EUROPEAN COMMISSION

Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

OPINION ON

the mixture of 5-chloro-2-methylisothiazolin-3(2H)-one and 2-methylisothiazolin-3(2H)-one

COLIPA n° P56

Scientific Committees
on consumer safety
on emerging and newly identified health risks
on health and environmental risks

The SCCS adopted this opinion at its 5th plenary meeting
of 8 December 2009

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 Evaluation Agency (EMEA), 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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ACKNOWLEDGMENTS

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Keywords: SCCS, scientific opinion, preservative, P56, mixture of 5-chloro-2-methylisothiazolin-3(2H)-one and 2-methylisothiazolin-3(2H)-one, directive 76/768/ECC, CAS 26172-55-4, 55965-84-9, EINECS 247-500-7

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on the mixture of 5-chloro-2-methylisothiazolin-3(2H)-one and 2-methylisothiazolin-3(2H)-one, 8 December 2009

TABLE OF CONTENTS

1. BACKGROUND

Submission I for the mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one in a ratio of 3:1 was submitted in January 1983 by COLIPA[1].

Submission II –VII was delivered in the year 1984.

The Scientific Committee on Cosmetology expressed on the 1 July 1986 an opinion concerning certain preservatives. The preservative mixture of 5-chloro-2-methyl-isothiazol-3(2H)-one and 2-methylisothiazol-3(2H)-one with magnesium chloride and magnesium nitrate was evaluated amongst the preservatives "*whose use in cosmetic products can be maintained for the time being, but concerning which the Committee would like to obtain additional data*".

Submission VIII was submitted in September 2001 and Submission IX was submitted in May 2002. These two submissions were mainly concerned obtaining permission to use other stabilisers than magnesium chloride and magnesium nitrate mentioned in the current regulation.

The Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP) adopted its opinion (SCCNFP/0670/03) at the 24th plenary meeting of 24-25 June 2003 with the conclusion, that "*the replacement of magnesium chloride and magnesium nitrate by copper sulphate or any other authorised cosmetic ingredient as a stabiliser system in the mixture of 5-chloro-2-methylisothiazolin-3(2H)-one and 2-methylisothiazolin-3(2H)-one does not alter the toxicological profile of this mixture*."

The term "*authorised"* raised some problems in implementing the opinion and the SCCP was asked for a clarification, which was given in the opinion (SCCP/0849/04) adopted at the 2nd plenary meeting the 7th December 2004.

Meanwhile, the Commission was requested by the Member states to ask Industry for a complete new dossier more in line with modern standards.

The current submission X, submitted in April 2006 is a full and updated dossier for the preservative mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one in a ratio of 3:1.

The preservative is currently regulated as a preservative in the cosmetic directive (76/768/EEC) in annex VI, part 1, entry 39 with the maximum authorized concentration 0.0015% (15 ppm) of a mixture in the ratio 3:1 of 5-chloro-2-methylisothiazolin-3(2H)-one and 2-methylisothiazolin-3(2H)-one.

2. TERMS OF REFERENCE

Does the SCCP consider, with the scientific data provided that the preservative mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one in a ratio of 3:1 is safe for the consumers, when used as a preservative up to a maximum authorised concentration of 0.0015 % in rinse-off cosmetic products?

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association

3. OPINION

3.1. Chemical and Physical Specifications

Several physicochemical properties are derived from earlier analyses of Kathon 886 and Acticide 14. Many of the studies were conducted using higher percentage CMI/MI mixtures than the 1.5% specified for cosmetics and the CMI/MI ratio varied.

3.1.1. Chemical identity

5-chloro-2-methylisothiazol-3(2H)-one (CMI) and 2-methylisothiazol-3(2H)-one (MI) combined formulations are marketed under several trade names, such as Kathon CG, Kathon 886, Kathon 886 WT. Kathon™ 886, Acticide LG, Acticide 14 L, Acticide 14P, Microcare IT, Microcare ITL etc.

Initially, all formulations were prepared as a mixture of two individual active ingredients CMI and MI and salts. However, Kathon™ 886 biocide is now defined as a combination of the two active ingredients produced by an integrated production process, resulting in an approximate total of 14% active ingredients, 16% magnesium nitrate, 10% magnesium chloride and 62% water. There is no indication as to when this change was made in the manufacturing process.

Only CMI/MI in a 3:1 ratio is permitted for the use in cosmetics. Kathon™ CG is a 1.5% dilution of Kathon™ 886 Biocide. It is not clear from the dossier which preparation of Acticide is for cosmetic use.

There is some confusion in the literature with the nomenclature. Both methylchloroisothiazolinone and methylisothiazolinone are also used to include the mixture, (e.g. US EPA Re-registration of Methylisothiazolinone (EPA738-R-98-012, 1998) states 'includes the active ingredients 5-chloro-2-methyl-3(2H)-isothiazolone and 2-methyl-4-isothiazolin-3-one').

3.1.1.1. Primary name and/or INCI name

Methylchloroisothiazolinone (and) methylisothiazolinone with magnesium chloride and magnesium nitrate (INCI name)

5-chloro-2-methylisothiazol-3(2H)-one and 2-methylisothiazol-3(2H)-one

3.1.1.2. Chemical names

5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one, 3:1 ratio

5-Chloro-2-methyl-4-isothiazolin-3-one
5-Chloro-2-methyl-3(2H)isothiazolone
5-Chloro-2-methyl-2H-isothiazol-3-one
4-Isothiazolin-3-one, 5-chloro-2-methyl-

2-Methyl-4-isothiazolin-3-one
2-Methyl-3(2H)isothiazolone
2-Methyl-2H-isothiazol-3-one
3(2H)-Isothiazolone, 2-methyl

3.1.1.3. Trade names and abbreviations

Chloromethylisothiazolione + Methylisothiazolinone (75% + 25%)
CMI/MI or MCI/MI
CIT/MIT
Kathon™ CG
Microcare IT
Microcare ITL
Acticide 14
Acticide LG

3.1.1.4. CAS / EINECS number

	CMI/M	Kathon™ CG (CMI/MI 3:1, Mg salts (current process))
CAS:	26172-55-4	55965-84-9
EINECS:	247-500-7	/

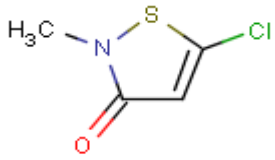
5-chloro-2-methyl-4-isothiazolin-3-one

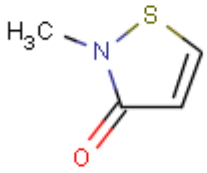
CAS: 26172-55-4
EINECS: 247-500-7

2-methylisothiazol-3(2H)-one

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

3.1.1.5. Structural formula





5-chloro-2-methylisothiazol-3(2H)-one 2-methylisothiazol-3(2H)-one

3.1.1.6. Empirical formula

5-chloro-2-methylisothiazol-3(2H)-one C_4H_4ClNOS
2-methylisothiazol-3(2H)-one C_4H_5NOS

3.1.2. Physical form

CMI/MI formulation (Kathon 886, current process) is supplied as a liquid at 20°C.

The two actives are the result of a chemical reaction and are not the result of blending two separately produced active ingredients. The reaction results in a 3 CMI:1 MI ratio.
There are no details for the blended formulations

3.1.3. Molecular weight

5-chloro-2-methylisothiazol-3(2H)-one	149.45
2-methylisothiazol-3(2H)-one	115.16

3.1.4. Purity, composition and substance codes

CMI and MI were characterised by NMR and IR

Kathon™ 886 Biocide (current process) is defined as a combination of the two active ingredients produced by an integrated production process, resulting in an approximate total of 14% active ingredients, 16% magnesium nitrate, 10% magnesium chloride and 62% water.

The current specifications for this are:

Kathon™ 886F (14% nominal)	**% Weight**
Chloromethylisothiazolinone:	10.6 – 10.8
Methylisothiazolinone:	3.43 – 3.47
Inert ingredients	
Magnesium nitrate:	16.5 – 17.1
Magnesium chloride:	9.30 – 9.43
Impurity (manufacturing by-products)	**% Weight**
Magnesium sulfate:	<0.1
ß-Aminocarbonyl ethane sulfonic acid:	<0.01 – <0.1
Ethyl acetate:	<0.05 – 0.08
Ethanol:	0.06 – 0.21
Acetic acid:	0.3 – 0.32
Ammonium chloride:	0.1
Ammonia acetate:	0.1
N-Methyl-chloro propionamide [MCPA]:	<0.01
N-Methyl di-chloro-acetamide [DCA]:	<0.05 - <0.1
4,5-Dichloro-2-Methyl-4-isothiazolin-3-one:	<0.01 – 50 to 100 ppm
Dimethyl nitrosamine:	<0.1, LOD
MMNP:	<0.3, LOD

The dossier does not state how the other formulations are prepared but it would suggest that they are also a direct mixture of two individual active ingredients CMI and MI and salts. These mixtures include Acticide LG, Acticide 14 L, Acticide 14P. As far as can be ascertained from the dossier, there are considerable variations in the ratio between the preparations Acticide LG would seem to be a 1.5% CMI/MI formulation.

Active ingredients *(product specifications)*	**% weight**
CMI	1.05%
MI	0.42 %
Inert ingredients *(product specifications)*	
Magnesium nitrate [Mg $(NO_3)_2$]	3 – 15 %
Water	75.5 % (nominal)
Impurities *(manufacturing by-products)*	

Magnesium chloride [$MgCl_2$]	6.6% w/w
Methyl-3-(methylnitrosamino)-propionamide	<0.0001 g/kg,
Acetic acid	0.5 – 1.7% w/w

Magnesium nitrate and magnesium chloride are present in varying amounts depending upon the source. Other stabilisers have been evaluated as safe (SCCNFP/0670/03), but not yet included in the cosmetics directive.

3.1.5. Impurities / accompanying contaminants

Analysis of 11 samples of various CMI/MI preparations (Kathon 886, Kathon 886 WT, Acticide LG, Acticide 14 L, Acticide 14P, Pinus Biozide PBK100, Pinus 6.94) revealed the following impurities:

2,2-Dichloro-N-methylacetamide:	<0.003 - 2.4 mg/g
3-Chloro-N-methylpropionamide:	0.0078 - 9.5 mg/g
4,4-Dichloro-2-methyl-4-isothiazolin-3-one:	0.0031 - 2.61 mg/g

Changes in manufacturing processes have reduced nitrosamines to below the limit of detection [<0.3ppm]. Thus nitrosamines, no longer, present an impurity issue.

Ref: L

3.1.6. Solubility

Kathon current process	
Solubility in Water ()	≥ 1000 g/L at 20 °C
Mixture of active ingredients	
Solubility in Water	367 g/L at 20 ºC
Solubility in Water	≥ 660 g/L at 30 ºC, pH 9
5-chloro-2-methyl-4-isothiazolin-3-one (CMI)	
Solubility in Water	706-751 g/L, 20 ºC
Solubility in Ethyl acetate	38.06 g/L, 10 °C
	52.55 g/L, 30 °C
Solubility in Hexane	1.39 g/L, 10 °C
	2.91 g/L, 30 °C
2-methyl-4-isothiazolin-3-one (MI)	
Solubility in Ethyl acetate	1.12 g/L, 10 °C
	1.89 g/L, 30 °C
Solubility in Hexane	5.87×10^{-3} g/L, 10 °C
	1.48×10^{-2} g/L, 30 °C

3.1.7. Partition coefficient (Log P_{ow})

Log Kow:
Kathon 886 (current process)
^{14}C-labeled CMI: K_p = 2.519 log K_p = 0.401
^{14}C-labeled MI: K_p = 0.326 log K_p = -0.486
Tests were conducted at 24**°**C and ambient pH.

