Amended Safety Assessment of Methylisothiazolinone as Used in Cosmetics

Status:	Final Amended Report
Release Date:	September 29, 2020
Panel Meeting Date:	September 14-15, 2020

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Lisa A. Peterson, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Christina L. Burnett, Senior Scientific Analyst/Writer, CIR.

1620 L Street, NW, Suite 1200 ♢ Washington, DC 20036-4702 ♢ ph 202.331.0651 ♢ fax 202.331.0088 ♢
cirinfo@cir-safety.org

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) reassessed the safety of Methylisothiazolinone, which functions as a preservative in cosmetics. The Panel reviewed relevant animal and human data provided in this safety assessment, and data from the previously published safety assessments of Methylisothiazolinone, and concluded that Methylisothiazolinone is safe for use in rinse-off cosmetic products at concentrations up to 100 ppm (i.e. 0.01%) and safe in leave-on cosmetic products when they are formulated to be non-sensitizing, which may be determined based on a quantitative risk assessment (QRA) or similar methodology.

INTRODUCTION

Methylisothiazolinone is reported to function in cosmetics as a preservative, according to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*).[1] In 2019, the Expert Panel for Cosmetic Ingredient Safety (Panel) published an amended safety assessment of Methylisothiazolinone with the conclusion that "Methylisothiazolinone is safe for use in rinse-off cosmetic products at concentrations up to 100 ppm and safe in leave-on cosmetic products when they are formulated to be non-sensitizing, which may be determined based on a quantitative risk assessment (QRA)."[2] This conclusion superseded the findings of the Panel's earlier safety assessment that was published in 2010.[3] At the September 2019 Panel meeting, during the re-evaluation of the mixture methylchloroisothiazolinone/ methylisothiazolinone (MCI/MI), the Panel reopened the amended safety assessment of Methylisothiazolinone to consider additional newly available data, with particular regard to inhalation toxicity.

In 2019, the Panel issued an amended safety assessment of the mixture MCI/MI (supplied as a ratio of 3:1), with the conclusion that the mixture "is safe in cosmetics when formulated to be non-sensitizing, based on the results of a QRA or similar methodology; however, at no point should concentrations exceed 7.5 ppm in leave-on products or 15 ppm in rinse-off products."[4]

Data from the original Methylisothiazolinone safety assessment that was published in 2010 and the amended safety assessment that was published in 2019 are summarized in *italics* in each appropriate section of this report.[2,3]

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Much of the data included in this safety assessment was obtained from the European Chemicals Agency (ECHA).[5] These data summaries are available on the ECHA website, and when deemed appropriate, information from the summaries has been included in this report.

CHEMISTRY

Definition and Structure

Methylisothiazolinone (CAS No. 2682-20-4) is the heterocyclic organic compound that conforms to the structure depicted in Figure 1.[1]

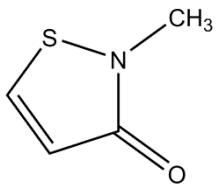

Figure 1. Methylisothiazolinone

Physical and Chemical Properties

Methylisothiazolinone has a molecular weight of 115.2 Da and a density of 1.02 g/ml at 25° C.[3] The ultraviolet/visible spectrum for a tradename Methylisothiazolinone product had peak wavelengths at 274 nm for a neutral solution, 266 nm for an acidic solution, and 274 nm for a basic solution. Additional properties are described in the original safety assessment.

Method of Manufacturing

Methylisothiazolinone is produced by the controlled chlorination of dimethyldithiodipropionamide in solvent.[3] Methylisothiazolinone is then neutralized and extracted into water followed by a solvent strip.

Composition and Impurities

The composition of technical grade Methylisothiazolinone was 96.8% Methylisothiazolinone, 0.1% 5-chloro-2-methyl-4-isothiazoline-3-one, 0.1% 4,5-dichloro-2-methyl-4-isothiazolinone-3-one, 0.2% N,N'-dimethyl-3,3'-dithiodipropionamide, 0.5% N,N'-dimethyl-3,3'-trithiodipropionamide, 0.1% N-methyl-3-chloropropionamide, 0.3% ammonium chloride, 0.2% water, 0.1% ethyl acetate, 0.1% acetic acid, and 1.5% unknown compounds.[3] *Impurities of a tradename Methylisothiazolinone product (9.5% active ingredient) included 79 - 103 ppm N,N'-dimethyl-3,3'-trithiodipropionamide, 44 - 79 ppm 5-chloro-2-methyl-4-isothiazolin-3-one, and 490 ppm N,N'-dimethyl-3,3'-dithiodipropionamide.*

USE

Cosmetic

The safety of the cosmetic ingredient addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in the FDA Voluntary Cosmetic Registration Program (VCRP) database. Data are submitted by the cosmetic industry in response to a survey, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.

According to 2019 VCRP survey data, Methylisothiazolinone (when not used with MCI) is used in a total of 915 formulations; the majority of the uses are in bath soaps and detergents (Table 1).[6] These uses have increased since the last review where 745 uses were reported; the majority of the uses reported then were in non-coloring hair conditioners and shampoos.[2] The maximum concentration of use range for Methylisothiazolinone in 2020 was reported to be 0.000002% to 0.00975% (0.02 ppm to 97.5 ppm), with 0.00975% reported in hair conditioners and 0.009% used in leave-on hair products.[7] In the amended safety assessment published in 2019, the maximum concentration of use range was reported to be 3.5 x 10^{-8}% to 0.01% (0.00035 ppm to 100 ppm), with 0.01% reported in multiple product categories, including eye makeup remover, hair shampoos and conditioners, and skin care products (both leave-on and rinse-off).

Methylisothiazolinone may be used in products that can come into contact with the eyes or mucous membranes; for example, it is reported to be used in bath soaps and detergents at up to 0.00755% (75.5 ppm) and in bath oils, tables and salts at up to 0.0090% (90 ppm).[7] Additionally, Methylisothiazolinone is used in cosmetic sprays and could possibly be inhaled; for example, it is reported to be used in hair sprays at up to 0.00095% (9.5 ppm). In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters > 10 μm, with propellant sprays yielding a greater fraction of droplets/particles < 10 μm compared with pump sprays.[8,9] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and thoracic regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[10,11]

Under regulations governing the use of cosmetic ingredients in the European Union, Methylisothiazolinone is listed under Annex V, the list of preservatives allowed in cosmetic products, with the restriction that it may only be used in rinse-of products at up to 0.0015% (15 ppm). [12] The most recent opinion on Methylisothiazolinone by the European Union's Scientific Committee on Consumer Safety (SCCS) has found that in leave-on cosmetic products (including "wet wipes"), no safe concentration has been adequately demonstrated for induction or elicitation of contact allergy.[13] In rinse-off cosmetic products, the SCCS has concluded that concentrations up to 0.0015% (15 ppm) Methylisothiazolinone are safe, in terms of induction of contact allergy, but recognized that there is no information available to evaluate the potential for this ingredient to elicit contact allergy. Furthermore, the SCCS states that Methylisothiazolinone should not be added to cosmetic products that already contains MCI/MI.

Non-Cosmetic

The uses of Methylisothiazolinone in paints and other non-cosmetic products were described in the original safety assessment.[2,3]

There is the potential for residential and occupational exposure when Methylisothiazolinone is used to preserve materials such as paints, cleaners and plastics. In April 2020, the US Environmental Protection Agency (EPA) released a draft risk assessment for MCI/MI.[14] Included were data and analyses of residential and occupational handler risks to inhalation of spray products containing Methylisothiazolinone and Methylisothiazolinone-preserved paints. Inhalation risks to these two groups were assessed using the Methylisothiazolinone maximum application rate of 400 ppm by weight. The human equivalent concentrations (HECs) for MCI/MI, derived from a no-observed-adverse-effect-concentration (NOAEC) of 0.34 mg/m^3 (inhalation) in rats, are calculated to be 0.11 and 0.038 mg/m^3, based upon an 8-h and 24-h time weighted average (TWA) exposure period, respectively. The inhalation margins of exposure (MOEs) for residential Methylisothiazolinone aerosol and vapor exposures range from 1.0 to 14,000, and the inhalation MOEs for occupational Methylisothiazolinone aerosol and vapor exposures range from 0.5 to 5800. Toxicological concern was noted when these values were less than the level of concern (LOC) of 10. Scenarios for residential handlers applying paint and occupational inhalation of paint vapors assuming long exposure durations had MOEs that had LOC below 10. Analyses of paint exposure

are not relevant to the assessment of cosmetic safety due to the exposure durations and concentrations of application being magnitudes greater than those of cosmetic use.

