a factor of 50 difference in sensitisation [induction] potential.
Thus on the basis of this data , the number of new sensitisations induced by exposure of people to MI, through the use of cosmetic and toiletry products, is predicted to be low.

Dose-elicitation studies of Methylisothiazolinone on individuals known to be allergic to Kathon® CG

In a study, 28 patients sensitised to MCI/MI were patch tested with MCI and MI, all individuals reacted to MCI at 300 ppm whereas only 2 reacted to MI at 300 ppm [one also reacted to 100 ppm MI].

Ref. : 35

Further studies showed that in subjects in 12 previously sensitised to MCI/MI [all reacted to a 150 ppm patch of MCI/MI] 3 reacted to MI at 115 ppm with weak reactions, recorded by the authors as 'doubtful'.

Ref. : 36

In a study, 85 subjects, from the clinics of the IVDK, identified as patch positive to MCI/MI were patch tested with MI at concentrations of 500 ppm a.i or 1000 ppm a.i. in water. The allergic status towards MCI/MI was compared with the responses to the MI patches. The results are shown in tables 2 and 3.

Ref. : 37

Table 2 : Reactions of the MCI/MI -positive test subjects to MI

No. Subjects	**Total MI negative**	**MI negative (500 ppm)**	**MI positive (500 +1000 ppm)**
85	58 [68%]	9 [11%]	27 [32%]

Table 3 : Reactions of the MCI/MI - positive test subjects to MI graded by response to MCI/MI

No. Subjects	**MI positive**	**MCI/MI (+) and MI positive**	**MCI/MI (++/+++) and MI positive**	**MCI/MI (++/+++) and MI negative**
85	27 [32%]	12 [14%1	1 1 [13%1	7 [8%]

In the 73 subjects where the intensity of the MCI/MI reaction was reported, there is a highly significant correlation [$p<0.01$] between the intensity of MCI/MI sensitisation and the reaction to MI. See table 4.

Table 4 : Relationship between the intensity of MCI/MI sensitisation and the reaction to MI

	MI (+/+++) positive	**MI (+/+++) negative**	**Total**
MCI/MI (++/+++) positive	11 [61%]	7 [39%]	18
MCI/MI (+) positive	12 [22%]	43 [78%]	55
Total	23	50	73

The results show that, at high concentrations of MI [500 to 1000 ppm], a proportion of the subjects with a known sensitivity to MCI/MI may also react to MI. Thus, from the available data, it cannot be excluded that patients previously sensitised to MCI/MI will react to products containing 100 ppm MI. However, the numbers are expected to be low and will be further reduced by the warning provided through ingredient labelling.

Importantly, based on the HRIPT data, the number of new sensitisations induced by exposure to MI through the use of cosmetic and toiletry products is expected to be low.

Sensitisation potential of degradation products

Degradation of MCI/MI involves opening of the isothiazolinone ring by nucleophilic attack on the ring sulphur. During the nucleophilic attack, the chlorine atom at position 5 of the isothiazolinone ring leaves, thus both MCI and MI will follow essentially the same metabolic/degradation pathways. Once the ring has opened the electrophilic reactivity and biological action is lost.
Confirmed by Bruze and Gruvberger who failed to find positive reactions when N-methylmalonamic acid, malonamic acid and malonic acid were tested in 10 MCI/MI sensitive patients. Further, inactivation of MCI/MI with sodium bisulphite destroys the sensitisation potential.