Acticide 14 (CMI/MI)
Log P_{ow}: 0.75 at 27 °C (Directive 92/69/EEC, A.8)
Log P_{ow}: 0.67 - 0.7 at 20 °C

pH value: 5 - 9

Effect of pH:
Log P_{ow} at pH 5 (20 °C): 0.70
Log P_{ow} at pH 7 (20 °C): 0.67
Log P_{ow} at pH 9 (20 °C): 0.69
Log P_{ow} at pH 7 (10 °C): 0.63
Log P_{ow} at pH 7 (30 °C): 0.71

The given pH refers to the aqueous part of the mobile phase (methanol/water 50/50).

Ref: M

3.1.8.	Additional physical and chemical specifications

Organoleptic properties:	Clear, light amber liquid
Melting point:	22.3 – 35.1 °C (manufacturer 1) 46.2 – 50.3 °C (manufacturer 2)
Boiling point:	100 °C (manufacturer 1) 106.5 °C (manufacturer 2)
Flash point:	Not applicable CMI/MI aqueous formulation (~ 75% water)
Vapour pressure:	0.00108 hPa at 20 °C (manufacturer 1) 20.8 hPa at 20 °C (manufacturer 2)
Density:	1.296 g/ml at 25 °C (manufacturer 1) 1.256 at 20 °C (manufacturer 2)
Viscosity:	11.4 Cp at 25.7 °C 8.4 Cp at 44.6 °C
pKa:	/
Refractive index:	/
UV spectrum:	λmax 273-274 nm

3.2. Function and uses

The "mixture of 5-Chloro-2-methyl-isothiazol-3(2H)-one and 2-Methylisothiazol-3(2H)-one with magnesium chloride and magnesium nitrate" is currently regulated as a preservative in the cosmetic directive (76/768/EEC) in annex VI, part 1, entry 39 with the maximum authorized concentration 0.0015% (15 ppm) of the mixture.

The submission describes Kathon™ CG as cosmetic grade at 1.5% active ingredient stabilized with magnesium nitrate. It is the formulated product sold to customers for cosmetic applications. Kathon™ CG is a cosmetic preservative. The main uses are rinse-off products, such as shampoos, conditioners, gels and surfactants. The EU use concentration is a maximum of 15 ppm a.i. for rinse-off and leave-on products. However, the manufacturers recommend a maximum level of 7.5 ppm a.i. (0.05% by weight of product as supplied) for leave-on products.
The manufacturers recommend CMI/MI as a preservative in shower gels, body washes, bubble baths, liquid soaps, shampoos, hair conditioners and wipes.

The CMI/MI mixtures have wide applications in household (domestic) and industrial products.

3.3. Toxicological Evaluation

The value of the acute, subchronic and reproductive toxicity studies is limited as the majority were carried out more than twenty years previously. The results are indicative of the possible toxicity as the studies were to the standards of the time. The major failings were that the test formulations are not properly characterised.

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

The table below summarises the acute toxicity studies

Test substance	Species	Result	Study date	Ref
Acute oral toxicity				
Kathon™ WT 1.5% a.i.	Rat	**male** LD_{50} > 5000 mg/kg bw (> 75 mg a.i./kg bw) **female** LD_{50} 3310 mg/kg bw (~ 49.6 mg a.i./kg bw)	1991	1
Acticide 14 (14% a.i)	Rat	**combined** LC_{50} Bliss' method 490 mg/kg bw (~ 69 mg a.i./kg bw); **combined** LC_{50} Litchfield & Wilcoxon's method 472 mg/kg bw (~ 66 mg a.i./kg bw)	1993 - 1994	2
Acticide 14 (14% a.i)	Rat	**male** LD_{50} 465mg/kg bw (69 mg a.i./kg bw) **female** LD_{50} 393 mg/kg bw (59 mg a.i./kg bw)	1997	3
Kathon CG 1.5% a.i.	Rat	**female** LD_{50} between 0.5 and 5.0 g/kg/bw	1980	4
Acute dermal toxicity				
Kathon CG	Rabbit	**female** LD_{50} >5000 mg/kg bw (> 75 mg/kg a.i mg/kg bw)	1980	4
Acticide 14 (14% a.i)	Rat	**Male & female** LD_{50} 1008 mg/kg bw (141 a.i. mg/kg bw)	1993 - 1994	5
Acute inhalation toxicity				
Kathon™ 886 F 13.9% a.i.	Rat	**Male & female** 4hr aerosol exposure LC_{50} 2.36 mg/L air, confidence limits of 1.60 to 4.82 and a slope of 2.2. (LC_{50} 0.33 mg a.i./L, confidence limits of 0.22 to 0.67, and a slope of 2.2.	1991	6

These were reliable acute toxicity studies, indicating that P56 has slight acute oral and inhalation toxicity and was considered non-toxic by single dermal application. No details of formulation were provided but were said to be GLP characterized. CMI:MI ratio was not given

3.3.2 Irritation and corrosivity

The table below summarises the irritation and corrosivity studies. This section consists of older studies that have been reformatted at a later date or are non-validated protocols. There are data gaps in the older studies. The Bovine Cornea Opacity-Permeability test (BCOP) is a non-validated screening test.

Test Substance	**dilution**	**Species/Test**	**Result**	**Study Date**	**Ref**
Skin Irritation					
Kathon™ MW 1.5%a.i with copper nitrate	undiluted	rabbit	Corrosive	1984	7
Acticide 14 14.2% a.i (CMI 10.2%/MI 4%)	undiluted	rabbit	Corrosive	1993	8
Mucous membrane Irritation					
Kathon™ RH 886T	100 ppm (0.01% a.i.)	rabbit	non-irritating	1975	9
Kathon™ CG	undiluted	rabbit	Severely irritating	1977	10

Test Substance	dilution	Species/Test	Result	Study Date	Ref
Microcare IT	1.5% a.i. 0.15% a.i. 0.015% a.i. 0.0015% a.i.	BCOP; (screening test)	Mild irritant Non irritant Non irritant Non irritant	2002	11
Respiratory Tract Irritation					
Kathon™ 886F	407µg/L	Rat	RD50 69 µg/L (9.4 µg a.i/L)	1991	12

P56, (5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one in a ratio of 3:1) is corrosive or irritating at high concentrations.

Comment
No adequate data is given to support safe use at a maximum authorised concentration of 0.0015 % in rinse-off cosmetic products. Nevertheless, the weight of evidences over several decades of consumer exposure to cosmetic products indicates that skin and/or mucous membrane irritation is not a problem under the conditions of use in leave-on and rinse-off products.

3.3.3. Skin sensitisation

Test substance	Species	Result	Study date	Ref
GPMT				
Kathon™ 886 Diluted to 1.5% a.i.	guinea pig	Non-sensitiser at 56 ppm a.i. [25µg a.i./cm2]	1977	13
Kathon™ 886F (14.05 % a.i.)	guinea pig, female	Diluted with saline to 30 ppm and 50 ppm. Not a sensitiser at 30 ppm [1.3 µg a.i/cm2] and 50 ppm [2.2 µg a.i/cm2]	2000	14
Acticide LG	guinea pig, female	Diluted with deionised water to 20%, challenge 7.5, 4,% (v/v). For intradermal treatment, 0.25% in 50 FCA;50 water Sensitiser	1991	15
Acticide 14, (14.2 %; 10.20 %CIT/ 4.00 % MIT)	guinea pig, male and female	Diluted with deionised water to 25%, challenge 10, 7.5,5, 2.5, 0.025 and 0.0025% (v/v). For intradermal treatment,, 5% in saline and FCA. Sensitiser at 10, 7.5,5, 2.5%, 0.025%. Highest tested non-sensitiser 0.0025% (v/v).	2000	16
Buelher				
Kathon™ various grade	guinea pig,	Sensitiser	1981,1979, 1979, 1985, 1984, 1988, 1988	17, 18, 19, 20, 21, 22
Open Epicutaneous				
CMI/MI (14.05% a.i)	guinea pig,	**Sensitiser. Threshold for induction $\geq$ 58 ppm a.i. [> 0.7 µg a.i./cm^2]**). Equivalent to 0.04 w/v % ethanol/water (constant content of 40v% ethanol)	2000	23
LLNA				
CMI/MI (14.05% a.i)	Mouse, female	Sensitiser EC_3 30 ppm [0.75 µg a.i./cm^2]	2000	25
CMI/MI (14.05% a.i)	Mouse, female	Sensitiser EC_3 70 ppm [~2µg a.i/cm^2] Vehicle acetone/olive oil (4:1 v/v)	2000	26
CMI	Mouse	Sensitiser EC_3 81 ppm [2.0 µg a.i./cm^2]		24
MI	Mouse	Sensitiser EC_3 25,150 ppm [620 µg a.i./cm^2]	1981	17
NMMA	Mouse	Non-sensitiser Vehicle acetone/olive oil (4:1 v/v)	2003	27
DCMI	Mouse	Sensitiser EC_3 > 67 ppm a.i. [1.7 µg a.i./cm^2] Vehicle acetone	2005	28
Pulmonary Hypersensitivity				
Kathon™ CG (1.53.a.i)		Not a respiratory sensitiser	1995	29

The only studies with a CMI/MI 3:1 formulation were the LLNA and the pulmonary hypersensitisation studies. The CMI:MI ratio was not given in the other studies.
The sensitization potential of CMI/MI in predictive animal tests, including the Buehler test and Magnusson-Kligman test in the guinea pig has been reported extensively (Andersen et. al., 1995; Basketter, 1996; Bruze et al., 1987a; Chan et al., 1983; Enslein et al., 1997; Schallreuler and Schulz, 1986 and Zissu, 2002. Chan et al., (1983) evaluated both the induction and elicitation dose response curves of CMI/MI in the Buehler test. Bruze et al., (1987a) evaluated both active ingredients of CMI/MI and demonstrated that CMI was significantly more responsive in the assay. These investigators also showed that a minor impurity, 4,5 dichloro-methyl-isothiazolone, of CMI/MI was as potent as the monochlorinated active (CMI) as a sensitizer (Bruze et. al. (1987b).
Development and validation of the local Lymph Node Assay (LLNA) in mice, required a battery of known positive and negative allergens as controls (summarized in Gerberick et. al., 1992 and 2004) and estimation of relative allergenic potency (Basketter et. al., 2000 and 2001). CMI/MI was often included in the battery of allergens tested in comparison with other predictive assays in laboratory models. CMI/MI, with other known allergens, has also been used to further evaluate the assay with respect to statistical handling of the data (Basketter et. al. 1999). Warbrick et al. (1999) used CMI/MI to demonstrate the effects of different vehicles in the LLNA. Responses with CMI/MI in acetone/olive oil vehicle were highest compared to other vehicles tested. Similar vehicle effects with CMI/MI were shown by Potter and Hazelton (1995).
During the validation of the LLNA with respect to isothiazolone class of chemistry (Potter and Hazelton, 1995), CMI was shown to be a significantly more potent allergen (EC3 = 2 µg/cm^2) compared to MI (EC3 = 200 µg/cm^2).
More recent investigations by Basketter et al., (2005) evaluated the LLNA and have shown that EC3 values of a number of allergens, including CMI/MI compare well with human data such as human predictive assays (HRIPT).
The data demonstrates that CMI/MI is an extreme sensitiser in animals.
A detailed evaluation of the potential of CMI/MI to cause contact allergy in humans is in section 3.3.11.

3.3.4. Dermal / percutaneous absorption

In vivo

Guideline:	/
Species:	Rabbit, albino
Group size:	2 female
Control	Test substance: ^{14}C-RH-886T prepared as ratio 3:1 of ^{14}C-RH-886 and ^{14}C-RH-056.
Batch:	/
Purity*:*	^{14}C-CMI/MI, specific activity 0.81 mCi/g equivalent to 1798 dpm/µg CMI/MI
Doses:	99.2 ppm w/v ^{14}C-CMI/MI
Sampling time:	0, 2, 4, 7, 24, 28, 30 48, 52, 55, and 72 h after first application
Method of Analysis:	liquid scintillation counting
GLP:	/
Study period:	1973

The rabbit skin was prepared by clipping the hair. The skin of one was intact, while the second was abraded. Dermal application of the ^{14}C-CMI/MI was made on 3 consecutive days. 0.5 ml of 99.2 ppm w/v ^{14}C-CMI/MI was applied at two sites of the skin and occluded for 24 hours. A 1 cm^2 gauze patch was used for each test site. Blood samples were taken from the ear. Untreated rabbits were controls, no other control information provided.