The EPA also assessed incidental oral and dermal post-application exposure for Methylisothiazolinone in textile and household cleaning products.[14] The induction point of departure (POD) for Methylisothiazolinone is based on the dermal sensitization induction threshold of 210 μg/cm^2, while the elicitation POD is 0.0105 μg/cm^2. In textile and household cleaning products, the chronic total dietary exposures do not show any risks; however, the dermal MOEs for elicitation are all of concern. As mentioned above, these analyses of exposures to textile and household cleaning products are not considered relevant to the assessment of cosmetic safety.

TOXICOKINETIC STUDIES

Absorption, Distribution, Metabolism, and Excretion (ADME)

The percutaneous absorption of [^{14}C]Methylisothiazolinone (99.88% radiochemical purity) was determined using rat skin mounted on diffusion cells.[3] Over a 24-h period, the rate of absorption was 0.0059, 0.0277, and 0.0841 μg equivalents/cm^2/h for 25, 75, and 150 ppm dose groups, respectively, and the mean amount of total applied radioactivity absorbed was 21.4%, 33.7%, and 51.2% for 25, 75, and 150 ppm dose groups, respectively. The total dose absorbed of aqueous solutions containing radiolabeled Methylisothiazolinone (96.90% radiochemical purity) in human epidermis was 29.8, 38.0, and 54.7% for 52.2, 104.3, and 313 μg Methylisothiazolinone/ml dose groups. The rate of absorption was 0.037 μg/cm^2/h over a 24-h exposure. In the same study, the total dose absorbed from shampoo, body lotion, and facial cream formulations containing 100 μg Methylisothiazolinone/ml was 29.5%, 8.98%, and 19.6%, respectively. The rates for absorption of Methylisothiazolinone in the formulations over a 24-h exposure ranged from 0.007 to 0.026 μg/cm^2/h. After oral dosing of 100 mg/kg radiolabeled Methylisothiazolinone (96.70% radio purity) in mice, total radioactive residues (TRR) were highest in the liver and lowest in the bone 1 h post-dosing. At 24 h post-dosing, TRR declined significantly in all tissues and the tissue-to-plasma ratio showed that the radiolabel partitioned preferentially from plasma to tissues. Blood had the highest tissue-to-plasma ratio at 48 h. TRR was higher in male tissues than female tissues overall. Most radiolabeled metabolites of Methylisothiazolinone (99.08% radio purity) were excreted in urine and feces by rats within 24 h of oral dosing. Tissue sampling at 96 h post-dosing found 1.9 - 3.6% of the radiolabel, mainly in blood. Total mean recovery of the radiolabel was 92 - 96%. Major metabolites in urine were N-methyl malonamic acid (NMMA), 3-mercapturic acid conjugate of 3-thiomethyl-N-methyl-propionamide, and N-methyl-3-hydroxyl-propamide. Another metabolism study of radiolabeled Methylisothiazolinone (96.90% radio purity) conducted on bile duct-cannulated rats had an 88% recovery of the dose at 24 h post oral dosing. The majority of the radiolabel was found in bile, urine, and feces. No intact Methylisothiazolinone was recovered and the main metabolites were NMMA and 3-mercapturic acid conjugate of 3-thiomethyl-N-methyl-propionamide.

TOXICOLOGICAL STUDIES

Acute Toxicity

Methylisothiazolinone at 97.5% was slightly toxic in rats in an acute dermal toxicity study.[3] The substance was corrosive to the skin. The LD_{50} was calculated to be 242 mg/kg body weight. In another acute dermal toxicity study, 9.69% Methylisothiazolinone was corrosive to rat skin, but no deaths occurred during the study. The LD_{50} was greater than 484.5 mg/kg body weight.

In acute oral toxicity studies, Methylisothiazolinone was slightly toxic in rats in concentrations ranging from 9.69% to 99.7%.[3] At 9.69%, the LD_{50}s for male and female rats were 274.6 and 105.7 mg/kg body weight, respectively. Rats that died during these studies had reddened intestines and/or stomach mucosa, clear or red/yellow fluid in the intestines and/or stomach; blackened intestines and distended stomachs. Studies in rats on body lotion, shampoo, and sunscreen formulations containing 100 ppm Methylisothiazolinone found no treatment-related effects and an LD_{50} greater than 2000 mg formulation/kg body weight. Slight toxicity, including gastrointestinal changes, was observed in mice that orally received 97.5% Methylisothiazolinone. The LD_{50} was 167 mg/kg body weight. An acute oral toxicity study of the metabolite NMMA in rats found the substance slightly toxic. The calculated oral LD_{50}s for NMMA in males and females were 3550 and 4100 mg/kg body weight, respectively.

Acute inhalation toxicity studies in rats found that 53.52% and 97.8% Methylisothiazolinone were slightly toxic after 4 h exposures.[3] The LC_{50}s were 0.35 and 0.11 mg/l, respectively. Rats that died during these studies had reddened lungs and distended gastrointestinal tracts. Mice exposed to 10 minutes of atomized 98.6% Methylisothiazolinone had up to 47% decrease in respiratory rates that equated to moderate responses for sensory irritation.[3]

Acute toxicity studies are summarized in Table 2. In a dermal study in rats, the LD_{50} for 49.0% Methylisothiazolinone was greater than 2000 mg/kg bw.[5] In oral studies, the LD_{50} for a 1% solution of Methylisothiazolinone in rats was 148.0 mg/kg, while the LD_{50} for a 50% solution of Methylisothiazolinone in rats was 232 - 249 mg/kg in males and 120 mg/kg in females. The LC_{50} of aerosolized 49.8% Methylisothiazolinone in rats was 0.422 mg/l in males and 0.354 mg/l in females.

Short-Term Toxicity Studies

Oral

In a 28-d oral toxicity study performed in accordance with Organization for Economic Co-operation and Development (OECD) test guideline (TG) 407, groups of 5 male and 5 female Wistar rats received 0, 10.03, 28.59, or 71.21 mg/kg bw Methylisothiazolinone in water daily via gavage.[5] The study included high-dose and control recovery groups that were observed for an additional 14 d following completion of the dosing period. Terminal studies included measuring organ weight and relative organ weight, and performing gross pathological and histopathological assessments. The number of mortalities were not reported. In males, the absolute and relative weights of the prostate in the low and high dose group, and the heart in the mid dose group were significantly reduced when compared to the control group. However, no lesions were found in the prostate. Absolute weight of the testes and epididymides was significantly less ($p < 0.05$) in the high dose recovery group when compared to the control recovery group; however, the relative weight of these organs was comparable to the control recovery group. Relative weight of the liver in the mid and high dose groups was significantly increased as compared to the control group; however, there was no significant variation in the high dose recovery group and no treatment-related lesions were observed in the liver. In females, the absolute weights of the organs in the treated animals were comparable to the controls, but there were statistically significant increases in relative weight of the kidneys in the low and mid dose groups. These observations were considered incidental as thee high dose group and high dose recovery group were comparable to the control groups. While pathological and histopathological changes were observed, the study summary did not detail the differences between the control and dose groups. The no-observed-adverse-effect level (NOAEL) was 28.6 mg/kg bw/d in males and females based on the combined assessment of clinical signs, mortalities, and pathological and histopathological findings; the lowest-observed-adverse-effect level (LOAEL) was 71.2 mg/kg bw/d in males and females was based on lethargy and mortality. No further details were provided.