Ref. : 38, 39

2.6. Reproductive toxicity

No data

2.7. Toxicokinetics (incl. Percutaneous Absorption)

In the absence of a percutaneous absorption study, 100% penetration is assumed in conducting the risk assessment

2.8. Genotoxicity

2.8.1 Mutagenicity/Genotoxicity in vitro

Bacterial Reverse Mutation Test

Study 1

Guideline : /
Species/strain : *Salmonella typhimurium*, TA98, TA100, TA1535, TA1537
Replicates : Not Indicated
Test substance : 2-methyl-4-isothiazolin-3-one in Saline Buffer Solution
Batch no : #MH32:72C (purity: not indicated)
Concentrations : 0.0001 - 100 μg/plate with and without metabolic activation
GLP : /
COLIPA P 94 has been investigated for gene mutation in *Salmonella typhimurium* . Liver S9 fraction from rats pre-treated with Aroclor 1254 was used as the exogenous metabolic activation system. No Indication is given regarding the concentration range selected. Negative and positive controls were in accordance with the OECD guideline.

Results

The test has been performed in 1982; purity is not given ; no dose-range finding assay is presented; no interpretation of data; only 1 page summary and 4 tables were included in submission I.

Conclusions
The study is unacceptable.

Ref. : 13

Study 2

Guideline	:	OECD 471 (1997)
	:	EEC Directive 92/69 Method B14
Species/strain	:	Salmonella typhimurium, TA98, TA100, TA 102 TA1535, TA1537
Replicates	:	2 experiments with and without metabolic activation
Test substance	:	2-methyl-4-isothiazolin-3-one in distilled water
Batch no	:	B-1103, (purity: 97.5 % a.i.)
Concentrations	:	5 -1000 μg/plate - Initial
	:	30 -600 μg/plate - Confirmatory
GLP	:	in compliance

COLIPA P94 has been investigated for gene mutation in Salmonella typhimurium using a plate incorporation protocol. Liver S9 fraction from rats pre-treated with Aroclor 1254 was used as the exogenous metabolic activation system. The concentration range was selected following a preliminary study which showed toxicity at and above 1000 μg/plate in the absence of activation. In the definitive assay, toxicity was noted at 1000 μg/plate in all Salmonella tester strains in the presence of activation and at 500 μg/plate in the absence of activation.
Negative and positive controls were in accordance with the OECD guideline.

Results
The test substance did not induce any significant, reproducible increases in the observed number of revertant colonies in any of the tester strains used, either in the presence or absence of activation system. The positive control agents gave the expected results.

Conclusions
Based on the reversion rate, it is concluded that the test agent COLIPA P 94 (2-methyl-4-isothiazolin-3-one) when tested in distilled water shows no evidence of mutagenic activity in this bacterial test system in the presence or in the absence of activation system.

Ref. : 14

***In Vitro* Mammalian Cell Gene Mutation Test**

OECD guideline	:	OECD 476 (1984)
Species/strain	:	Chinese Hamster Ovary cells/ HGPRT Locus
Replicates	:	2 independent tests with and without metabolic activation
Test substance	:	2-methyl-4-isothiazolin-3-one in distilled water
Batch no	:	B-1103, (purity: 97.5 % a.i.)
Concentr. scored	:	0.5 - 25.0 μg/ml - Initial

	:	5.0 - 40.0 μg/ml - Confirmatory
GLP	:	in compliance

COLIPA P 94 has been investigated for induction of gene mutations at the HPRT locus in Chinese hamster ovary (CHO) cells. Liver S9 fraction from Aroclor 1254-induced rats was used as the exogenous metabolic activation system. The maximum concentration was determined on the basis of a preliminary study which showed toxicity at and above 50 μg/ml. Negative and positive controls were in accordance with the OECD guideline.

Results
No statistically or biologically significant increase in mutant frequency was observed over the concurrent solvent controls for any dose. No indication for a positive dose-response trend was noted in either the initial or confirmatory tests in the presence or absence of activation.

Conclusions
Based on the mutation frequency rate, it is concluded that the test agent COLIPA P 94 (2-methyl-4-isothiazolin-3-one) when tested in distilled water does not demonstrate mutagenic potential on the HPRT gene of CHO cells.