Results

Blood samples assayed from both treated rabbits did not exceed the average control count rate (23.8 ± 1.24 cpm) + 2x standard deviation (26.3cpm). No blood sample showed a concentration of RH-886T greater than 0.0045 ppm.

Conclusion
^{14}C- RH-886T was applied to the intact and abraded skin of rabbits over a 3 day period. No radioactivity was found in the blood at any time point.

Ref.: 30

Comment
This was an old study with data gaps. The chemical identity of the test substance is not defined.

Guideline:	/
Species:	Rat, Sprague-Dawley, Charles River
Group size:	12 male, 6 per group
Test substance:	^{14}C-Kathon 886 Solution 1: ^{14}C-CMI (labelled in the carbonyl position, RH-651) and MI (unlabelled, RH-573); Solution 2: CMI (unlabelled RH-651) and ^{14}C-MI (labelled in the carbonyl position, RH-573)
Batch:	Solution 1: Lot N° 395.0201; Solution 2:.Lot N° 395.0101
Purity*:*	Solution 1: Specific activity 13.72 mCi/g, radio-purity >98% by TLC; Solution 2: Specific activity 10.47 mCi/g, radio-purity >98% by TLC.
Doses:	0.2 ml ^{14}C-CMI/MI in water, each containing 2000 ppm a.i.
Sampling time:	24, 48, 72 and 96 h after application
Method of Analysis:	liquid scintillation counting
GLP:	/
Study period:	1982

0.2 ml of ^{14}C labelled material was applied to the skin and occluded for 24 hours. A contoured glass ring was used for each test site. Dermal application of the 14C-label was for one 24 hour period. 2 rats /group were killed 24, 48 and 96 h after application for sampling and analysis of blood, testes and urine.

Results
Following dermal application of ^{14}C -CMI or ^{14}C -MI, total recovery of ^{14}C -label in this study ranged from 74 to 91% of administered dose.
Washing removed 3 to 7% of the ^{14}C -CMI dose after the 24 hr exposure period, while 51 to 59% of ^{14}C -label (expressed as ^{14}C -CMI equivalents) was retained in the skin application site for 96 hr observation period. Since ^{14}C-label at the application site was constant over the 96 hr period, it was likely to be unavailable for systemic absorption. At 96 hr post dose, <1% of the ^{14}C -label was present in the skinned carcass and 15% of ^{14}C -label was found in the excreta (predominantely in the urine). Concentrations of ^{14}C -label (derived from ^{14}C -CMI and expressed in CMI equivalents) in whole blood and testes are shown below:

Time	Whole Blood ppb*	Testes ppb*
24 hr	140	11
28 hr	35	8
96 hr	31	1

Washing removed 8 to 28% of the ^{14}C -MI dose after the 24 hr exposure period, while 30 to 68% of ^{14}C -label (expressed as ^{14}C -CMI equivalents) was retained in the skin application site for 96 hr observation period. Since ^{14}C -label in the skin application site was constant over the 96 hr period, this material was likely unavailable for systemic absorption. At 96 hr post dose, <1% of the ^{14}C -label was present in residual carcass and 23% of ^{14}C -label was

found in the excreta (predominantly in the urine). Concentrations of ^{14}C -label (derived from ^{14}C -MI and expressed in MI equivalents) in whole blood and testes are shown below:

Time	Whole Blood ppb*	Testes ppb*
24 hr	12	7
28 hr	10	7
96 hr	16	Not detected

Conclusion
Following dermal application (24 hr exposure) of CMI or MI, approximately 15 and 24% of the dose, respectively, was systemically available. Within 72 to 96 hr most of the absorbed radioactivity was excreted (predominantly in the urine).

Ref.: 31

Guideline:	OECD 417
Strain:	Rat, Crl:CD®BR
Group size:	40 male, 3 to 5 rats for each of the 8 studies
Test substances:	A: Kathon Biocide ^{14}C-RH-651 (5-chloro-2-methyl-4-isothiazolin-3-one), 4.22 mCi/g specific activity, 14.6% a.i.;(11% ^{14}C-CMI, 3.6 % MI) B: Kathon Biocide ^{14}C-RH-573 (2-methyl-4-isothiazolin-3-one), 1.73 mCi/g specific activity, 14.5% a.i., (11% CMI, 3.5 % ^{14}C-MI) Kathon Biocide 14.5% a.i.
Batch:	A: 555.0101; B: 555.0201; Kathon Biocide unlabelled: DB16-41
Purity*:*	A: 99.5% radio-purity, B: 98.1% radio-purity
Doses:	35µ aliquots^{14}C-Kathon in water, 25 or 2500 ppm a.i.
Sampling time:	0, 3, 6, 24, 48 and 96 h
Diffusion cells:	Franz cells
Receptor fluid:	Tyrode's buffer, pH 7.4, 37 C: 6/8 contain gentamicin
Method of Analysis:	HPLC, TLC, liquid scintillation counting
GLP:	in compliance
Study period:	1989

Freshly excised rat skin sections were mounted in Franz diffusion cells. Six of the eight studies used bathing solutions (Tyrode's buffer, pH 7.4, 37 C) containing gentamicin (0.5 mg/cell, approximately 70 µg/ml) to control bacterial growth. A single 35 µl aliquot of ^{14}C -CMI/MI or CMI/ ^{14}C -MI, diluted in water, was applied onto the skin at 25 or 2500 ppm. At various times after application, the skin sections were wiped with cotton swabs moistened with distilled water (wipes were analyzed for ^{14}C-label), and the amount of ^{14}C-label found both bound to or in the skin and penetrating the skin into the bathing solution was measured. ^{14}C-label found bound to or in the skin, plus ^{14}C-label that penetrated through the skin, was considered to be bioavailable.

Results

Kathon ^{14}C-component	Time h	Upper cell rinse	Wipe-off	Bath and rinse	Skin	Total ^{14}C-recovery
^{14}C-CMI	0	4 ± 6	69 ± 22	0.1 ± 0.1	19 ± 24	93 ± 8
2500 ppm	3	2 ± 1	27 ± 20	0.4 ± 0.3	71 ± 27	101 ± 7
	6	3 ± 3	3 ± 1	3 ± 3	96 ± 3	105 ± 3
	24	2 ± 1	2 ± 0.3	10 ± 0.4	92 ± 1	106 ± 1
	48	1 ± 2	4 ± 2	9 ± 6	91 ± 10	104 ± 3
	96	3 ± 1	1 ± 0.1	13 ± 4	90 ± 4	107 ± 0.4
^{14}C-CMI	0	3 ± 3	69 ± 9	1 ± 0.4	19 ± 1	91 ± 9
25 ppm	3	1 ± 1	3 ± 2	0.5 ± 0.2	117 ± 5	121 ± 3
	6	3 ± 2	2 ± 0.3	0.2 ± 0.2	118 ± 8	122 ± 7
	24	1 ± 1	2 ± 0	1 ± 0.5	115 ± 2	121 ± 4
	48	1 ± 2	1 ± 1	1 ± 1	103 ± 5	107 ± 9
	96	1 ± 3	2 ± 2	1 ± 1	102 ± 4	106 ± 2

Kathon ^{14}C-component	Time h	Upper cell rinse	Wipe-off	Bath and rinse	Skin	Total ^{14}C-recovery
^{14}C-MI 2500 ppm	0	0.3 ± 0.3	82 ± 3	0.1 ± 0.1	2 ± 0.5	84 ± 3
	3	4 ± 5	60 ± 38	0.4 ± 0.4	26 ± 41	91 ± 9
	6	0.2 ± 0.4	74 ± 2	1 ± 1	4 ± 1	79 ± 3
	24	1 ± 0.1	77 ± 5	0.3 ± 0.2	3 ± 1	81 ± 6
	48	2 ± 2	58 ± 47	5 ± 5	30 ± 39	95 ± 2
	96	3 ± 2	4 ± 1	23 ± 2	58 ± 4	89 ± 5
^{14}C-MI 25 ppm	0	10 ± 5	132 ± 4	1 ± 1	1 ± 0.2	143 ± 9
	3	1 ± 2	111 ± 4	1 ± 0	2 ± 1	115 ± 3
	6	0 ± 0	85 ± 30	1 ± 1	15 ± 16	95 ± 16
	24	13 ± 2	16 ± 0.2	24 ± 2	49 ± 1	102 ± 2
	48	12 ± 3	6 ± 3	55 ± 6	28 ± 0.4	100 ± 1
	96	23 ± 1	6	61 ± 25	16 ± 2	111 ± 5

At 96 hr exposure, 1 to 13% of the Kathon Biocide ^{14}C-CMI (25 or 2500 ppm respectively) was absorbed across rat skin (systemically available). Most of the ^{14}C-label (90-102%, 2500 or 25 ppm respectively) was bound to the skin.
At 96 hr exposure 23 to 61% of ^{14}C -label derived from Kathon Biocide ^{14}C-MI (25 or 2500 ppm respectively) was absorbed across rat skin. Much of the ^{14}C-label (16 to 58%, 25 or 2500 ppm respectively) was bound to the skin.
It was not possible to determine if the ^{14}C in the tissue at 96h, from either CMI or MI, was the parent compound and/or metabolites nor if it was permanently bound or available for further absorption.
HPLC and thin layer chromatographic analyses of bathing solutions collected 24 or 96 h after 2500 ppm CMI/MI application provided evidence that the parent components of Kathon Biocide (CMI and MI) were not in the receptor fluid suggesting biotransformation and/or degradation in the skin. The parent compounds were stable in spiked receptor fluid.

Conclusion
In this rat skin *in vitro* absorption study, MI was absorbed across the skin barrier to a greater extent than CMI. Both compounds were bound to the skin but it was not determined if this bound material was systemically available.

Ref.: 32

In-vitro human skin

Guideline:	OECD draft 428
Tissue:	Human (*post mortem*) skin
Group size:	6 membranes from 3 different donors
Diffusion cells:	glass diffusion cells, 2.54 cm^2 membrane area
Skin integrity:	trans-dermal electrical resistance; at least 10 kΩ
Test substances:	(4,5-^{14}C)-CMI (RH-651, 5-Chloro-2-methyl-4-isothiazolin-3-one; 48 µCi/g specific activity), and non-radiolabelled MI (RH-573, 51.1% a.i) CMI 3:MI 1 (225 µg/ml ^{14}C-CMI / 75µg/ml MI w/v in water). Batch: CMI/MI Sublot 1065.000101; MI: Lot N^o 41814, Pail 1
Purity*:*	98.7% radio-purity
Doses:	22.5, 75.0 and 225 µg/ml CMI, 20 µl/cm^2
Diffusion cells:	Franz cells
Receptor fluid:	water
Sampling time:	0, 1, 2, 4, 8, 12, 24 h
Method of Analysis:	liquid scintillation counting
GLP:	in compliance
Study period:	May –Aug 2005

Six intact membranes from at least three different subjects were selected for each dose. The 3 aqueous dilutions were applied to the epidermis in the cells and occluded for the 24h exposure period. Receptor fluid samples (0.5 ml) were taken at specified intervals; the volume was maintained with fresh receptor fluid after sampling time. At the end of the

exposure period, the distribution of CMI in the test system was measured and reported as ^{14}C-CMI equivalents.