Subchronic Toxicity Studies

Oral

No toxic effects were observed when 97.5% Methylisothiazolinone was administered to rats in drinking water for 13 wk at concentrations of 0, 75, 250, or 1000 ppm.[3] Dogs that were fed diets prepared with 51.4% Methylisothiazolinone for 3 mos had a NOAEL of 1500 ppm. In a subchronic study, rats fed the metabolites NMMA and malonic acid (MA), up to 220 ppm and 44 ppm in the diet, respectively for 3 mos had no effects observed in body weight, food consumption, hematology, clinical chemistry, urinalysis, ophthalmology, or gross pathologic changes. Beagle dogs that received up to 500 ppm NMMA and 100 ppm MA in their diets for 3 mos had no systemic toxicity.

In a 90-d oral toxicity study performed in accordance with OECD TG 408, groups of 10 male and 10 female Wistar rats received 0, 7.52, 15.05, or 30.09 mg/kg bw Methylisothiazolinone in water daily via gavage.[5] The study included high-dose and control recovery groups that were observed for an additional 28 d following completion of the dosing period. The animals were observed for mortalities, clinical signs of toxicity, ophthalmological changes, and feed consumption. Hematology values and clinical chemistry measurements were taken. Sperm were analyzed for motility, number, and morphology (results reported in the section below). Terminal studies included measuring organ weight and relative organ weight, and performing gross pathological and histopathological assessments. No treatment-related mortalities, clinical signs of toxicity, ophthalmological changes, or changes in feed consumption were observed. There were no significant treatment-related changes in hematological values or clinical chemistry. No significant adverse effects were reported in terminal studies. The NOAEL was 30.09 mg/kg bw/d in males and females based on no treatment-related mortality or clinical signs of toxicity.

Inhalation

While there are no published inhalation data on Methylisothiazolinone, a 13-wk repeated-dose inhalation study on MCI/MI was performed in accordance with OECD TG 413.[15] Groups of 16 Crl:CD(SD)BR rats per sex were exposed to 14% MCI/MI (11% MCI/3% MI). The rats were exposed whole body for 6 h/d, 5 d/wk, at aerosol concentrations of 0, 0.34, 1.15, or 2.64 mg active ingredient (a.i.)/m^3, with an aerosol particle size of 1.1 to 1.4 µm (mean mass median diameter (MMAD), which is defined as the diameter at which 50% of the particles by mass are larger and 50% are smaller). During the exposure period, the rats were observed for clinical signs of toxicity, and body weight and ophthalmologic evaluations were made. At study termination, hematology, clinical chemistry, gross pathology, and histopathologic evaluations were conducted. No statistically significant effects were observed in the hematology, gross pathology, or ophthalmologic evaluations at any concentration. At 2.64 mg/m^3, rats of both sexes had signs consistent with exposure to a sensory irritant, including chromorhinorrhea, rhinorrhea, eye squint, bradypnea, and dyspnea. Decreased body weight gains, decreased male spleen weights, and decreased serum protein in females were also observed in rats exposed to 2.64 mg/m^3. No treatment-related clinical signs of toxicity, body weight effects, or organ weight effects were observed in the 0.34 or 1.15 mg/m^3 dose groups. Treatment-related histopathologic findings consisting of slight to moderate incidences of eosinophilic droplets in the anterior respiratory mucosa of the nasal turbinates and slight rhinitis in the lining of the anterior portion of the nasal cavity were observed in the 2.64 mg/m^3 dose group. At 1.15 mg/m^3, rhinitis was observed in rats of both sexes. No treatment-related histopathologic effects were observed in the 0.34 mg/m^3 dose group. All histopathologic changes were minor,

potentially reversible, and generally reflective of minimal tissue response to a very mild, low-grade respiratory irritant. Based on the occurrence of rhinitis, the LOEL was 1.15 mg/m^3 a.i. The NOAEC was 0.34 mg/m^3 a.i.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

In a teratogenicity study, Methylisothiazolinone was administered by daily single oral doses to pregnant rats at doses of 5, 20, or 60 (reduced to 40) mg/kg body weight/d on gestation days 6 - 19. Females in the high dose group had clinical signs of rales, gasping, and labored breathing and at necropsy had red areas in the glandular portion of the stomach and lungs. No treatment-related effects were observed in the fetuses. The maternal and developmental NOAELs were 20 mg/kg/d and 40 mg/kg/d, respectively. In a teratogenicity study of Methylisothiazolinone in rabbits, pregnant females received daily single oral doses of 3, 10, or 30 mg/kg/d Methylisothiazolinone on gestation days 6 - 28. Maternal effects in the 30 mg/kg/d group included decreased defecation and dark red areas in the stomach. The maternal NOAEL was 10 mg/kg/d. No treatment-related effects were observed in the fetuses and the developmental NOAEL was determined to be 30 mg/kg/d. A 2-generation reproduction toxicity test found that Methylisothiazolinone in drinking water at concentrations up to 1000 ppm was not a reproductive toxicant.[3]

In the 90-d oral toxicity study described above, no adverse effects were observed on the male rat reproductive system after Wistar rats received up to 30.09 mg/kg bw Methylisothiazolinone in water.[5]

The teratogenic potential of 49.8% Methylisothiazolinone was studied in Wistar rats in accordance with OECD TG 414.[5] Groups of 25 pregnant rats received 33.4, 49.8, or 74.7 mg/kg of the test material in water via gavage once daily on days 6 through 15 of gestation. Slight maternal toxic effects, including depressed body weight gains and feed consumption, were observed at 49.8 mg/kg and 74.7 mg/kg. A significant increase in the number of visceral anomalies were observed at 74.7 mg/kg, which were likely due to maternal toxicity. No teratogenic effects on fetuses attributed to the test material could be verified. The NOAEL and LOAEL for maternal toxicity were 33.4 mg/kg bw/d and 49.8 mg/kg bw/d, respectively; the NOAEL and LOAEL for embryotoxicity were 49.8 mg/kg bw/d and 74.7 mg/kg bw/d, respectively.

GENOTOXICITY

Methylisothiazolinone (up to 1000 µg/plate) and the metabolite NMMA (up to 5000 µg/plate) were not mutagenic in the Ames test when tested with and without metabolic activation. In a Chinese hamster ovary (CHO) cell assay, 97.5% pure Methylisothiazolinone was non-mutagenic when tested with and without metabolic activation (0.5 - 40.0 µg/ml). However, another CHO assay that studied Methylisothiazolinone at 97.5% active ingredient (0.0785 - 5000 µg/ml) found significant increases in cells with chromosome aberrations, with and without metabolic activation. The aberrations were accompanied by significant cytotoxicity, which may have caused a false positive in this assay. Methylisothiazolinone was non-mutagenic in an unscheduled DNA synthesis assay and in a micronucleus test.[3]

Genotoxicity studies are summarized in Table 3. Methylisothiazolinone (49.0% - 49.8%) was not mutagenic in an Ames study, chromosome aberration study, or in a mammalian cell gene mutation assay, nor was it mutagenic in an in vivo micronucleus assay in mice.[5]

CARCINOGENICITY

Studies of the carcinogenicity of the sole ingredient Methylisothiazolinone were not available; however, a 2-yr drinking water study in rats concluded that the mixture MCI/MI tested up to 300 ppm was not a carcinogen.[3]

OTHER RELEVANT STUDIES

Neurotoxicity

An acute in vitro neurotoxicity study of Methylisothiazolinone (up to 300 µM) in embryonic rat cortical neurons and glia observed widespread neuronal cell death within 24 h in the cortical cultures. Gliotoxicity was low. A 14-h in vitro neurotoxicity study of Methylisothiazolinone (up to 3.0 µM) from the same laboratory concluded that prolonged exposure to Methylisothiazolinone and related isothiazolinones may damage developing nervous systems. However, no evidence of neurotoxicity has been observed in vivo.[3]