Ref. : 15

***In Vitro* Mammalian Chromosome Aberration Test**

Guideline	:	OECD 473 (1997)
	:	Annex V to Directive 67/548 EEC. Method B10
Species/strain	:	Chinese Hamster Ovary (CHO) cells
Replicates	:	Duplicate cultures, one experiment only
Test substance	:	2-methyl-4-isothiazolin-3-one in sterile deionised water
Batch no	:	B-1103, (purity: 97.5 % a.i.)
Concentr. scored	:	1.25, 2.5, 5.0, 9.53 and 12.7μg/ml without metabolic activation in the initial test.
	:	2.5, 5.0, 9.53, 12.7 and 16.9 μg/ml with metabolic activation in the initial test.
	:	1.25, 2.5, 3.75 and 7.5 μg/ml without metabolic activation in the confirmatory test.
	:	1.25, 2.5, 5.0 and 7.5 μg/ml with metabolic activation in the confirmatory test.
GLP	:	in compliance

COLIPA P 94 has been investigated for induction of chromosomal aberrations in CHO cells. Liver S9 fraction from rats pre-treated with Aroclor 1254 was used as the exogenous metabolic activation system. The test concentrations were selected based on a preliminary dose-range finding study. Negative and positive controls were in accordance with the OECD guideline.

Exposure periods were as follow: The test agent was added ± 24 h after cells seeding :

Test # 1	:	initial assay	without S9 mix	:	3 h exposure + 20 h recovery
			with S9 mix	:	3 h exposure + 17 h recovery

Test # 2	:	confirmatory	without S9 mix	:	17.8 h exposure + 2 h recovery
			with S9 mix	:	3 h exposure + 17 h recovery

Results
pH & Osmolarity: No influence of these parameters have been observed (Osmolarity of McCoy's 5 medium : 299 mOsm/kg water. Osmolarity of P 94 at 5000 µg/ml: 316 mOsm/kg water).

Tests # 1 - initial assay - without S9 mix

Toxicity
In the dose-range finding assay, from 16.9 µg/ml and above, severe signs of toxicity were noted (floating dead cells, reduction in the number of visible mitotic cells, reduction in the cell monolayer confluence,…)

In the initial assay, results are :

Dose tested for CA	:	1.25	2.5	5.0	9.53	12.7 µg/ml
% cell counts reduction	:	2 %	1 %	39 %	42 %	57 %
% MI reduction	:	0 %	0 %	0 %	8 %	23 %

Chromosome aberrations (CA)
A statistically and biologically significant increase in the number of cells with aberration was observed at 9.53 and 12.7 µg/ml as compared to the corresponding solvent control.
(Neg Ctrl MacCoys medium: 1.5 % ; water : 1.0%, Positive ctrl : 72 % of aberrant cells).

Dose tested for CA	:	1.25	2.5	5.0	9.53	12.7 µg/ml
% aberrant cells	:	0.5 %	0.5 %	3.5 %	15.5 %	30.0 %

(9.53µg/ml : 11/200 triradial & 7/200 quadriradial figures ; 12.7 µg/ml : 8/200 triradial & 20/200 quadriradial figures A+B of 50 cells).

Aneuploidy
No significant increase in the number of polyploid cells or endoreduplication was observed.

Positive control
Showed a strong distinct increase of aberrant cells.

Tests # 2 - confirmatory assay - without S9 mix

Toxicity
In the dose-range finding assay, from 10,0 µg/ml and above, severe signs of toxicity were noted : floating dead cells, reduction in the number of visible mitotic cells, reduction in the cell monolayer confluence,…

In the confirmatory assay, results are :

Dose tested for CA	:	1.25	2.5	3.75	7.5	µg/ml
% cell counts reduction	:	0 %	0 %	29 %	42 %	
% MI reduction	:	0 %	0 %	0 %	32 %	

Chromosome aberrations (CA)
A statistically and biologically significant increase in the number of cells with aberration was observed at 3.75 and 7.50 µg/ml as compared to the corresponding solvent control.
(Negative contril MacCoys medium: 0.5 % ; water : 2.5 %, Positive control : 44 % of aberrant cells).