Results
Minimal absorption of CMI occurred within the first 2 hr of exposure, thereafter, the absorption rates ranged from 0.004 to 0.079 µg/cm^2/h over the 24 h. CMI absorption was fastest between 4-8 hr after dosing and then slowed possibly due to depletion of the applied reservoir. After 24h exposure, 16.9, 22.4 and 36.2% of the applied doses (22.5, 75.0 and 225 µg/ml, respectively) were absorbed.
Systemic availability of CMI was potentially 79.2, 73.2, and 84.5% of the applied doses (22.5, 75.0 and 225 µg/ml, respectively), including ^{14}C -label retained in the epidermis. All values expressed as percent of total CMI dose applied (mean ± SD)

Dose	Donor chamber	Skin wash at 24 h	*Stratum corneum*	Remaining epidermis	Absorbed	Total recovery (equivalents)
225 µg/ml	6.27 ± 1.00	4.99 ± 1.80	2.67 ± 1.98	48.3 ± 20.8	36.2 ± 15.5	98.5 ± 4.49
75 µg/ml	6.37 ± 3.71	7.73 ± 4.21	4.74 ± 3.38	50.8 ± 15.5	22.4 ± 12.4	92.1 ± 2.46
22.5 µg/ml	5.39 ± 2.31	11.3 ± 4.83	6.16 ± 1.05	62.3 ± 19.6	16.9 ± 15.5	102 ± 8.3

At the end of the study, given the reactivity of CMI, it was not possible to determine if the ^{14}C -material was the parent compound or ring opened degradation/metabolic products nor whether it was permanently bound in the tissue or available for further absorption.

Conclusion
Absorption of CMI across the epidermis was 16.9, 22.4, and 36.2% of the respective doses in aqueous solutions (22.5, 75.0 and 225 µg/ml) following a 24 hr occluded exposure, in the presence of MI (CMI: MI ratio of 3:1). Potential systemic availability of CMI, including ^{14}C -label retained in the epidermis, was 79.2, 73.2, and 84.5% of the applied doses.

Ref.: 33

In-vitro human skin

Guideline:	OECD draft 428
Tissue:	Human (*post mortem*) skin
Group size:	6 membranes from 3 different donors/application
Diffusion cells:	glass diffusion cells, 2.54 cm^2 membrane area
Skin integrity:	trans-dermal electrical resistance; at least 10 kΩ
Test substances:	(4,5-^{14}C)-CMI (RH-651, 5-Chloro-2-methyl-4-isothiazolin-3-one; 48.91 µCi/g specific activity), and non-radiolabelled MI (RH-573, 99.0% a.i) 3:1 w/v in water.
Batch:	CMI/MI Sublot 1065.0003; CMI: Lot N° 1065.00; MI: 033055A
Purity*:*	98.7% radio-purity
Test applications:	Aqueous solution, shampoo, body lotion and facial cream
Doses:	CMI/MI ratio 3:1 (11.37µg/ml CMI / 3.75µg/ml MI w/v in water).
Diffusion cells:	Franz cells
Receptor fluid:	purified water
Sampling time:	0, 1, 2, 4, 8, 12, 24 h
Method of Analysis:	liquid scintillation counting
GLP:	in compliance
Study period:	July- Oct 2005

In vitro dermal absorption of radioactive 5-Chloro-2-methyl-4-isothiazolin-3-one (^{14}C-CMI) was evaluated in human epidermis from an aqueous solution and 3 formulations (shampoo,

body lotion and facial cream) at a concentration of 11.25 µg CMI/ml in the presence of non-radiolabelled 2-methyl-4-isothiazolin-3-one in a ratio of 3:1, CMI to MI.
Six intact membranes from at least three different subjects were selected for each application type. The aqueous solution and 3 formulations were applied to the epidermis at a rate of 20 µl/cm^2 (aqueous solution and shampoo) and 20 mg/cm^2 (body lotion and facial cream) and occluded for the 24h exposure period. Receptor fluid samples (0.5 ml) were taken at specified intervals; the volume was maintained with fresh receptor fluid after sampling time. At the end of the exposure period, the distribution of CMI in the test system was measured and reported as ^{14}C-CMI equivalents.

Results
Minimal absorption of CMI occurred within the first 4 h of exposure; thereafter, the rate of absorption was 0.0009 µg/cm^2/hr at concentrations tested over the 24 h period. After 24h, 7.36% of the applied CMI in aqueous solution containing 11.25 µg/ml, was absorbed across the epidermis during 24 h occluded exposure. Systemic availability of CMI was potentially 58.7% of the applied dose including ^{14}C-label retained in the epidermis.
At the end of the study, given the reactivity of CMI, it was not possible to determine if the ^{14}C -material was the parent compound or ring opened degradation/metabolic products nor whether it was permanently bound in the tissue or available for further absorption.

When CMI (11.25 µg/ml)/MI (ratio of 3:1) was formulated in a shampoo, body lotion and facial cream, 3.53, 2.82 and 1.11% of the applied CMI dose (24 hr, occluded exposure) was absorbed across the epidermis, respectively. Systemic availability of CMI, including ^{14}C-label retained in the epidermis, was potentially 48.4, 51.6 and 46.6% from the applied shampoo, body lotion and facial cream formulations respectively.

All values expressed as percent of dose recovered (mean ± SD)

Dose 11.25 µg/ml	Donor chamber	Skin wash 24 h	Stratum corneum	Remaining epidermis	Absorbed	Total recovery (equivalents)
aqueous	2.02 ± 0.31	18.6 ± 4.10	12.9 ± 3.74	51.3 ± 6.23	7.36 ± 3.22	92.2 ± 2.07
shampoo	2.21 ± 1.02	25.4 ± 6.45	17.0 ± 4.79	44.9 ± 5.95	3.53 ± 2.22	93.1 ± 3.42
body lotion	2.04 ± 1.35	17.1 ± 7.74	7.29 ± 4.84	48.8 ± 7.97	2.82 ± 2.19	78.0 ± 6.21
facial cream	1.48 ± 0.26	24.0 ± 6.49	11.3 ± 5.67	45.5 ± 9.17	1.11 ± 1.05	83.4 ± 7.78

Conclusion
Absorption rates of CMI, in either aqueous solution or in formulations, were minimal within the first 4 hours of exposure. The absorption rates of CMI (11.25 µg/ml) across the human epidermis were slower in formulations (0.0002 to 0.0004 µg/cm^2/hr during 24 hr occluded exposure) compared with the rate of absorption of CMI (11.25 µg/ml concentration) in aqueous solution (0.009 µg/cm^2/hr during 24 h occluded exposure). Absorption of CMI across the epidermis was 7.36, 3.53, 2.82 and 1.11% of the respective doses in aqueous solution, shampoo, body lotion and facial cream following a 24 hr occluded exposure, in the presence of MI (CMI: MI ratio of 3:1). Potential systemic availability of CMI, including ^{14}C -label retained in the epidermis, was 58.7, 48.4, 51.6 and 46.6% of the applied doses.
Ref.: 34

Comment
No details of the formulations were provided.

In-vitro human skin

Guideline: OECD draft 428
Tissue: Human (*post mortem*) skin
Group size: 6 membranes from 3 different donors/application
Diffusion cells: glass diffusion cells, 2.54 cm^2 membrane area

Skin integrity:	trans-dermal electrical resistance; at least 10 kΩ
Test substances:	(4,5-^{14}C)-MI ^{14}C-MI (2-methyl-4-isothiazolin-3-one, RH-573)
Batch:	1063.0005 aqueous dilutions; 1063.0008 shampoo, body lotion and facial cream
Purity*:*	98.7% radio-purity
Test applications:	Aqueous solutions, shampoo, body lotion and facial cream
Doses:	Aqueous solution 300, 100 and 50µgMI/ml and 100ml formulations.
Diffusion cells:	Franz cells
Receptor fluid:	purified water
Sampling time:	0, 1, 2, 4, 8, 12, 24 h
Method of Analysis:	liquid scintillation counting
GLP:	in compliance
Study period:	Jan -Aug 2005

In vitro dermal absorption of radioactive 2-methyl-4-isothiazolin-3-one (^{14}C-MI) was evaluated in human epidermis from three aqueous solutions (313, 104.3, and 52.2 µg MI/ml) and from three formulations (shampoo, body lotion and facial cream) at a concentration of 100 µg MI/ml. The aqueous solutions were applied to the epidermal membranes at a rate of 20 µl/cm^2, while the three formulations were applied to the epidermal membranes at a rate of 20 mg/cm^2. All applications were occluded for an exposure period of 24 hr. Receptor fluid samples (0.5 ml) were taken at specified intervals; the volume was maintained with fresh receptor fluid after sampling time. At the end of the exposure period, the distribution of MI in the test system was measured and reported as ^{14}C-MI equivalents.

Results

MI in aqueous solution was readily absorbed across the human epidermis following a 24 h occluded exposure – 29.8, 38.0 and 54.7% of applied dose at MI concentrations of 52.2, 104 and 313 µg/ml, respectively.

Systemic availability of MI was potentially 65.3, 60.1 and 75.5% of the applied dose (52.2, 104 and 313 µg/ml, respectively) including ^{14}C-label retained in the epidermis.

When MI (100 µg/ml) was formulated in a shampoo, body lotion and facial cream, 29.5, 9.0 and 19.6% of the applied dose was absorbed across the epidermis (24 hr, occluded exposure), respectively. Systemic availability of MI was potentially 49.7, 25.9 and 36.1% of the applied dose (100 µg/ml) including ^{14}C-label retained in the epidermis.

At the end of the study, given the reactivity of MI, it was not possible to determine if the ^{14}C -material was the parent compound or ring opened degradation/metabolic products nor whether it was permanently bound in the tissue or available for further absorption.

All values expressed as percent of dose recovered (mean ± SD)

MI Dose	Donor chamber	Skin wash at 24 h	*Stratum corneum*	Remaining epidermis	Absorbed	Total recovery (equivalents)
313 µg/ml	12.9 ± 3.09	7.03 ± 3.68	1.55 ± 0.74	10.8 ± 3.99	54.7 ± 12.0	86.9 ± 7.51
104.3 µg/ml	10.5 ± 2.68	15.0 ± 6.92	4.27 ± 2.31	22.1 ± 9.38	38.0 ± 12.1	89.9 ± 3.70
52.2 µg/ml	10.7 ± 3.97	14.1 ± 4.87	4.48 ± 2.39	35.5 ± 7.16	29.8 ± 10.1	94.7 ± 3.41
100 µg/ml shampoo	7.95 ± 4.06	29.7 ± 10.6	4.06 ± 2.78	20.2 ± 7.78	29.5 ± 13.4	91.4 ± 4.14
100 µg/ml body lotion	4.07 ± 1.32	69.4 ± 7.00	3.86 ± 1.29	16.9 ± 3.20	8.98 ± 3.10	103 ± 5.88
100 µg/ml facial cream	8.69 ± 2.62	49.1 ± 10.8	2.11 ± 0.78	16.5 ± 2.25	19.6 ± 10.0	96.0 ± 6.44

Conclusion

Absorption rates of MI, in either aqueous solution or in formulations, were minimal within the first 6 hours of exposure. The absorption rates of MI (100 µg/ml) across the human epidermis were slower in formulations (0.007 to 0.026 µg/cm^2/hr during 24 hr occluded exposure) compared with the rate of absorption of MI (104 µg/ml concentration) in aqueous

solution (0.037 µg/cm^2/hr during 24 h occluded exposure). Absorption of MI across the epidermis was 29.8, 38.0 and 54.7% of the respective doses in aqueous solutions (52.2, 104.3, 313 µg/ml) following a 24 hr occluded exposure. Potential systemic availability of MI, including ^{14}C -label retained in the epidermis, was 65.3, 60.1 and 75.5% of the applied doses. Absorption of MI from cosmetic formulations was 29.5, 9.0 and 19.6% of the respective doses from shampoo, body lotion and facial cream and potential systemic availability of MI, including ^{14}C -label retained in the epidermis, was 49.7, 25.9 and 36.1% of the applied doses.