DERMAL IRRITATION AND SENSITIZATION

In EpiDerm™ skin constructs, 1.7% Methylisothiazolinone applied for 3 or 60 min was non-corrosive.[3] *In the same study, 51.5% Methylisothiazolinone was non-corrosive in the 3-minute exposure but corrosive at the 60-min exposure. Undiluted 97.8% Methylisothiazolinone was corrosive to intact rabbit skin after an exposure period of 1 h. Rabbit dermal irritation studies of Methylisothiazolinone at 9.69% and 10% concluded the chemical was non-irritating. A single 24-h application of 100 ppm Methylisothiazolinone in 40 volunteer subjects did not produce skin irritation. Respective skin irritation studies in body lotion, shampoo, and sunscreen formulations containing 100 ppm Methylisothiazolinone also found Methylisothiazolinone to be nonirritating.*

In a guinea pig maximization test, 0.076% w/v Methylisothiazolinone was a weak sensitizer and a follow-up study found that 0.015% Methylisothiazolinone produced no sensitization. [3] *An investigation using the Buehler method found that 99.8% Methylisothiazolinone was a sensitizer at concentrations ≥ 1000 ppm. Another maximization test that evaluated the sensitization potential of 99.7% Methylisothiazolinone concluded that the chemical was not a sensitizer at concentrations up to 800 ppm. Methylisothiazolinone was a sensitizer at concentrations ≥ 1.5% in an open epicutaneous test. Results from a local lymph node assay (LLNA) indicated that 99.8% Methylisothiazolinone produced sensitization at > 10,000 ppm. In one LLNA, the effective concentration inducing a stimulation index (SI) of 3 (EC_3) for Methylisothiazolinone was calculated to be 25,150 ppm. In another LLNA, the calculated EC_3 was 0.86% (8600 ppm). In a study using both the LLNA and cytokine profiling to assess Methylisothiazolinone, the EC_3 for Methylisothiazolinone diluted in acetone/olive oil was 0.4% (4000 ppm), and it was 2.2% (22,000 ppm) when diluted in propylene glycol (a moderate skin allergen); however, the cytokine profile of 0.5% Methylisothiazolinone in acetone/olive oil was not typical for respiratory allergens, and the authors concluded that Methylisothiazolinone was not likely to cause sensitization of the respiratory tract. The metabolite NMMA did not induce hypersensitivity in a LLNA up to and including 30% concentration*.

A re-evaluation of the LLNA results reported in the published literature in an editorial article indicates that Methylisothiazolinone should be categorized as a strong sensitizer, and not a moderate sensitizer as previously reported.[2]

In a cumulative irritation/ sensitization study of Methylisothiazolinone in 80 subjects, the sensitization threshold was determined to be at or around 1000 ppm.[3] *A human repeated insult patch test (HRIPT) in 98 subjects tested with 100 ppm Methylisothiazolinone concluded that Methylisothiazolinone did not induce skin sensitization in humans. A series of HRIPTs evaluating the sensitization of 50% Methylisothiazolinone at concentrations of 200, 300, 400, 500, or 600 ppm concluded that Methylisothiazolinone up to 600 ppm was not a dermal sensitizer.*

In sensitization studies conducted in 11 Methylisothiazolinone-allergic patients, the lowest eliciting dose in a patch test was 1.47 µg Methylisothiazolinone/cm^2 (49 ppm). No reactions were observed at 0.441 µg Methylisothiazolinone/cm^2 (15 ppm) or lower, nor were there any reactions in the controls. In a HRIPT of 100 ppm Methylisothiazolinone, with or without various glycols, no evidence of induced allergic contact dermatitis was observed in any of the subjects.[2]

Dermal irritation and sensitization studies are summarized in Table 4. In a rabbit irritation study, 49.0% Methylisothiazolinone in water was corrosive.[5] Methylisothiazolinone was sensitizing in a guinea pig maximization test and in a local lymph node assay (LLNA) when tested at up to 10.0%; however, it was not a sensitizer in another LLNA at up to 4.5%. In human sensitization studies, dose-dependent sensitization was observed to Methylisothiazolinone at up to 2500 ppm in a cumulative irritation study and human repeated insult patch tests (HRIPTs).

Phototoxicity

Methylisothiazolinone at 100 ppm was not phototoxic or photosensitizing in guinea pig studies. No phototoxic effects were observed in a study of 200 ppm Methylisothiazolinone in 12 female subjects.[3] *A photosensitization study of 200 ppm Methylisothiazolinone in 32 subjects did not produce photoallergic reactions.*

OCULAR IRRITATION STUDIES

A bovine cornea study classified Methylisothiazolinone (neat) as mildly irritating. Ocular irritation studies in body lotion, shampoo, and sunscreen formulations containing 100 ppm Methylisothiazolinone found the formulations non-irritating in rabbit eyes.[3]

Human

In an ocular irritation study, 12 human subjects received 100 ppm Methylisothiazolinone in buffered physiological saline as a single 10 µl drop in the eye on 5 consecutive days.[5] An ophthalmologist performed eye examinations and the subjects subjectively rated the irritation. Mild pink in the bulbar and palpebral conjunctiva and slight lacrimation were noted 30-60 seconds after instillation of the test material, but not after 60 min and the results were comparable to the control subjects. No more than slight/mild stinging/burning/pain were reported for both the test material and the control. Three adverse events were reported by 2 subjects: one subject reported mild bilateral ocular discharge and stinging, which were possibly related to the test material, and the other subject reported mild bilateral ocular discharge which was unlikely related to the test material. The test material was considered safe and well tolerated in this study.

CLINICAL STUDIES

Retrospective and Multicenter Studies

In a clinical study of 22 patients tested with fractions isolated from a tradename mixture of MCI/MI, only 2 patients had positive reactions to Methylisothiazolinone.[3] *Sensitization may have been due to cross-reactions to MCI. Methylisothiazolinone was determined to be a weak sensitizer in a study of 12 patients. Eighty-five patients with pre-determined sensitization to MCI/MI were tested epicutaneously to 500 or 1000 ppm Methylisothiazolinone. The results show that at high concentrations of Methylisothiazolinone (500 to 1000 ppm), 32% of the subjects with known sensitivity to*

MCI/MI reacted to Methylisothiazolinone. In a repeat open application test (ROAT), 7 patients (64%) reacted to 0.105 and 0.21 µg Methylisothiazolinone/cm^2 and 2 patients (18%) reacted to 0.0105 µg Methylisothiazolinone/cm^2.

Incidences of contact allergy to Methylisothiazolinone, tested separately from MCI/MI, appear to be increasing in Europe since the start of the use of Methylisothiazolinone as a stand-alone ingredient.[2]

Methylisothiazolinone was named Allergen of the Year for 2013 by the American Contact Dermatitis Society due to the rise of use of the preservative and the increased incidences of contact allergy being reported, especially in the European Union.[2] *A standard series of patch testing includes the mixture MCI/MI, which may miss 40% of contact allergy to Methylisothiazolinone alone due to the relatively low concentration of Methylisothiazolinone in the mixture. Recommendations have been made to test for Methylisothiazolinone contact allergy separate from the MCI/MI, although there currently is no consensus of about the concentration of Methylisothiazolinone that should be tested.*

A selection of the numerous baseline and retrospective studies on Methylisothiazolinone that have become available in the published since 2014 are summarized in Table 5. These studies show that sensitization to Methylisothiazolinone is still found world-wide.[16-26] In a study from 14 centers in 11 European countries, the prevalence of contact allergy to Methylisothiazolinone decreased by 50% from 2015 to 2027.[26] Of note, the share of cosmetic products (leave-on in particular) eliciting allergic contact dermatitis is decreasing.