Dose tested for CA	:	1.25	2.5	3.75	7.5	µg/ml
% aberrant cells	:	0 %	3.5 %	28 %	34 %	

(3.75 µg/ml : 36/200 triradial figures ; 7.5 µg/ml : 32/200 triradial figures extrapolated from a cell count A+B of 50).

Aneuploidy
No significant increase in the number of polyploid cells or endoreduplication was observed.

Tests # 1 - initial assay - with S9 mix

Toxicity
In the dose-range finding assay, from 16.9 µg/ml and above, severe signs of toxicity were noted : floating dead cells, reduction in the number of visible mitotic cells, reduction in the cell monolayer confluence,…

In the initial assay, results are :

Dose tested for CA	:	2.5	5.0	9.53	12.7	16.9 µg/ml
% cell counts reduction	:	0 %	10 %	27 %	56 %	70 %
% MI reduction	:	15 %	24 %	28 %	27 %	52 %

Chromosome aberrations (CA)
A statistically and biologically significant increase in the number of cells displaying aberrations was observed at 12.7 and 16.9 µg/ml as compared to the corresponding solvent control.
(Negative control MacCoys medium: 0 % ; water : 1.0 %, Positive control : 32 % of aberrant cells).

Dose tested for CA	:	2.5	5.0	9.53	12.7	16.9 µg/ml
% aberrant cells	:	0.5 %	0.5 %	3.0 %	11 %	12 %

(11 µg/ml : 7/200 triradial & 7/200 quadriradial figures ; 12.7 µg/ml : 7/125 triradial & 2/125 quadriradial figures A+B of 125 cells).
Aneuploidy
No significant increase in the number of polyploid cells or endoreduplication was observed.

Positive control
Showed a strong distinct increase of aberrant cells.

Tests # 2 - confirmatory assay - with S9 mix

Toxicity
In the dose-range finding assay, from 12.5 µg/ml and above, severe signs of toxicity were noted : floating dead cells, reduction in the number of visible mitotic cells, reduction in the cell monolayer confluence.

In the confirmatory assay, results are :

Dose tested for CA	:	1.25	2.5	5.0	7.5 µg/ml
% cell counts reduction	:	103 %	86 %	166 %	102 %
% MI reduction	:	0 %	21 %	25 %	48 %

Chromosome aberrations (CA)
A statistically and biologically significant increase in the number of cells displaying aberrations was observed at 7.5 µg/ml as compared to the corresponding solvent control.
(Negative control MacCoys medium: 1.0 % ; water : 1.5 %, Positive control : 40 % of aberrant cells).

Dose tested for CA	:	1.25	2.5	5.0	7.5 µg/ml
% aberrant cells	:	0 %	1.5 %	1.5 %	9.0 %

(7.5 µg/ml : 9/200 triradial figures ; 3/200 tetraradial figures).

Aneuploidy
No significant increase in the number of polyploid cells or endoreduplication was observed.

Positive control
Showed a strong distinct increase of aberrant cells.

Conclusions
In addition of the trend for a dose-effect relationship in the number of cells with aberrations, qualitatively, a large number of breaks, triradial and quadriradial figures were scored. Such rearrangements are associated with a biological relevance of the induced aberrations observed. COLIPA P 94 may be considered as being positive for inducing structural chromosome aberrations in Chinese hamster ovary cells in the absence or in the presence of metabolic activation system when tested at concentrations that induce toxic effects.

Therefore COLIPA P 94 (or one of its metabolites) should be considered as a clastogen qualitatively speaking. Frequency and types of aberration evidenced should be considered as an alert regarding the clastogenic potential of this substance.