Ref.: 35

General Comment on dermal absorption

Assessment of percutaneous absorption is difficult with 2 reactive active ingredients. Both substances, CMI and MI, were bound to the skin, but it was not determined if this bound material was systemically available. For CMI and MI on their own, ^{14}C was found to be minimally absorbed during the first 4-6 h after application. The mean ^{14}C moiety from aqueous solutions absorbed across human skin over 24 h varies from 7-56% with very high standard deviations. The ^{14}C moiety of MI was absorbed across the skin barrier to a greater extent than the ^{14}C moiety of CMI. Given the reactivity of both CMI and MI, it was not possible to determine if the absorbed ^{14}C moieties were either parent compounds or ring opened degradation/metabolic products nor whether they were permanently bound in the tissue or available for further absorption.

For these reasons, combined with large variations seen between the submitted studies, a reliable value for dermal absorption could not be determined. Therefore, 100% dermal absorption will be used to calculate the Margin of Safety.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity

A 21 day dermal rabbit study, (1972) was provided, but was not considered as there were data gaps including incomplete characterisation of the test material.

Ref.: 36

3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

Oral studies

Guideline:	/
Species/strain:	Rat, Charles River CD
Group size:	120 (15/dose/sex)
Test substance:	RH-886 Technical (5-chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-4-isothiazolin-3-one, CMI/MI, 75.3% a.i.) RH 35,375 (N-methyl malonamic acid, NMMA) RH 00,345 (malonic acid, MA)
Batch:	RH-886: Lot N° SW 72/0571 RH 35,375: Lot N° MH 24:28A
Purity:	/
Vehicle:	diet
Dose levels:	0 (control), 40-80, 132-260, 400-800 ppm CMI/MI, 33-66 ppm NMMA+6.7-13.4 ppm MA, 110-220 ppm NMMA+22-44 ppm MA
Route:	Diet
Exposure:	90 days

GLP: /
Study period 1975

Rats were exposed to CMI/MI or NMMA combined with MA in a powdered commercial diet. CMI/MI concentrations were increased over the 13-week period (initial concentration up to week 2, intermediate concentration week 3-4, final concentration week 5 to 13). Concentrations in the control and CMI/MI groups were: 0/0/0, 40/57/80, 132/187/260, 400/570/800 ppm. For the NMMA+MA groups, the concentrations were 33/47/66 ppm NMMA/6.7/9.5/13.4 ppm MA and 110/156/220 ppm NMMA/22/31/44 ppm MA.

Results
There were no mortalities and no effects on body weight or food consumption.
In each group, some animals showed slight alopecia or reddened raw or scabbed areas on the skin. There were no other differences in general behaviour or appearance.
There were no treatment-related changes in haematological, biochemical, urinary parameters nor any pathology.

Conclusion
No systemic toxicity was observed up to and including the highest dose of either CMI/MI (800 ppm, equivalent 29.1 mg a.i./kg/day] or its metabolites, N-methyl malonamic acid and malonic acid tested in combination [13 to 15 mg/kg/day N-methyl malonamic acid/2.6 to 3.0 mg/kg/day malonic acid].
The No Observed Effect Level (NOEL) in this study was estimated to be greater than or equal to the highest dose tested (29.1 mg a.i. /kg/day).

Ref.: 37

90-day repeated oral dose and one-generation reproduction toxicity in rodents

Guideline:	/
Species/strain:	Rat, COBS CD (SD) BR
Group size:	250 (15/sex/dose subchronic; 10/sex/dose reproduction)
Test substance:	Kathon 886 NAR (5-chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-4-isothiazolin-3-one, 15.5% a.i CMI/MI)
Batch:	Lot N° SW81-0138
Purity:	99.9 area% (HPLC)
Vehicle:	water
Dose levels:	0, 25, 75 or 225 ppm a.i.
Route:	drinking water
Exposure:	90 days (subchronic), approximately 5 months (15 weeks up 21 postnatal day 21, reproduction)
GLP:	in compliance
Study period	1981

Kathon 886 NAR, with magnesium salts, is given as 15.1% a.i. but there is no indication of the CMI/MI ratio.
Dose levels were selected based on a range-finding study that indicated a no-observed-effect-level (NOEL) of 200 ppm a.i. after two weeks of CMI/MI administration in the drinking water.
Rats were exposed to CMI/MI via their drinking water at concentrations of 25, 75 or 225 ppm a.i. for three months [equivalent to an average intake of 2.38, 6.28 and 16.3 mg/kg/day in males and 4.06, 10.8, and 24.7 mg/kg/day in females]. Two additional groups of rats (25/sex/group) were given tap water or tap water containing the inorganic ions present in the CMI/MI solution, at a concentration equal to that in the high dose group (225 ppm). This solution is referred to as the ion control solution.

13 week Toxicity Results
There were no mortalities in either sex at any dose level. There were no treatment-related effects on body weight up to the mid dose. A significant decrease in body weight was seen in males at the high dose during the first two weeks of the study. Food consumption was significantly decreased in males at all dose levels and in females mid and high dose groups during the first few weeks of dosing. Water consumption was significantly decreased at all concentrations.
No overt clinical signs or ophthalmic were seen in any group throughout the 13 week toxicity or the reproductive phase. No adverse effects were seen in the examinations.
No haematological treatment-related changes were seen in either sex at any dose level. There was a significant decrease in globulin and an increase in the albumin/globulin (A/G) ratio in males in both the high concentration and in the ion control groups, after 13 weeks of treatment. A significant decrease in total protein was also seen high concentration group, but was not seen in the ion control group. Females in the high concentration group showed a modest (40%) increase in SGOT (AST, aspartate aminotransferase) levels after 13 weeks of dosing. No changes were observed at any dose in mixed-function oxidase activities of the liver.
At the end of 13 weeks of treatment, there was a significant increase in relative liver weight in males and in relative kidney weight in females at the high concentration but without any correlative changes in organ pathology.
Histology revealed a local irritation of the glandular mucosa of the stomach in 7 of 15 males and 5 of 15 females at the high concentration. These subtle low level changes did not occur at the low or mid concentration nor were they present in either control group. No other compound related changes were seen. Reproductive organs at all doses were comparable to the controls.

Reproductive Results
Reproductive capability was similar in all groups. Litter size and survival at birth was also similar in all groups. One dam at the high concentration lost the entire litter by day 4 due to a lactation problem. This is not uncommon and was not considered treatment related. Pups of the other high concentration group dams, except one, survived and thrived to day 21.

Conclusion
The No Observed Effect Level (NOEL) in this study was 75 ppm a.i. [equivalent to 6.28 and 10.8 mg/kg body weight/day in males and females, respectively], based primarily on irritation of the glandular stomach at the high dose. The No Observed Adverse Effect Level (NOAEL) was 225 ppm a.i. [equivalent to 16.3 and 24.7 mg/kg body weight/day in males and females, respectively], the highest dose tested, since no adverse effects were observed on the histopathology of any tissues or organs distant from the site of dosing. No adverse effects were observed on reproductive capability of male and female rats and no effects were observed on foetal health or pup survival (to day 21) up to and including the high dose [equivalent to 16.3 and 24.7 mg/kg body weight/day in males and females, respectively].
Ref.: 38

Guideline:	/
Species/strain:	Dog, beagle
Group size:	48 (4/dose/sex)
Test substance:	RH-886 Technical (, CMI/MI) RH 35,375 (N-methyl malonamic acid, NMMA) RH 00,345 (malonic acid, MA)
Batch:	RH-886: Lot N° SW 72/0571 and SW 73/05459-6713 RH 35,375: Lot N° MH 24:28A
Purity:	/
Vehicle:	diet

Dose levels:	0, 84, 280 and 840 ppm a.i. CMI/MI, 150 NMMA+30 MA, 500 NMMA+100 MA
Route:	Diet
GLP:	/
Study period	1975

RH-886 Technical is described in the dossier as 56% active ingredient and a "calcium chloride complex". However, the two batches of RH-886 in this study were RH-886 T with 75.3% active ingredient, the second batch RH 886, 73% a.i. CMI/MI was at concentrations of 84, 280 and 840 ppm a.i., resulting in doses equivalent to 2.7, 8.9, 26.9 mg a.i./kg/day CMI/MI
N-methyl malonamic acid (NMMA) is the major ring opened metabolite of CMI/MI.
Dogs were exposed to the test compounds in a powdered commercial diet for three months. There were no deaths at any dose level of CMI/MI or NMMA combined with MA. Body weight and food consumption were comparable to the controls. No treatment related differences in general behaviour, appearance or in neurological changes.
Haematology, biochemistry and urinalysis revealed no treatment-related changes. Organ weights were within the normal parameters. There were no treatment related gross or microscopic pathological effects.

Conclusion
No systemic toxicity was observed up to and including the highest dose of CMI/MI or NMMA combined with MA [16 to 17 mg/kg/day N-methyl malonamic acid/3.2 to 3.4 mg/kg/day malonic acid].

Ref.: 39

Guideline:	OECD 409 (1981)
Species/strain:	Dog, Beagle
Group size:	116 (58 males and 58 females)
Test substance:	ACTICIDE 14, 14 % (10.2% 5-chloro-2-methyl-2H-isothiazol-3-one (CIT): 3.8% 2-methyl-3H-isothiazol-3-one (MIT)
Batch:	58008-608
Purity:	/
Vehicle:	diet
Dose levels:	0, 101, 363 and 555 mg a.i. /kg diet
Route:	Diet
Exposure:	90 days
GLP:	in compliance
Study period	23 Jan - 25 April 1996

Acticide 14 was 10.2% CMI: 3.8% MI, which is not a 3:1 ratio. In addition, the pH was 2.7 and there were no salts.
The nominal values of the doses were 150, 500 and 750 mg a.i. /kg diet. The values given under "Doses" are calculated values. Because of the poor analytical recovery of ACTICIDE 14 from the test diet, the "worst case" figures were used to calculate the actual concentration of Acticide 14 consumed by the animals. The data taken was that recorded in Week 2 when dietary levels of 101, 363 and 555 ppm a.i. were obtained.

Results
There were no deaths during the study. In the high dose group, there was a dose-related decrease in weight gain, resulting in losses in absolute body weight. This was attributed to statistically significantly reduced food consumption. Poor food consumption was also noted in a number of animals in the mid dose group over the 13 week treatment period. This appeared to be related to the palatability, since a short period of meat supplementation improved food consumption in the thinnest animals. This indicated that there was no central depression of appetite.

Haematology, clinical chemistry, ophthalmoscopy and organ weight were comparable to the controls. There were no macroscopic or microscopic findings indicative of toxicity.

Conclusion
Acticide 14 did not cause any organ or systemic toxicity in the diet at dose levels up to 555 mg a.i./ kg diet active ingredient (nominal concentration of 750 mg a.i./kg diet active ingredient), which is equivalent to up to 30 mg a.i./kg bw/day.

Ref.: 40

Dermal Studies

Guideline:	/
Species/strain:	Rat, Sprague-Dawley
Group size:	40 (10 dose/sex))
Test substance:	Acticide 14 (10.2% CMI / 4% MI)
Batch:	/
Purity:	/
Vehicle:	distilled water
Dose levels:	0, 0.75, 3.75 and 18.75 mg/kg/day bw
Dose volume:	60 µl/kg bw
Route:	dermal
Exposure:	once daily for 91 days
GLP:	in compliance
Study period	Sept - Dec 1993

There are no further details about the Acticide formulation, which is not at 3 CMI:1 MI ratio.
Method: The hair was removed by clipping prior to first application and then as needed. Application was to intact skin. A semi-occlusive dressing applied for 6 hours.
Results: One control group male and female and one high-dose male were found dead during the first 2 weeks of the study. The high-dose death was considered to be incidental since a relationship between the cause of death and test article toxicity could not be established. There were no significant effects on body weight and food consumption.
A dose-response related erythema was seen but no other treatment-related clinical changes. There were no treatment related changes in haematological, biochemical, urinary, ophthalmic parameters or organ weight. There were no macroscopic treatment-related lesions apart from the skin alterations. There were no histopathological lesions in the other organs and tissues suggestive of systemic target organ toxicity due to the test article.
Investigation of local skin reactions showed minimal individual skin reactions in 2 females low dose, 3 males and females in the mid group and in the majority of all high-dose animals of both sexes. Reactions included dose-related slight to moderate erythema and desquamation, slight oedema and atonia as well as eschar formation. These reactions were generally mild in the males and more pronounced in the females. The only microscopic treatment-related findings were related lesions such as inflammation, parakeratosis and acanthosis at the treated skin sites.