Case Studies

Three cases of allergic contact dermatitis were reported in patients that had come into contact with coolant solutions containing biocides.[3] *Patch testing in 2 of the patients revealed ++ and +++ reactions to Methylisothiazolinone, respectively. An investigator in this study developed eczematous dermatitis while isolating coolant components and had a ++ reaction to Methylisothiazolinone during patch testing. Another case study reported hand eczema in a diesel mechanic that was exacerbated with the use of moist toilet paper. The diesel oil and the toilet paper the man came in contact with both contained tradename mixtures of MCI/MI biocides. Positive reactions to Methylisothiazolinone were observed with patch testing. Two cases of occupational contact allergy and dermatitis were reported in patients exposed to compounds containing the biocide Methylisothiazolinone. Patch testing revealed +++ reactions to Methylisothiazolinone. Four out of 14 workers at a Danish paint factory were observed with contact dermatitis after exposure to paint additives containing 7-10% Methylisothiazolinone. Positive reactions were observed in all 4 patients during patch testing. Numerous other reports of contact allergy, particularly to toilet wipes and water-based wall paint containing Methylisothiazolinone, have been reported.*

A sampling of case studies that report adverse effects to Methylisothiazolinone from various exposures is summarized in Table 6. Cases include reports of Methylisothiazolinone sensitization from a wide range of materials, including personal care products, paints, photographic processing agents, glues, eye glass frames, and cleaners.[27-36]

QUANTITATIVE RISK ASSESSMENT

Cosmetics Europe and the CIR Science and Support Committee (SCC) conducted QRAs of Methylisothiazolinone in response to the increased incidences of contact sensitization to Methylisothiazolinone in Europe.[2] *The QRA, which used a conservative no expected sensitization induction level (NESIL) of 15 µg/cm^2/d that was derived based on a weight of evidence (WoE) evaluation of data from 5 HRIPTs and 4 LLNAs, predicted that consumer exposures to 100 ppm Methylisothiazolinone in skin leave-on products and cosmetic wet wipes could induce skin sensitization, while exposures to the same concentration in rinse-off products and hair care leave-on products would not induce skin sensitization.*

SUMMARY

In 2019, the Panel published an amended safety assessment of the preservative Methylisothiazolinone with the conclusion that this ingredient "is safe for use in rinse-off cosmetic products at concentrations up to 100 ppm and safe in leave-on cosmetic products when they are formulated to be non-sensitizing, which may be determined based on a QRA." This conclusion superseded the findings of the Panel's earlier safety assessment that was published in 2010. At the September 2019 Panel meeting during the re-evaluation of the mixture MCI/MI, the Panel reopened the amended safety assessment of Methylisothiazolinone to consider additional newly available data, with particular regard to inhalation toxicity.

According to 2019 VCRP survey data, Methylisothiazolinone (when not used with MCI) is reported to be used in a total of 915 formulations; the majority of the uses are in bath soaps and detergents. Use of Methylisothiazolinone (without MCI) has increased since 2014, where 745 uses were reported; the majority of the uses reported then were in non-coloring hair conditioners and shampoos. The maximum concentrations of use for Methylisothiazolinone in 2020 is reported to range from 0.000002% to 0.00975%, with 0.00975% reported in hair conditioners and 0.009% used in leave-on hair products. In the amended safety assessment published in 2019, the maximum concentration of use range was reported to be 3.5 x 10^{-8}% to 0.01%, with 0.01% reported in multiple product categories including eye makeup remover, hair shampoos and conditioners, and skin care products (both leave-on and rinse-off).

The US EPA has released a draft risk assessment for MCI/MI that included analysis of residential and occupational handler risks to inhalation of spray products containing Methylisothiazolinone and Methylisothiazolinone-preserved paints. The inhalation MOEs for residential aerosol exposures ranged from 15 to 14,000, and were not of toxicological concern because the values were greater than the LOC of 10. The MOEs for occupational aerosol exposures ranged from 4.4 to 5800; certain exposure scenarios were of toxicological concern when the LOC was below the value of 10. Scenarios for residential handlers applying paint and occupational inhalation of paint vapors assuming long exposure durations had MOEs that had LOC below 10. The US EPA also assessed incidental oral and dermal exposure in textile and household cleaning products and found that exposures across routes are not aggregated. These analyses of exposures to paints and textile and household cleaning products are not considered relevant to the assessment of cosmetic safety.

In a dermal study in rats, the LD_{50} for 49.0% Methylisothiazolinone was greater than 2000 mg/kg bw. In oral studies, the LD_{50} for a 1% solution of Methylisothiazolinone in rats was 148.0 mg/kg, while the LD_{50} for a 50% solution of Methylisothiazolinone in rats was 232 - 249 mg/kg in males and 120 mg/kg in females. The LC_{50} of aerosolized 49.8% Methylisothiazolinone in rats was 0.422 mg/l in males and 0.354 mg/l in females.

In a 28-d oral toxicity study in rats tested with 0, 10.0, 28.6, or 71.2 mg/kg bw Methylisothiazolinone, the NOAEL was 28.6 mg/kg bw/d and the LOAEL was 71.2 mg/kg bw/d based on lethargy and mortality. When Methylisothiazolinone was tested at up to 30.09 mg/kg bw in a 90-d oral toxicity study in rats, the NOAEL was 30.09 mg/kg/d based on no treatment-related mortality or clinical signs of toxicity.

In the 90-d oral toxicity study, no adverse effects were observed on the male rat reproductive system after rats received up to 30.09 mg/kg bw Methylisothiazolinone in water. In a study that investigated the teratogenic potential of 49.8% Methylisothiazolinone in rats, no teratogenic effects on fetuses attributed to the test material could be verified. The NOAEL and LOAEL for maternal toxicity were 33.4 mg/kg bw/d and 49.8 mg/kg bw/d, respectively; the NOAEL and LOAEL for embryotoxicity were 49.8 mg/kg bw/d and 74.7 mg/kg bw/d, respectively. In a 13-wk inhalation study of 14% MCI/MI in rats that followed OECD TG 413, MCI/MI was tested at up to 2.64 mg a.i./m^3. Based on the occurrence of rhinitis, the LOEL was 1.15 mg/m^3. The NOEL was 0.34 mg/m^3.

Methylisothiazolinone (49.0% - 49.8%) was not mutagenic in an Ames study, chromosome aberration study, or in a mammalian cell gene mutation assay. Additionally, it was not mutagenic in an in vivo micronucleus assay in mice.

In a rabbit irritation study, 49.0% Methylisothiazolinone in water was corrosive. Methylisothiazolinone was sensitizing in a guinea pig maximization test and in an LLNA when tested at up to 10.0%; however, it was not a sensitizer in another LLNA at up to 4.5%. In human sensitization studies, dose-dependent sensitization was observed to Methylisothiazolinone at up to 2500 ppm in a cumulative irritation study and HRIPTs. Methylisothiazolinone (100 ppm in saline) was considered safe and well tolerated in an ocular irritation study of human subjects.

A sampling of the numerous baseline and retrospective studies on Methylisothiazolinone that have become available in the published literature since 2014 indicate that sensitization to Methylisothiazolinone is still found world-wide. A selection of case studies that report adverse effects to Methylisothiazolinone from various exposures included reports of Methylisothiazolinone sensitization from a wide range of materials, including personal care products, paints, photographic processing agents, glues, eye glass frames, and cleaners. In a study from 14 centers in 11 European countries, the prevalence of contact allergy to Methylisothiazolinone decreased by 50% from 2015 to 2027. Of note, the share of cosmetic products (leave-on in particular) eliciting allergic contact dermatitis is decreasing.