Ref. : 16

2.8.2. Mutagenicity/Genotoxicity *in vivo*

In Vivo Erythrocyte Micronucleus test

Guideline	:	OECD 474 (1997)
	:	Dir. 92/69//EEC B12
Species/strain	:	Mouse, Crl:CD-1 mice
Group size	:	5 male + 5 female

Test substance	:	2-methyl-4-isothiazolin-3-one in sterile deionised water
Batch no	:	B-1103, (purity: 97.5 % a.i.)
Dose levels	:	10 , 50 and 100 mg/kg bw, single intragastric gavage
Sacrifice times	:	24 and 48 hours after dosing
GLP	:	in compliance

COLIPA P 94 has been investigated for induction of micronuclei in the bone marrow cells of CD-1 mice. The substance was administered once by gavage at 0, 10, 50 and 100 mg/kg bw and the bone marrow harvested after 24 and 48 hours. Negative and positive controls were in accordance with the OECD guideline.
Maximum Tolerated Dose (MTD) : the top dose of P 94 was chosen on the basis of patterns of lethalities or severe toxicity observed previously during an acute oral toxicity study in male and female mice. The top dose selected is 100 mg/kg bw.

Results

Reactions to treatment
Some mortalities and reactions to treatment were observed for the high dosage group : lethargy and labored breathing was recorded in 2 females 4 hours after dosing; these 2 females were found death 24 hours after dosing.
No other signs of clinical toxicity were observed in any of the remaining females or in the male dosage group.

Mean values of micronucleated PCE
No statistically significant or biologically relevant increase in the incidence of micronucleated polychromatic cells over the concurrent vehicle control values were observed. This holds true for both 24 h and 48 h sacrifice time.

PCE/NCE ratio
Groups of mice treated with COLIPA P 94 did not exhibit a statistically significant reduction of the PCE/NCE ratio compared to the vehicle control for any of the sacrifice times. This may reflect either a lack of toxic effects of this compound on the erythropoiesis or that the test substance did not reach the target organ (bone marrow).
Conclusions
Under the conditions of the test, it can be concluded that there was no evidence of induced chromosomal or other damage leading to the micronucleus formation in polychromatic erythrocytes treated mice after 24 and/or 48 hours after single gavage of COLIPA P94 up to doses at which signs of clinical toxicity were recorded only in 2 female mice.
COLIPA P94 did not show any toxicity on PCE/NCE ratio due possibly to the fact that the compound has not reached the target cells.

Ref. : 17

General Conclusions

Methylisothiazolinone in sterile deionised water has been tested in procaryotic cells for gene mutation in several tester strains. The results of the bacterial gene study demonstrated the absence of mutagenic effects in bacteria at the gene level.

The *in vitro* test for mammalian gene mutation assay is negative in the presence or in the absence of activation.
The *in vitro* test for clastogenicity in Chinese Hamster Ovary cells (CHO) is considered clearly positive in repeated experiments.
The mouse *in vivo* micronucleus test is negative (questionable due to the fact that there is no indication on bone marrow toxicity).
On the base of the available adequate studies the compound is classified as clastogenic *in vitro*.

2.9. Carcinogenicity

No data

2.10. Special investigations

No data

2.11. Safety evaluation

NOT APPLICABLE

CALCULATION OF THE MARGIN OF SAFETY

Maximum amount of ingredient applied	I (mg)	=	mg
Typical body weight of human		=	60 kg
Maximum absorption through the skin	A (%)	=	%
Dermal absorption per treatment	I x A	=	
Systemic exposure dose (SED)	I x A / 60	=	
No observed adverse effect level (mg/kg) (species, study)	NOAEL	=	

Margin of Safety	NOAEL / SED	=

2.12. Opinion

The SCCNFP is of the opinion that the information submitted is insufficient to allow an adequate risk assessment of this substance to be carried out.

The genotoxicity/mutagenicity studies are inadequate in general; the *in vitro* test for clastogenicity has been considered clearly positive in independent studies.

Consequently, and before any further consideration, the following are required :

* more detailed information concerning the physico-chemical properties of Methylisothiazolinone (e.g. LCMS analysis, pH, stability and degradation products);

* information on the material used in the tests (batch numbers, purity and impurities);

* an *in vitro* percutaneous absorption study;

* relevant and adequate genotoxicity/mutagenicity studies.