Group		Control	Low	Mid	High
Dose mg/kg/day		0	0.75	3.75	18.75
Mean total score (on scale 1 - 4)	Males	0.0	0.0	0.0	0.3
	Females	0.0	0.0	0.1	0.4
Eschar formation (%)	Males	0	0	0	60
	Females	0	2	7	75
Exfoliation (%)	Males	0	0	0	0
	Females	0	0	0	0

NOAEL:	<= 0,104 mg/kg/day (a.i.)
NOAEL (male rat):	0.104 mg a.i./kg/day
NOAEL (female rat):	not observed.

LOAEL: >= 0,104 mg/kg/day (a.i.)

Ref.: 42

Guideline: /
Species/strain: Rabbit, New Zealand
Group size: 48 (6/dose/sex)
Test substance: Kathon 886 MW (12.1% CMI/ 2.5% MI), 14.6% active ingredient
Batch: Lot N° 3433 Purity:/
Vehicle: Deionized water
Dose levels: 0, 100, 200 and 400 ppm a.i. (0, 0.67, 1.3 and 2.7 % Kathon MW)
Route: dermal
Exposure: 1 ml/kg once daily, (13 x 5 days)
GLP: in compliance
Study period 1978

Kathon 886 MW is the magnesium formulation but as supplied is not at the 3:1 ratio.
Method: The hair was removed by clipping prior to first application and then as needed. The dose groups were further divided, 3 with intact skin and 3 with lightly abraded skin. The abrasion was done prior to first application and then weekly. The application area was left uncovered.
Results: There were 12 deaths (4 high dose, 5 mid dose and 3 low dose). There were no significant effects on body weight, food consumption. There was ocular and nasal discharge in most treated animals. Controls and all dose groups exhibited poor eating behaviour and diarrhoea.
A dose-response related erythema was seen.

Dose	Response week	Post mortem
High	2	Moderate to severe erythema , slight oedema
Mid	5	Slight to moderate, 1/7 slight oedema
Low	7	Slight to moderate erythema , 1/9 slight oedema

Haematology, biochemistry and urinalysis revealed no treatment-related changes. Post mortem showed organ weights comparable to the control. There was no evidence of systemic toxicity at autopsy.
A retrospective examination of the animals suggested that the health status was not ideal. The deaths were considered to be an aggravation of an endemic pulmonary disease in the rabbits induced by stress associated with dermal irritation and not systemic toxicity of CMI/MI.

Ref.: 41

Inhalation studies

Guideline: OECD 413
Species/strain: Rat, Charles River CRL: CD(SD)BR
Group size: 128 (16/dose/sex)
Test substance: Kathon™ 886 MMPA Process (5-chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-4-isothiazolin-3-one, CMI/MI), 14% a.i.
Batch: Lot N° SW 82/0169
Purity: /
Vehicle: water (Milli-U)
Dose levels: 0.0, 0.34, 1.15 and 2.64 mg ai/m³
Dose volume: 10 ml/kg bw
Route: aerosol
Exposure: 6 hours per day, 5 days per week for 13 consecutive weeks
GLP: in compliance

Study period July – October 1982

Kathon 886 MMPA Process contains the magnesium salts.
There were no deaths. There were no effects on body weight at low or mid dose. At the high dose, decreased body weight, body weight gains and food consumption were significant.
The high exposure group showed signs consistent with those produced by exposure to a sensory irritant – chromorhinorrhea, rhinorrhea, eye squint, bradypnea, dyspnea.
There were no treatment related changes in haematological, urinary or ophthalmic parameters. High dose females had decreased serum protein levels. High dose males had decreased spleen weights. This was not observed in females and did not correlate with any histopathological change. Other than the respiratory tract, there were no treatment-related macroscopic or histopathological lesions indicating systemic target organ toxicity
At the high dose, slight to moderate eosinophilic droplets in the anterior respiratory mucosa of the nasal turbinates and very slight to slight rhinitis in the lining of the anterior portion of the nasal cavity were noted. Both changes were minor, potentially reversible physiologic responses to an upper respiratory tract irritant. Additional changes included very slight to slight hyperplasia of the squamous epithelium in the nasal turbinates and varying degrees of sinusitis in the paranasal sinuses. These were described as being secondary effects and not direct-treatment related. At the mid-dose, the only effect observed was a very slight incidence of rhinitis.

Conclusion The No Observed Effect Level (NOEL) was 0.34 mg/m³, based on minimal irritation of the respiratory tract at 1.15 mg/m³. No adverse effects on the histopathology of any tissues/organs distant from the site of dosing were noted up to and including the highest dose tested (2.64 mg a.i./m³).

Ref.: 43

General comment
The value of these repeated dose studies is limited as the test formulations are not properly characterised or are not at the 3:1 ratio and there are other data gaps.

3.3.5.3. Chronic (> 12 months) toxicity

See 3.3.7. Carcinogenicity

3.3.6. Mutagenicity / Genotoxicity

In Vitro studies

Substance: test	***Findings***	***Study date***	***Ref***
CMI/MI: reverse mutation test in *Salmonella typhimurium* and *Saccharomyces cerevisiae*	Positive in strain TA100	1981	46
CMI/MI: reverse mutation test in *Salmonella typhimurium*)	Positive in strain TA100 without S9-mix only	1981	47
CMI/MI: reverse mutation test in *Salmonella typhimurium*)	Positive in strain TA100 without S9-mix only	1981	48
CMI/MI: reverse mutation test in *Salmonella typhimurium*	Positive in strain TA100 (only strain tested)	1991/1992	49
CMI/MI: reverse mutation test in *Salmonella typhimurium*	Positive in strains TA98, TA100, TA102, TA1535 and TA1537 without S9-mix; and TA100 and TA 102 with S9-mix	1994	50
CMI/MI: reverse mutation test in *Salmonella typhimurium*	Positive in strain TA-100, negative in *E. coli*	1982	51
CMI: reverse mutation test in *Salmonella typhimurium*	Positive in strain TA-100 without S9-mix only	1981	52

Substance: test	***Findings***	***Study date***	***Ref***
MI: reverse mutation test in *Salmonella typhimurium*	Negative	1982	53
MI: reverse mutation test in *Salmonella typhimurium*	Negative	1999	54
NMMA: reverse mutation test in *Salmonella typhimurium* and *Escherichia coli*	Negative	2005	55
CMI/MI: gene mutation test in mammalian cells (mouse lymphoma cells)	Positive	1981	56
CMI/MI: gene mutation test in mammalian cells (mouse lymphoma cells)	Positive	1993/1994	58
CMI/MI*: In vitro* unscheduled DNA synthesis (UDS) assay [primary rat hepatocytes]	Negative	1990/1991	59
CMI/MI: Mammalian cell chromosome aberration test (Chinese hamster lung cells)	Negative	1982	61

Under *in vitro* conditions CMI/MI induced an increase in the number of revertants in the 4 gene mutation assays in bacteria performed. Increases were predominantly seen in *Salmonella* strain TA100; increases in other strains were only demonstrated in one test. CMI induced an increase in the number of revertants in TA100 only in the absence of S9-mix. Both MI and NMMA were negative in the gene mutation assay in bacteria. In two gene mutation assays in mammalian cells CMI/MI treatment resulted in an increase in the mutant frequency at the *tk* locus of mouse lymphoma cells both in the absence as presence of S9-mix. Unscheduled DNA synthesis was not observed after treatment of hepatocytes with CMI/MI. Clastogenicity was tested in a poorly performed *in vitro* chromosome aberration test; CMI/MI did not induce an increase in the number of cells with chromosome aberrations.

In Vivo studies

Substance: test	***Treatment***	***Findings***	***Study date***	***Ref***
CMI/MI: Rat chromosome aberration test (bone marrow cells)	Single dose or treatment on 5 consecutive days by oral gavage or in the feed Up to 28 mg a.i./kg	Negative, No indication of exposure	1973	62
CMI/MI: Mouse chromosome aberration test (bone marrow cells)	Single dose or treatment on 5 consecutive days by oral gavage or in the feed Up to 30 mg a.i./kg.	Negative No indication of exposure	1982	63
CMI/MI: Mouse chromosome aberration test (bone marrow cells)	Single dose by oral gavage Up to 20 mg a.i./kg.	Negative	1991	67
CMI/MI: Mouse chromosome aberration test (bone marrow cells)	Single dose by oral gavage Up to 30 mg a.i./kg.	Negative	1992	68
CMI/MI: Mouse micronucleus assay	Single dose or treatment on 5 consecutive days by oral gavage Up to 30 mg a.i./kg	Negative	1983	64
CMI/MI: Mouse micronucleus assay	Single dose by oral gavage Up to 50 mg/kg bw	Negative	1996/1997	65
CMI/MI: Mouse micronucleus assay	Treatment on 2 consecutive days by oral gavage up to 4,17 mg a.i./kg	Negative, No indication of exposure	1994	66
CMI/MI: Unscheduled DNA synthesis (UDS) study in the rat	Single dose by oral gavage Up to 500 mg/kg bw	Negative	1997	69
CMI/MI: Unscheduled DNA synthesis (UDS) study in the rat	Single dose by oral gavage Up to 60 mg/kg bw	Negative	1994	70

Substance: test	***Treatment***	***Findings***	***Study date***	***Ref***
CMI/MI: *Drosophila melanogaster* sex-linked recessive lethal test	Treatment in the feed or by injection. Up to 86 µg a.i./kg (feed) or 258 µg a.i./kg (injection)	Negative	1982	71
Other related studies:				
CMI: *In vitro* and *in vivo* DNA binding study to DNA from mouse lymphoma cells and rat testes cells		No binding of ^{14}C-label, derived from ^{14}C-CMI, to DNA fraction *in vitro* or *in vivo*	1983	72
CMI/MI: Tissue distribution study in mouse	Single dose by oral gavage Up to 100 mg base-eq/kg bw of ^{14}C-labelled test compound	CMI/MI and / or metabolites found in blood, bone marrow and liver	2002	90
CMI/MI: Tissue distribution study in mouse	Single dose by oral gavage Up to 22.5 mg base-eq/kg bw of ^{14}C-labelled test compound	CMI/MI and / or metabolites found in blood, bone marrow and liver	2004	91

The positive mutagenic effect of CMI/MI found *in vitro* in gene mutation assays in bacteria as well as mammalian cells was not confirmed in the sex-linked recessive lethal test in *Drosophila melanogaster* nor in two Unscheduled DNA synthesis (UDS) studies in the rat. As under *in vitro* conditions, both in *in vivo* chromosome aberration tests and in micronucleus tests, CMI/MI appeared not clastogenic.
CMI/MI does also not have relevant clastogenic potential in an *in vivo* micronucleus test in mice described by Richardson *et. al*
The negative *in vivo* mutagenicity tests are supported by the results of the carcinogenicity studies that show that CMI/MI did not produce an increase of the type or incidence of tumours.