DISCUSSION

This safety assessment is on the preservative Methylisothiazolinone as used in cosmetics. In response to concerns of reports of adverse events observed in infants following inhalation exposure to humidifier disinfectants that contained the preservative mixture Methylchloroisothiazolinone/Methylisothiazolinone (MCI/MI), the Panel moved to reopen the safety assessment of Methylisothiazolinone in September 2019. A search of inhalation toxicity data for Methylisothiazolinone (separate from the combination of MCI/MI) did not yield any additional inhalation data; however, studies were detailed in the MCI/MI report. The Panel reviewed a 13-wk repeated-dose inhalation study of MCI/MI in rats and determined that the data mitigated concern for the use of Methylisothiazolinone at the reported concentrations in cosmetic products that could be incidentally inhaled following cosmetic use. The Panel also reviewed a draft risk assessment for MCI/MI produced by the US EPA and determined that the analyses of exposures to paints, textile, and household cleaning products were not relevant to the assessment of cosmetic safety due to exposure duration and concentrations of application being magnitudes greater than those of cosmetic use.

As discussed in the previous report on Methylisothiazolinone, the Panel reviewed the results of QRAs performed by Cosmetics Europe and the CIR Science and Support Committee. Those results supported the safety of the use of Methylisothiazolinone in rinse-off product categories at concentrations up to 100 ppm. However, the QRA indicated that Methylisothiazolinone use in several leave-on product categories, such as wet wipes, would be safe only at concentrations lower than 100 ppm. Using the QRA results, the Panel reaffirmed the limitation of 100 ppm Methylisothiazolinone in rinse-off products. However, they also determined that the original limitation for leave-on products needed to be modified, and

that leave-on cosmetic products should be formulated to contain Methylisothiazolinone at concentrations below 100 ppm and to be non-sensitizing, as demonstrated, for example, by QRA estimates of safe exposures (typically expressed in $\mu g/cm^2/d$) for the relevant cosmetic product category.

The Panel's recommendations for Methylisothiazolinone in rinse-off and leave-on cosmetic products are intended to prevent the induction of sensitization to Methylisothiazolinone. However, the Panel cautioned that following these recommendations may not necessarily prevent the elicitation of allergic reactions in individuals who are already allergic to Methylisothiazolinone. Individuals sensitized to Methylisothiazolinone should avoid products that contain Methylisothiazolinone.

The Panel discussed the issue of incidental inhalation exposure from hair sprays and fragrance preparations. The limited data available from inhalation studies, including acute exposure data on Methylisothiazolinone and subchronic exposure data on MCI/MI, suggest little potential for respiratory effects at relevant doses. Methylisothiazolinone is reportedly used at concentrations up to 0.00095% in cosmetic products that may be aerosolized. The Panel noted that 95% – 99% of droplets/particles would not be respirable to any appreciable amount. Coupled with the small actual exposures expected in the breathing zone and the absence of significant signs of toxicity in acute, short-term, subchronic, chronic, reproductive and developmental animal studies, and genotoxicity studies reviewed by the Panel, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that Methylisothiazolinone is safe for use in rinse-off cosmetic products at concentrations up to 100 ppm (i.e. 0.01%) and safe in leave-on cosmetic products when they are formulated to be non-sensitizing, which may be determined based on a QRA or similar methodology.

TABLES

Table 1. Frequency and concentration of use according to duration and type of exposure for Methylisothiazolinone.

	# of Uses	*Max Conc of Use (ppm)*	*# of Uses*	*Max Conc of Use (%)*
	2019[6]	**2020[7]**	**2014[2]**	
Totals†	**915**	**0.02-97.5**	**745**	**0.000000035-0.01**
Duration of Use				
Leave-On	559	1.9-90	478	0.000000035-0.01
Rinse Off	345	0.02-97.5	260	0.00000025-0.01
Diluted for (Bath) Use	11	2.3-90	7	0.0002-0.01
Exposure Type				
Eye Area	28	NR	22	0.00019-0.01
Incidental Ingestion	1	NR	1	0.0048
Incidental Inhalation-Spray	3; 278[a]; 168[b]	9.5	3; 268[a]; 114[b]	0.00018-0.01; 0.0002-0.01[a]
Incidental Inhalation-Powder	168[b]	NR	114[b]	NR
Dermal Contact	679	0.02-90	544	0.000000035-0.01
Deodorant (underarm)	NR	NR	NR	0.0095
Hair - Non-Coloring	224	1-97.5	190	0.000004-0.01
Hair-Coloring	NR	0.1-80	NR	0.000056-0.0095
Nail	3	NR	5	0.0002-0.006
Mucous Membrane	124	0.51-90	103	0.0000009-0.01
Baby Products	5	3	6	0.0002-0.0075

NR = Not reported

† Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

[a.] It is possible these products may be sprays, but it is not specified whether the reported uses are sprays.

[b.] Not specified whether a powder or a spray, so this information is captured for both categories of incidental inhalation.

Table 2. Acute toxicity of Methylisothiazolinone

Concentration	Dose	Species/Strain	Method	Results	Reference
		Dermal			
49.0%; no vehicle used	2000 mg/kg bw; no control dose	5 male and 5 female Wistar rats	Acute dermal toxicity study in accordance with OECD TG 402; 24-h patch was occluded	LD_{50} > 2000 mg/kg bw; strong irritation of the treated skin was observed	[5]
		Oral			
50% solution of active ingredient in distilled water	150, 180, 225, or 300 mg active ingredient/kg	CD(BR) rats; 6 males each in 180, 225, and 300 mg/kg dose groups and 6 females each in 150, 180, and 225 mg/kg dose groups (36 rats total)	Animals received test material in a single 10 ml/kg dose via gavage	LD_{50} was 232-249 mg/kg in males and 120 mg/kg in females	[5]
49.0% in water	110.3, 165.6, 247.9, 371.9, or 558.1 mg active ingredient/kg bw	6 male and 6 female Wistar rats per dose group	Acute oral toxicity study in accordance with OECD TG 401 via gavage	LD_{50} was 285.5 mg/kg bw for both sexes	[5]
1% w/v solution in water	100, 126, 160, 200, or 251 mg/kg	3 male and 2 female Sherman-Wistar rats per dose group	Animals received a single dose via gavage	LD_{50} was 148.0 mg/kg for both sexes	[5]
		Inhalation			
49.8%; vehicle not reported	Calculated atmospheric concentrations were 0, 0.127, 0.252, or 0.504 mg active ingredient/l	5 male and 5 female Wistar rats	Acute inhalation toxicity study in accordance with OECD TG 403; animals were exposed nose-only to aerosol for 4 h	LC_{50} was 0.422 mg/l in males, 0.354 mg/l in females	[5]

Table 3. Genotoxicity studies of Methylisothiazolinone

Concentration	Dose	Species/Strain/Cell	Method	Results	Reference
		In Vitro			
49.0% in DMSO	3.9, 11.8, 35.3, 105.8, or 317.5 µg/plate, with and without metabolic activation	*S. typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537	Ames study in accordance with OECD TG 471	Not mutagenic	[5]
49.8%; vehicle not reported	0.0013, 0.0025, or 0.005 mg/ml, with and without metabolic activation	Human lymphocytes	Chromosome aberration study in accordance with OECD TG 473	Not mutagenic	[5]
49.8%; vehicle not reported	0.125-2.490 mg/ml; with and without metabolic activation	Chinese hamster ovary cells	Mammalian cell gene mutation assay in accordance with OECD TG 476	Not mutagenic	[5]
		In Vivo			
49.8% in 0.9% NaCl	0, 49.8, 74.4, 99.6 mg/kg bw	5 male and 5 female NMRI mice per dose group	Micronucleus assay in accordance with OECD TG 474; single oral gavage treatment	Not genotoxic	[5]