2.13. References

1. Rohm and Haas report No. 98R-212. (1999) RH-573 Technical; Acute oral toxicity study in male and female rats.
2. Rohm and Haas Report No 00R-004 (2000) Neolone™ 950 Preservative Acute Oral Toxicity Study in Male and Female rats.
3. Rohm and Haas report No 00R-005 (2000) Neolone™ 950 Preservative Acute Dermal Toxicity Study in Male and Female rats.
4. Rohm and Haas Report No 99R-135 (2000) RH-573 Technical: Three-Month Drinking Water Toxicity Study in Rats
5. Rohm and Haas Report No 96R-123, RH-573T - Skin Irritation Study in Rabbits
6. Rohm and Haas Report No OOR-006 (2000) NeoloneTm 950 Preservative (100 ppm aqueous solution) Skin Irritation Study in Rabbits.
7. Rohm and Haas Report No OOR-007 (2000) NeoloneTm 950 Preservative (100 ppm aqueous solution) Eye Irritation Study in Rabbits.
8. Rohm and Haas Report No. 92RC-097A. (1994) RH-573 - Evaluation of 21-Day Cumulative Irritation Potential in Humans
9. Rohm and Haas Report No. 88R-052. (1989) RH-24,573: Delayed Contact Hypersensitivity Study in Guinea Pigs.
10. Rohm and Haas Report No. 99RC-0138. (2000) A Patch Test to Determine the Skin Irritation and Sensitisation Propensities of Kordek TM 50C (study conducted at 100 ppm active ingredient)
11. Rohm and Haas Report No. 00RC-099A. (2000) Repeat Insult Patch Study with 2-methylisothiazolin-3-one at an Aqueous Concentration of 200 ppm Active Ingredient.
12. Rohm and Haas Report No. OORC-099B. (2000) Repeat Insult Patch Study with 2-methylisothiazolin-3-one at an Aqueous Concentration of 300 ppm cove Ingredient.
13. Rohm and Haas report n° 81R-301. (1982) 2-Methyl-4-isothiazolin-3-one Microbial Mutagen Test
14. Rohm and Haas report n° 99R-062. (1999) Kordek 573T Salmonella typhimurium Gene Mutation Assay
15. Rohm and Haas report n° 99RC-265. (1999) Test .for Chemical Induction of Gene Mutation at the HPGRT Locus in Cultured Chinese Hamster Ovary (CHO) Cells With and Without Metabolic Activation With a Confirmatory Assay.
16. Rohm and Haas report n° 99RC-133. (2000) Mutagenicity Test on Kordek 573T Measuring Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells
17. Rohm and Haas report n° 99R-132. (2000) Kordek 573T Micronucleus Assay in CD-1 Mouse Bone Marrow Cells
18. Basketter, D.A., Rodford, R., Kimber, I., and Wahlberg, J.E. Skin sensitisation risk assessment: a comparative evaluation of 3 isothiazolinone biocides. *Contact Dermatitis* (1999): 40: 150 - 154
19. Cardin, C.W., Weaver, J.E. and bailey, P.T. Dose response assessments of Kathon biocide. (II) Threshold prophetic patch testing. *Contact dermatitis* (1986): 15: 10 -16