CMI on its own was positive *in vitro* in a gene mutation test in bacteria without S9-mix only. However, a DNA binding study *in vivo* was negative indicating that the possibility that CMI alone has genotoxic potential *in vivo* is low.
Both MI and NMMA were negative under *in vitro* conditions and consequently not tested *in vivo.*

3.3.7. Carcinogenicity

One mammalian cell transformation test (mouse embryo fibroblast test) was negative.
Ref.: 60

Combined chronic toxicity/carcinogenicity test: 24-month repeated oral (drinking water) dose in rodents

Guideline:	OECD 453
Species/strain:	Rat, Charles River CRL:CD®BR
Group size:	850, 450 male, 400 female (90/male/dose, 80/female/dose)
Test substance:	Kathon™ 886 (5-chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-4-isothiazolin-3-one, CMI/MI) 14.2% active ingredient
Batch:	Lot N° 48014
Purity:	/
Vehicle:	water
Dose levels:	30, 100 and 300 ppm a.i.
Control:	water alone; water with magnesium salt
Route:	drinking water
Exposure:	2 year
GLP:	in compliance

Study period Sept 1990 – Sept 1992

In the study report, Kathon 886 is given as 14.2% active ingredient with a pH between 2-3. In the document with compiled batch information for Kathon the active ingredient is 13.2, [10.13 % CMI/ 3.85% MI] with 15.4% magnesium nitrate and 9.0% magnesium chloride.
The dose levels were equivalent to: 0, 0, 2.0, 6.6, 17.2 mg a.i./kg bw/day in males and 0, 0, 3.1, 9.8, 25.7 mg a.i./kg bw/day in females. The animals were monitored daily. Blood and urine were evaluated at intervals throughout the study. Ten animals/sex/group were killed and *post mortems* carried out at 12 and 18 months. The remainder were killed at 24 months for full analysis.

Results
There were no deaths. There were no treatment-related effects on body weight or body weight gain at doses or food consumption up to and including the mid dose group. A treatment-related and concentration-dependent decrease in water consumption was seen in both sexes in all treated groups throughout the study. These decreases ranged from 0-22% at low dose 3-30% at mid dose and 15-40% at high dose. These decreases appear to be due to the unpalatability of the CMI/MI and not its inorganic stabilizer salts since the water consumption in the salt control was comparable to the tap water control throughout the study. Based on the average daily water consumption, the high dose was judged to be a maximum tolerated dose. The decreases in body weight and body weight gain were seen in high dose animals throughout the study and may be secondary to decreased water consumption.
No treatment-related clinical effects were recorded. No treatment-related ophthalmic, haematological. biochemical or urinary changes were noted. Organ weights were comparable to the control.
No effects on type or incidence of neoplasms were seen at up to and including the high dose (males: 17.2 a.i.; females 25.7 mg a.i./kg/day). Slight to moderate forestomach hyperplasia was seen at both mid and high dose groups. Gastric irritation was the primary effect observed. No adverse effects on the histopathology of any other tissues/organs were observed away from the site of dosing. No systemic effects were observed.

Conclusion
Kathon™ 886 in the drinking water for 24 months produced no treatment-related effects on the type or incidence of neoplasms in rats at concentrations up to and including 300 ppm a.i. (17.2 to 25.7 mg/a.i./kg/day). CMI/MI is not considered carcinogenic.
The No-Observed-Effect Level (NOEL) in this study was 30 ppm a.i. (2.0 to 3.1 mg a.i./kg/day), based primarily on gastric irritation of the stomach at 100 and 300 ppm a.i.. The No-Observed-Adverse-Effect Level (NOAEL) was 300 ppm a.i. (17.2 to 25.7 mg/a.i./kg/day), since no evidence of systemic toxicity was observed at any dose and there was no adverse effects on the histopathology of any tissues/organs distant from the site of dosing at any dose.

Ref.: 74

Dermal Study

Guideline:	/
Species/strain:	Mouse, Charles River CD-1
Group size:	120 males (40/dose)
Test substance:	Kathon™ CG (5-chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-4-isothiazolin-3-one, CMI/MI), 1.5% active ingredient
Batch:	Lot N° MH31:9E (prepared from Lot SW 78/4014, 14.7% a.i.)
Purity:	/
Vehicle:	deionized water
Positive control:	3-methylcholanthrene 1000 ppm a.i. in acetone.
Dose levels:	0, 400 ppm a.i.

Dose volume: 25µl
Route: dermal
Exposure: topically 3 times per week for 30 months
GLP: /
Report date 1983

Lot SW 78/4014 was a semi-works-manufactured formulation formulated into the 1.5% a.i end-use formulation Lot MH 31:9E. The CMI/MI ratio was not given.
All positive controls (3-methylcholanthrene) died within 16 months. By the end of the study period, 10/40 control and 7/40 Kathon treated animals survived. Deaths in the Kathon treated group occurred mainly between in the period of 8 –28 months, compared with the controls of 12 –28 months.
There were no effects on body weight. There was brown staining eschar and/or desiccation, flaking of skin at application site of Kathon™ CG treated mice
All positive controls showed squamous cell carcinoma of the skin with metastases to lungs, kidney and spleen.
No treatment-related neoplasms were observed in Kathon-treated mice when compared with the control mice. Histology of the treated skin of the Kathon animals showed focal or multifocal epidermal necrosis, hyperplasia, hyperkeratosis, eschar, dermal inflammation, and increased dermal collagen. No other adverse histopathological effects of tissues/organs were seen. There was no evidence of systemic toxicity.

Conclusion
Due to the lack of positive histopathological findings, the test substance was not considered to be carcinogenic.

Ref.: 73

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

Test substance	Species	Result	Study date	Ref
Kathon 886F 11.1%CMI/3.7 % MI a.i	Rat Drinking water	Parental P1 NOAEL 2.8 –4.4 mg a.i/kg bw/day P1 Reproductive NOEL 22.7-28.0 mg a.i/kg bw/day P2 Reproductive NOEL 35.7-39.1 mg a.i /kg bw/day [highest dose tested]	1998	80
Acticide 14 13.9%a.i.	Rat Gavage	Reproductive NOAEL was >/= 10 mg a.i./kg bw/day Parental NOAEL was >/=10 mg a.i./kg bw/day F1 NOAEL was >/= 2.5 mg a.i/kg bw/day F2 NOAEL >/= 2.5 mg a.i./kg bw/day	1998	81

Guideline: OECD 416
Species/strain: Rat, Crl: CD®BR strain
Group size: 26 males and 26 females /dose
Test substance: Kathon 886F (5-chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-4-isothiazolin-3-one, CMI/MI), 14.76% active ingredient

Batch: Lot N° 0157A001
Purity: /
Vehicle: deionized water
Positive control: none
Dose levels: 0, 0 (salt control), 30, 100, or 300 ppm a.i. of CMI/MI
Route: oral
GLP: Yes
Report date 1998

Rats were dosed with CMI/MI in drinking water through two generations at concentrations of 0 (control), 0 (magnesium salt control), 30, 100 or 300 ppm a.i. For the P1 generation, this was equivalent to 0, 2.8-4.4; 8.5-11.8, and 22.7-28.0 mg a.i./kg bw/day; and in the P2 generation 0, 4.3-5.5, 13.4-16.0, and 35.7-39.1 mg a.i./kg bw/day.

There were no treatment related effects on survival, food consumption or overt signs of toxicity. A decrease in bodyweight gain was noted initially in the P1 generation. This was thought to be linked to reduced water consumption since significant dose-related reduction in water consumption was seen at all concentrations in both the P1 and P2 generations, during the premating, gestation and lactation stages.

Treatment-related histopathological changes were seen in the stomach in the P1 and P2 generation. These included erosions of the glandular mucosa, oedema and inflammation in the submucosa of the glandular and nonglandular stomach, with hyperplasia and hyperkeratosis of the nonglandular stomach at the 100 and 300ppm a.i. Other histopathological changes were seen but were not dose dependent.
The oestrus cycle in P1 or P2 females at any treatment level was comparable with the controls, as was the sperm motility, morphology, testicular sperm count or caudal epididymal reserves of P1 or P2 males.

All other endpoints (gestation index, gestation length, number of pups per litter or treatment-related gross findings in F1 or F2 pups) were similar to those in the controls in either generation.

The study authors considered that rats exposed to CMI/MI in the drinking water through two generations had a No Observed Adverse Effect Level (NOAEL) of 30 ppm a.i. (2.8-4.4 mg/kg/day in the P1 animals; 4.3-5.5 mg/kg/day in the P2 animals) for parental animal toxicity, based on the gastric irritation of stomach at higher doses. The No Observed Effect Level (NOEL) for reproductive toxicity was 300 ppm a.i. (22.7-28.0 mg/kg/day in the P1 animals; 35.7-39.1 mg/kg/day in the P2 animals), the highest dose tested. There were no effects on fertility or foetal development at any dose level.

3.3.8.2. Teratogenicity

Test substance	Species	Result	Study date	Ref
Kathon™ 886 13.9% a.i.	Rat Gavage	Developmental NOEL for CMI/MI was >15 mg a.i./kg bw/day during organogenesis (highest dose tested).	1980	75
Acticide 14 10.2%CMI/4% MI	Rat Gavage	NOAEL maternal toxicity: <= 3.95 . mg/kg bw a.i. NOAEL teratogenenicity : >= 19.6 . mg/kg bw a.i. NOAEL embryotoxicity: >= 19.6 mg/kg bw a.i.	1994	76
Acticide 14 10.2%CMI/4% MI	Rabbit Gavage	Developmental NOAEL was > 5.49 mg a.i./kg bw/day NOAEL for maternal toxicity and foetal toxicity was 1.41 mg a.i./kg bw/day	2002	78
Kathon MW 13.9% a.i	Rabbit Gavage	NOEL maternal toxicity: 2 mg/kg bw Developmental NOEL: 8 mg a.i./kg bw/day [highest dose based on severe maternal toxicity at 20 mg/kg/day]	1991	77

General comment on reproductive toxicity

In the two generation reproductive toxicity and the teratogenicity study, the chemical characterisation of Kathon™ 886 was CMI/MI 3:1 ratio with magnesium salts. The other studies were not considered for the calculation of the MoS due to the inadequate chemical characterisation of the test substances.

Therefore, the NOAEL of 2.8 mg a.i./kg bw/day Kathon™ 886 from the parental P1 two generation reproductive toxicity study,(Ref 80), based on significantly reduced water intake

and irritation/erosion of the stomach wall was used for the calculation of the Margin of Safety.

3.3.9. Toxicokinetics

Toxicokinetic studies in rats, *in vivo* and using different CMI/MI administration routes, using either ^{14}C-labelled CMI or ^{14}C-labelled MI, showed that systemically it was rapidly absorbed and metabolized. CMI, MI and/or their metabolites did not bioaccumulate in tissues. CMI and MI were not detected in urine, faeces or bile. The metabolites were eliminated mainly within 24 h, only low levels of ^{14}C were found in blood and highly vascular tissue in the body after 168 h.
N-methyl-malonamic acid (NMMA), a ringed-opened metabolite, was the major metabolite of both CMI and MI. It appeared to have low systemic toxicity. It was eliminated through urine > faeces > bile. Other metabolites of CMI (or MI) were Phase I reductive and oxidative cleavage metabolites and Phase II metabolites consisting of glutathione-derived conjugates of Phase I metabolites of CMI(or MI) , in addition to glutathione conjugates.

Ref.: 82

Comment
This reference is a 1997 compilation of 5 separate studies that were performed between 1972 – 1986 to comply with the OECD 417 Guideline, 1992. The value of this compilation is questionable, as the quality of the studies was variable and did not meet modern standards.

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

Human

Guideline:	/
Group size:	2 male, 23 female
Test substance:	Kathon™ CG (5-chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-4-isothiazolin-3-one, CMI/MI), 1.5% active ingredient
Batch:	/
Purity:	/
Vehicle:	aqueous
Dose levels:	15 ppm a.i.
Dose volume:	0.2ml
Route:	dermal
Exposure:	24h, 0.75 µg/cm^2 topically
GCP:	/
Report date:	1982

The inner surface of the forearms was wiped with alcohol, and stripped with Scotch tape. The test solution was applied to Parke Davis Readi-Band (Webril) patches (2 cm^2), that were in place for 24 hours. Dermal responses were then recorded. Irradiation of one arm with UV-A (wavelength unspecified, 4,400 µW/ cm^2) was for 15 minutes. Both skin sites (irradiated and not irradiated) were examined immediately after irradiation and again at 24, 48 and 72 hr. The irradiated site was also examined 1 week later for "tanning effects".
No phototoxic effects were observed with CMI/MI under these test conditions.