Table 4. Irritation and sensitization studies of Methylisothiazolinone

Concentration/Dose/Vehicle	Test System	Method	Results	Reference
		Irritation – Animal		
49.0% in water	3 New Zealand White rabbits; sex not reported	Dermal irritation study in accordance with OECD TG 404; patches were semi-occluded and were of 4 h duration; test material was not diluted	Corrosive; moderate dermal irritation and eschar formation was observed; primary dermal irritation index was 2.9, erythema score was 2, edema score was 1; erythema and edema were not fully reversible within 14 d	[5]
		Sensitization – Animal		
49.0% in water; 1st induction was 0.1%, 2nd induction was 10%, challenge was 1%	Female Dunkin-Hartley guinea pigs; 10 test and 5 control animals	Guinea pig maximization test in accordance with OECD TG 406; challenge patch was occluded	Sensitizing; erythema observed in all treated animals at up to 72 h post-challenge patch, no reactions in control group	[5]
0.75%-4.5% in water	Groups of 5 female CBA/J mice	LLNA in accordance with OECD TG 429; positive control group received 25% α-hexylcinnamalde-hyde in DMSO; negative control was tissue culture water	Not sensitizing; the SI values were less than 3 at all concentrations; controls yielded expected results	[5]
50.5% in ethanol/water (1:1, v/v) tested at 2.5%, 5%, and 10% (w/v)	Groups of 4 female CBA mice	LLNA in accordance with OECD TG 429	Sensitizing; SI values were 1.9, 6.5 and 16.0 at 2.5%, 5.0%, and 10.0%, respectively	[5]
		Sensitization- Human		
51.4% active ingredient tested at 1000, 1500, 2000, or 2500 ppm in water	Groups of 12 male and female subjects; total completed through challenge was 43	Cumulative irritation study for 21 consecutive days except Sundays, total of 18 patches; challenge patches were performed 2 wk after the final irritation patch; 0.2 ml of test material was applied on the back of each subject with occlusive 2 cm^2 patches; SLS was the positive control and distilled water was the negative control	Sensitizing with number of sensitizing reactions increasing with increasing concentration of active ingredient; irritation scores of the test material were below that of the SLS control	[5]
500, 750, 1000, 1500, or 2000 ppm in aqueous solution	115 male and female subjects divided into 5 groups	HRIPT; induction phase consisted of daily patches for 14 d followed by a challenge phase conducted after a 2-wk rest period; 0.15 ml of test material was applied on the back of each subject with occlusive patches; SLS was the positive control, negative control was physiological saline	Minimal sensitization was observed in the 500 ppm dose group, but a clear dose-response relationship was not observed; irritation responses were observed in a dose-dependent manner	[5]
300 ppm active ingredient with 300 ppm propylene glycol in water	98 subjects completed study	HRIPT; 0.2 ml test material was applied on the back of each subject with 2 cm^2 occlusive patches; induction phase consisted of a total of nine-24 h patches for over 3 wk followed by a challenge phase conducted after a 2-wk rest period	Not sensitizing	[5]
400 ppm active ingredient with 400 ppm propylene glycol in water	13 subjects completed study	HRIPT; 0.2ml test material was applied on the back of each subject with 2 cm^2 occlusive patches; induction phase consisted of a total of nine-24 h patches for over 3 wk followed by a challenge phase conducted after a 2-wk rest period	Not sensitizing	[5]
600 ppm active ingredient with 600 ppm propylene glycol in water	108 subjects completed study	HRIPT; 0.2ml test material was applied on the back of each subject with 2 cm^2 occlusive patches; induction phase consisted of a total of nine-24 h patches for over 3 wk followed by a challenge phase conducted after a 2-wk rest period	Not sensitizing	[5]
500.1 ppm active ingredient in water	109 subjects completed study	HRIPT; 0.2ml test material was applied on the back of each subject with 2 cm^2 occlusive patches; induction phase consisted of a total of nine-24 h patches for over 3 wk followed by a challenge phase conducted after a 2-wk rest period	Not sensitizing	[5]

Table 4. Irritation and sensitization studies of Methylisothiazolinone

Concentration/Dose/Vehicle	Test System	Method	Results	Reference
300 ppm active ingredient with 300 ppm propylene glycol in water	98 subjects completed study	HRIPT; 0.2ml test material was applied on the back of each subject with 2 cm^2 occlusive patches; induction phase consisted of a total of nine-24 h patches for over 3 wk followed by a challenge phase conducted after a 2-wk rest period	Not sensitizing	[5]
500 ppm active ingredient with 500 ppm propylene glycol in water	101 subjects completed study	HRIPT; 0.2ml test material was applied on the back of each subject with 2 cm^2 occlusive patches; induction phase consisted of a total of nine-24 h patches for over 3 wk followed by a challenge phase conducted after a 2-wk rest period	Sensitizing	[5]

OECD TG - Organization for Economic Co-operation and Development test guideline
LLNA – local lymph node assay
SI – stimulation index
HRIPT – human repeated insult patch test

Table 5. Baseline and retrospective studies of Methylisothiazolinone

Number of Patients	Clinical Testing Type	Location and Time Span	Results	Reference
79 out of 9037 patients which had allergic reactions to allergens identified with wet wipes	Retrospective review of patients tested with the North American Contact Dermatitis Group coded with wet wipes as source of allergen; 0.2% Methylisothiazolinone aq.	North America; January 1, 2011 to December 31, 2014	Out of the reactions associated with wet wipes, 59% had positive reactions to Methylisothiazolinone	[17]
4857 patients	Patch tested with screening series of 70 allergens, including 0.2% Methylisothiazolinone aq.; patches were Finn chambers	13 centers in North America; January 1, 2013 to December 31, 2014	10.9% (527) patients had positive reaction to Methylisothiazolinone	[16]
1142 patients	Retrospective study of patch test cases of children with known atopic dermatitis	United States; January 1, 2015 to December 31, 2015	3.2% (14/429) patients had positive reactions to Methylisothiazolinone	[22]
2787 patients	Retrospective study of patients tested with allergen including 0.2% Methylisothiazolinone aq.; taches were Finn chambers or Allergeaze test chambers	Australia; January 1, 2011 to December 31, 2017	14.5% (404) patients had positive reactions to Methylisothiazolinone	[21]
139 patients	Retrospective study of patients with Methylisothiazolinone-induced allergic contact dermatitis; European baseline series, targeted complementary series, and personal products used; 200, 500, or 2000 ppm Methylisothiazolinone; patches were IQ chambers	France; January 2010 to December 2015	Relapses observed in 64% of patients and were severe in 18%; rinse-off cosmetics were responsible for 27% of the relapses	[23]
2028 patients	Testing in consecutive dermatitis patients; Methylisothiazolinone tested at 0.2% aq.	Italy; January 2012 to December 2014	5.2% (106) patients had positive reactions to Methylisothiazolinone overall; prevalence of Methylisothiazolinone sensitization increases from 2.3% in 2012 to 6.9% in 2014	[24]
99 patients	Retrospective study of patients that underwent cutaneous allergy testing for perianal and/or genital symptoms; patch testing with British Society for Cutaneous Allergy standard series with additional series in some patients; patches were IQ Ultra chambers or Finn chambers; 0.2% Methylisothiazolinone	Ireland; January 2013 to December 2015	5% (5) patients had positive reactions to Methylisothiazolinone	[25]
264 patients with suspected eyelid allergic contact dermatitis	Prospective study of patients tested with an eyelid series, the European baseline series, the French additional series, and personal products; additional testing with additional series and repeated open application tests were performed if necessary; concentration of Methylisothiazolinone tested not reported	France; September 2014 to August 2016	10.2% (27) patients had positive reactions to Methylisothiazolinone; these results may include reactions to MCI/MI	[19]
798 patients	Testing in consecutive dermatitis patients with diagnosed Methylisothiazolinone contact allergy; Croatian baseline series that included 0.2% Methylisothiazolinone aq. and 0.01% MCI/MI aq.; patches were 8 mm Finn chambers	Croatia; November 2, 2015 to November 3, 2016	13.2% (105) patients had positive reactions to Methylisothiazolinone	[20]
324 patients	Retrospective study of patients tested with European baseline series, including 0.2% Methylisothiazolinone aq.; patches were IQ Ultra changers	Turkey; January 2016 to June 2018	8.02% (26) patients had positive reaction to Methylisothiazolinone	[18]
317 positive patients out of 8157 tested	Cross-sectional survey of patients tested with European baseline series in accordance to guidelines of the European Society of Contact Dermatitis; 0.2% Methylisothiazolinone occluded for 2 days on upper back; results compared with reference year 2015	14 centers in 11 European countries; May 1, 2016 to October 31, 2017	4.72% patients in 2016 and 2.96% patients in 2017 had positive reactions to Methyliso-thiazolinone; 5.97% patients in 2015 had positive reactions; prevalence of contact allergy to Methylisothiazolinone decrease by 50% from 2015 to 2017	[26]