20. Fewings, J. and Menne, T. An update of the risk assessment for methylchloroisothiazolinone / methylisothiazolinone (MCFMI) with focus on rinse-off products. *Contact Dermatitis* (1991): 41: 1 - 13.
21. Friedmann, P.S., Moss, C., Shuster, S., Simpson, J.M. Quantitation of sensitization and responsiveness to dinitochlorobenzene in normal subjects *Br JDermatol* (1983): 25 (suppl 109): 86 - 88.
22. Flyvholm, M-A., Hall, B. M., Agner, T., Tiedemann, E., Greenhill, P., Vanderverken, W., Freeberg, F.E. and Menne, T. Threshold for occluded formaldehyde patch test in formaldehyde sensitive patients. *Contact Dermatitis (1997):* 36: 26 - 33.
23. Kimber, I., Gerberick, G.F. and Basketter, D.A. Threshold in Contact Sensitization: Theoretical and Practical Considerations. *Food and Chemical Toxicology (1999):* 37: 553 - 560.
24. Marzulli, F.N. and Maibach, H.I. The use of graded concentrations in studying skin sensitizers: Experimental contact sensitisation in man. *Fd Cosmet Toxicol (1974): 12:* 219 - 227.
25. Robinson, M.K., Gerberick, G.F., Ryan, C.A., McNamee, P., White, I.R. and Basketter, D.A. The importance of exposure estimation in the assessment of skin sensitisation risk. *Contact Dermatitis (2000):* 42: 251 - 259.
26. Upadhye, M. R. and Maibach, H. I. Influence of area of application of allergens on sensitisation in contact dermatitis. *Contact Dermatitis (1992):* 27: 281 - 286.
27. White, S.I., Friedmann, P.S., Moss, C., and Simpson, J.M. The effect of altering the area of application and dose per unit area on sensitisation by DNCB. *British Journal of Dermatology (1986): 115:* 663 - 668.
28. Chan, P. K., Baldwin, R. C., Parsons, R. D., Moss, J. N., Stiratelli, R., Smith, J. M. and Hayes, A. W. [1983] Kathon Biocide: Manifestation of Delayed Contact Dermatitis in Guinea Pigs is Dependent on the Concentration for Induction and Challenge. Journ. Investigat. Dermatol: 81: 409 - 411
29. Potter, D. W. and Hazelton, G. A. [1995]. Evaluation of Auricular Lymph Node Cell Proliferation in Isothiazolone-Treated Mice. Fundamental and Applied Toxicology 24 165 - 172.
30. Wiemann, C. and Hellwig, J. [2001]. Methylisothiazolinone 20% - Open Epicutaneous Test in Guinea Pigs
31. Wiemann, C. and Hellwig, J. [2001].]. Chloromethylisothiazolinone/ Methylisothiazolinone 3:1 Open Epicutaneous Test in Guinea Pigs
32. Cosmetic Ingredient Review (CIR) Expert Panel: Final Report on the Safety Assessment of Methylisothiazolinone and Methylchlororisothiazolinone, J. Amer. College Toxicol., Vol. 11 (1), 1992.
33. Fewings, J. and Menne, T.: An Update of the Risk Assessment for methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) with Focus on Rinse-Off Products, Contact Dermatits, Vol. 41, 1999.
34. Repeat Insult Patch Studies with 2-Methylisothiazolin-3-one at Aqueous Concentrations of 100 to 600 ppm Active Ingredient : Rohm and Haas Report Numbers: 99RC-01 38, OORC -099A, OORC-099B, OORC-099C, OORC099D, OORC-099E, OORC-099F; conducted: 1999-2001.
35. Bruze, M., Dahlquist, I., Fregert, S., Gruveberger, B. and Persson, K. Contact allergy to the active ingredientsof Kathon® CG. Contact Dermatitis 1987: 16: 183 - 188
36. Bruze, M., Dahlquist, I. And Gruvberger, B. [1989] Contact allergy to dichlorinated methylisothiazolinone. Contact dermatitis 20: 219 - 239.

37. Schnuch, A. [1999] Testing the Frequency of Sensitization to MI in MCI/MI (Kathon CG) sensitized subjects. Un-published study conducted for Rohm and Haas.
38. Bruze, M. and Gruvberger, B. Patch Testing with degradation products of Kathon CG. Contact Dermatitis 1989: 21: 124
39. Gruvberger, B. and Bruze, M. Can chemical burns and allergic contact dermatitis from higher concentrations of methyl chloroisothiazolinone/methylisothiazolinone be prevented? Am. J. Contact Derm 1998 31: 11-14.