Ref.: 102

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

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3.3.11. Human data

Contact allergy to Methylchloroisothiazolinone/methylisothiazolinone, recent clinical data

A recent overview of the use of MCI/MI and contact allergy to it is available (Thyssen et al 2007).
Since the late 1970s in Europe and early 1980s in the USA, preservatives containing MCI/MI have been widely used (Law et al, 1984). In 1980–1982, the first cases of occupational allergic contact dermatitis to MCI/MI were described and later also found in consumers (Bjorkner et al, 1986; Gruvberger., 1997). The MCI/MI-containing preservatives Kathon® CG and Kathon® 886 were added to the baseline patch test series and cutting oil patch test series, respectively, in southern Sweden (Bjorkner et al, 1986).
In the 1980s, a number of reports of allergic contact dermatitis to MCI/MI were published (de Groot et al, 1985; Foussereau J. 1990; O'Driscoll and Beck. 1988). The prevalence of contact allergy markedly increased in unselected eczema patients with prevalence rates between 3% and 8% (Bjorkner et al, 1986: 14: 85–90; Hannuksela, 1986: 15: 211–214; Tosti, 1988), which was largely attributed to cosmetic leave-on products. Cosmetic products contained a concentration of MCI/MI that was within the recommended levels of use (30 ppm) (Fewings et al, 1999).
Since the 1990s, the content of MCI/MI in the EU has been limited to 15 ppm in cosmetic products (Reinhard, 2001). However, the Cosmetics, Toiletries and Fragrance Association recommended a use concentration of no more than 7.5 ppm in cosmetic leave-on products and 15 ppm for cosmetic rinse-off products (Cosmetic Ingredient Review Panel of the Cosmetic, Toiletry Fragrance Association. 1992). It has been suggested that such reduction should reduce the risk of induction of sensitization as well as elicitation of contact dermatitis in MCI/MI-sensitized individuals from the use of rinse-off products (Fewings et al, 1999). However, the frequency of patch test reactions to MCI/MI from a 10-year multicentre data remained high at 2–2.5% (Wilkinson et al., 2002). An epidemiological study from a Spanish group found that 4.04% of tested individuals had contact allergy to MCI/MI (García-Bravo et al, 2004). It has been shown that the elicitation threshold for a MCI/MI-containing solution is less than 2 ppm in sensitized individuals (Zachariae et al, 2006).

The results of patch testing 6958 patients were collected from 9 dermatology centres in the UK during the period 2004–2005. Methylchloroisothiazolinone/methyl-isothiazolinone had a high positivity rate with a mean of 2.0% (1-5-2.5%) (Jong et al, 2007).
During an allergy screening programme involving 9320 children aged 7 and 16 years, 12.6% reported symptoms of chronic/recurrent eczema. From this group, a representative sample of 229 eczema children underwent patch testing. 43.8% of 7 year olds with eczema were patch test positive with 6.3% having contact allergy to MCI/MI. 52.6% of teenagers were patch test positive with 0.8% to MCI/MI. (Czarnobilska et al, 2009).
Clinical and patch test data of 19 793 patients patch tested in 2005/2006 in the 31 participating departments from 10 European countries (the European Surveillance System on Contact Allergies' (ESSCA) www.essca-dc.org) were descriptively analysed and aggregated to four European regions. A number of allergens showed limited variation across the four regions, but differences observed with other allergens may hint at underlying differences in exposures: MCI/MI 4.1% in Southern Europe versus 2.1–2.7% in the other regions. (Uter et al, 2009).
Contact allergy to MCI/MI remains high in European patients with eczema under current use and exposure conditions.

3.3.12. Special investigations

Pulmonary hypersensitivity

Guideline:

Species:	Guinea pig, Dunkin Hartley
Group:	40, 8 males/dose
Substance:	Kathon CG/ICP 1.53% active ingredient
Batch	Lot N° J87008
Purity:	99.6%
Induction route:	inhalation
Dose:	3 groups exposed to 320 mg/m^3 CMI/MI, (4.8 mg ai/m^3)
Control:	aqueous magnesium salt solution
Vehicle:	aqueous
Positive Control:	trimellitic anhydride, (TMA)
GLP:	in compliance
Study period:	July-Aug 1994, report 1995

Five daily induction exposures of 80 min inhalation of CMI/MI (3 groups at 4.8 mg ai/m^3), magnesium salt control or a positive control (trimellitic anhydride, TMA; 91 mg ai/m^3) were administered to groups of eight guinea pigs.
Challenge exposures were on Day14 and 28 after the last induction. However, inductions were staggered to allow correct challenge times. One-hour inhalation challenge exposures of 0.17, 0.35 and 0.72 mg ai/m^3 CMI/MI in aqueous solution and individual respiratory rates were monitored continuously during the challenge and during a 10-hour post-challenge recovery period.

Results
There were no clinical signs of toxicity noted during the induction or challenge phases in either the control-exposed or the CMI/MI-exposed guinea pigs. All of the positive control animals upon challenge to TMA displayed a change in respiratory rate and a change in the respiratory waveform indicative of an immediate pulmonary hypersensitivity response. The data indicate that induction at 4.8 mg ai/m^{3}of CMI/MI did not result in an immediate or delayed pulmonary hypersensitivity response in guinea pigs when subsequently challenged with an aerosol of the test substance at 0.17, 0.35 and 0.72 mg ai/m^3.

Conclusion
Under the conditions of this study, CMI/MI induction at 4.8 mg ai/m^3 did not result in an immediate or delayed pulmonary hypersensitivity response in guinea pigs when subsequently challenged with an aerosol of the test substance at 0.17, 0.35 and 0.72 mg ai/m^3 nor CMI/MI did produce respiratory sensitisation.

Ref.: 29

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Indicative daily exposure rinse-off products (approximate value, including retention factors)	=	1.5 g/day
Concentration CMI/MI	=	0.0015%
Daily exposure CMI/MI	=	0.0225 mg
Dermal absorption	=	100%
Typical body weight of human	=	60 kg
Systemic exposure dose (SED) rinse off	=	0.00038 mg/kg bw
No observed adverse effect level (NOAEL) (two-generation reproductive study, parental P1)	=	2.8 mg/kg bw

Margin of Safety	NOAEL / SED	=	7368

A worst case scenario of 100% absorption has been assumed for the calculation of MoS.

3.3.14. Discussion

As a dossier this was poorly put together with no links between the numbered references in the submission and the references provided. The toxicological studies were useable but did not meet modern standards as the majority were carried out more than twenty years previously. This resulted in confusion with inconsistent nomenclature and poor chemical characterisation.
Despite the numerous short comings of the dossier, the weight of evidence over several decades of exposure to both industrial products and cosmetics indicate CMI/MI has low general toxicity and that skin sensitisation is the main problem.

Physico-chemical properties
Only preparations with 5-chloro-2-methylisothiazol-3(2H)-one (CMI) and 2-methylisothiazol-3(2H)-one (MI) in the ratio of 3:1 are permitted for the use in cosmetics. Initially, CMI/MI formulations were prepared as a mixture of two individual active ingredients CMI and MI and salts. However, Kathon™ 886 Biocide is now defined as a combination of the two active ingredients produced by an integrated production process, resulting in an approximate total of 14% active ingredients, 16% magnesium nitrate, 10% magnesium chloride and 62% water. This seems to be a change in the manufacturing process, but there is no indication as to when this change was made.
The CMI/MI formulations described in the dossier show variations in the CMI/MI ratio and several physicochemical parameters.

Toxicity
The value of the acute, subchronic and reproductive toxicity studies is limited as the test formulations are not properly characterised and there are other data gaps. For the calculation of the Margin of Safety, the parental P1 NOAEL of 2.8 mg a.i./kg bw/day from the Kathon MW two generation reproductive toxicity study was used.

Skin/eye irritation and sensitisation
P56, (5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one in a ratio of 3:1) is corrosive or irritating at high concentrations. No adequate data is given to support safe use at a maximum authorised concentration of 0.0015 % in rinse-off cosmetic products. However, the weight of evidences over several decades of consumer exposure to cosmetic products indicates that skin and/or mucous membrane irritation is not a problem under the conditions of use in leave-on and rinse-off products.
The data demonstrates that CMI/MI is an extreme sensitiser in animals and an extreme contact allergen in humans. The chloro-isomers including CMI appear to be more allergenic than MI as seen in the LLNA.
Contact allergy to CMI/MI remains high in European patients with eczema under current use (rinse-off and leave-on products) and exposure conditions. Induction and elicitation would be less likely in a rinse-off product than when the same concentration is present in a leave-on product. The main exposure from the general consumer will be from cosmetic products, but there may be also exposure from other consumer products and occupational settings.

Percutaneous absorption
Assessment of percutaneous absorption is difficult with 2 reactive active ingredients. Both substances, CMI and MI, were bound to the skin, but it was not determined if this bound material was systemically available. For CMI and MI on their own, ^{14}C was found to be minimally absorbed during the first 4-6 h after application. The mean ^{14}C moiety from aqueous solutions absorbed across human skin over 24 h varies from 7-56% with very high standard deviations.. The ^{14}C moiety of MI was absorbed across the skin barrier to a greater extent than the ^{14}C moiety of CMI. Given the reactivity of both CMI and MI, it was not

possible to determine if the absorbed ^{14}C moieties were either parent compounds or ring opened degradation/metabolic products nor whether they were permanently bound in the tissue or available for further absorption.

Therefore, 100% dermal absorption was used to calculate the Margin of Safety.

Mutagenicity and Carcinogenicity
The results of the *in vitro* mutagenicity tests were equivocal. In bacterial reverse mutation tests, CMI/MI was positive in *Salmonella* strain TA100 and but predominantly negative in other strains commonly tested strains. CMI/MI was mutagenic in the mouse lymphomagene mutation assays in mammalian cells both with and without metabolic activation. Effects at the *Tk* locus was seen both with and without S-9, but were enhanced with S9-mix. CMI/MI was not genotoxic in the *in vitro* unscheduled DNA synthesis (UDS) assay nor an induction in cells with were chromosome aberrations induced in cultured human peripheral blood lymphocytes Chinese hamster lung cells were found.
However, the *in vivo* studies provided indicated that CMI/MI does not have relevant mutagenic potential *in vivo*. The positive mutagenic effect of CMI/MI found *in vitro* in gene mutation assays was not confirmed in the sex-linked recessive lethal test in *Drosophila melanogaster* nor in two Unscheduled DNA synthesis (UDS) studies in the rat. CMI/MI did also not show any increase in cells with micronuclei in mice nor did it induce chromosomale aberrations changes in rat bone marrow cells under the conditions of the assays.

CMI/MI was not considered to be carcinogenic, since there was no increase in the type or incidence of tumours.

CMI on its own was positive *in vitro* in a gene mutation test in bacteria without S9-mix only. However, a DNA binding study *in vivo* was negative indicating that the possibility that CMI alone has genotoxic potential *in vivo* is low.

Both MI and NMMA were negative under *in vitro* conditions and consequently not tested *in vivo.*

4. CONCLUSION

The mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one in a ratio of 3:1 is well recognised as an important skin sensitiser at current conditions of use and applications. Hitherto, it has been used in both leave-on and rinse-off products in Europe.

Induction and elicitation would be less likely in a rinse-off product than when the same concentration is present in a leave-on product.

On the basis of the data submitted, the SCCS is of the opinion that the mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one in a ratio of 3:1 does not pose a risk to the health of the consumer when used as a preservative up to a maximum authorised concentration of 0.0015 % in rinse-off cosmetic products, apart from its sensitising potential.

5. MINORITY OPINION

Not applicable

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