Table 6. Case reports

Suspected Sensitizing Material	Patient(s)	Presentation	Patch Test Results	Reference
Multiple personal care products, and wall paint containing Methylisothiazolinone	51-yr-old atopic woman	Pruritic eczema dermatosis of the face, ears, cheeks, neck, forearms, elbow folds, and back that evolved over a time span of 6 yr	++ reaction to Methylisothiazolinone and + reaction to 2-n-octyl-4-isothazolin-3-one in the European contact allergen series	[27]
Hair care products (gel and conditioner) containing Methylisothiazolinone	60-yr-old man	Allergic contact dermatitis presenting over 3 yr, and involving dorsal hands, forearms, torso, and face	+++ reaction to Methylisothiazolinone and a ++ reaction to MCI/MI in the North American Contract Dermatitis Group standard series and preservatives series	[28]
Photograph developing stabilizing agents containing isothiazolinones including Methylisothiazolinone	61-yr-old man	Itchy erythematous and vesicular lesions presenting for 1 yr on the dorsa of the hands, progressively extending to the neck, neckline and face	+ and ++ reactions to MCI/MI (200 ppm), + and ++ reactions to Methylisothiazolinone (2000 ppm), and + and ++ reactions to octylisothiazolinone (1000 ppm) on Day 2 and Day 3, respectively, when tested with the European baseline, additive series, photographic chemical series, dyes, and personal photographic developing chemicals; patches were IQ Ultra chambers that were occluded for 2 d	[29]
Wall paint containing isothiazolinones	66-yr-old man	Pruritic, erythematous and edematous lesions on the face following sleeping in a freshly painted house; prior to the allergic contact dermatitis, patient was under treatment for plaque psoriasis	Positive reactions were observed on Day 2 and Day 4 to Methylisothiazolinone, MCI/MI in the TRUE Test series and to 2 of the 4 paints that were used in the house, which contained MCI/MI	[30]
Wall paint and façade renders containing isothiazolinones	26-yr-old man	Persistent dry cough and rhinitis, followed a few days later by eczematous eruptions on face, eyelids, chest, nape of neck, and elbow folds	When tested with European baseline series, preservatives series, and occupational products, ++ and +++ reactions were observed on Day 2 and Day 4 to Methylisothiazolinone (2000 ppm aq.), MCI/MI (200 ppm aq.) and indoor façade render ("as-is"); + and ++ reactions were observed to water-based paint ("as-is")	[31]
Eye lash extensions	34-yr-old atopic woman	Immediate pain when product was directly applied; within 12 h, pruritic, edematous, and eczematous rash developed around eyes; after 4 mos, patient still had periorbital eczema	+++ reactions on Day 3 to Methylisothiazolinone at 62, 250, and 2000 ppm as well as to the eyelash products; ++ reaction to Methylisothiazolinone at 125 ppm, and + reaction to Methylisothiazolinone at 31 ppm	[32]
Adhesive labels containing Methylisothiazolinone	28-yr-old woman with history of atopic dermatitis	Hand eczema of 1-yr duration	+ reaction to Methylisothiazolinone (0.2% aq.) on Day 2 and Day 3 when tested with the German baseline series	[33]
Eyeglass frames containing Methylisothiazolinone	48-yr-old man	Eczema on ulnar aspects of both hands on the right lower leg; one month later, severe facial dermatitis	+++ and ++ reactions to Methylisothiazolinone and ++ and ++ reactions to eyeglass frame scrapings on Day 3 and Day 7 following testing with TRUE Test, additional allergen series, and personal products; further testing showed the glass frames contained 11.8 µg/g Methylisothiazolinone	[34]
Facial sponges containing Methylisothiazolinone	38-yr-old woman	Vesicular pulpitis on fingers of both hands; facial dermatitis	++ and + reactions to Methylisothiazolinone (0.02% aq.) in the European baseline series supplemented with baseline allergens; further testing showed the facial sponge contained 387 ppm Methylisothiazolinone	[35]
Household detergent containing Methylisothiazolinone	60-yr old non-atopic woman and a 36-yr-old atopic woman	Eyelid and facial dermatitis	Both patients had had positive reactions to 0.2% Methylisothiazolinone aq. (+ and ++, respectively) and 0.002% MCI/MI aq. (++ each) following patch tests with the Belgian baseline and additional series; patients had used a household detergent containing 200 ppm Methylisothiazolinone to clean eyeglasses	[36]

REFERENCES

1. Nikitakis J, Kowcz A. Web-Based International Cosmetic Ingredient Dictionary and Handbook. http://webdictionary.personalcarecouncil.org/jsp/Home.jsp. Washington, DC: Personal Care Products Council. Last Updated: Accessed: 02/19/2020.

2. Burnett CL, Boyer I, Bergfeld WF, et al. Amended Safety Assessment of Methylisothiazolinone as Used in Cosmetics. *Int J Toxicol.* 2019;38(Suppl 1):70S-84S.

3. Burnett CL, Bergfeld WF, Belsito DV, et al. Final Report of the Safety Assessment of Methylisothiazolinone. *Int J Toxicol.* 2010;29(Suppl 3):187S-213S.

4. Burnett CL, Bergfeld WF, Belsito DV, et al. Amended Safety Assessment of Methylchloroisothiazolinone and Methylisothiazolinone as Used in Cosmetics. Washington, DC2020. (Available from the Cosmetic Ingredient Review.: https://www.cir-safety.org/ingredients.)

5. European Chemicals Agency (ECHA). 2-methyl-2H-isothiazol-3-one. https://echa.europa.eu/registration-dossier/-/registered-dossier/23868/7/1. 2020. Accessed. 01/16/2020.

6. US Food and Drug Administration (FDA) Center for Food Safety & Applied Nutrition (CFSAN). 2019. Voluntary Cosmetic Registration Program (VCRP) - Frequency of Use of Cosmetic Ingredients. College Park, MD Obtained under the Freedom of Information Act from CFSAN; requested as "Use Data Methylisothiazolinone When Used in Cosmetics Without Methylchloroisothiazolinone" October 11, 2019; received November 11, 2019.

7. Personal Care Products Council. 2020. Concentration of Use by FDA Product Category: Methylisothiazolinone (used without Methylchloroisothiazolinone). Unpublished data submitted by Personal Care Products Council

8. Johnsen MA. The Influence of Particle Size. *Spray Technology and Marketing.* 2004;14(11):24-27.

9. Rothe H. Special Aspects of Cosmetic Spray Evalulation. 2011. Unpublished data presented at the 26 September Expert Panel for Cosmetic Ingredient Safety meeting. Washington, D.C.

10. Bremmer HJ, Prud'homme de Lodder LCH, Engelen JGM. Cosmetics Fact Sheet: To assess the risks for the consumer; Updated version for ConsExpo 4. Bilthoven, Netherlands 2006 2006. RIVM 320104001/2006. http://www.rivm.nl/bibliotheek/rapporten/320104001.pdf. Accessed 8/24/2011. Pages 1-77.

11. Rothe H, Fautz R, Gerber E, et al. Special aspects of cosmetic spray safety evaluations: Principles on inhalation risk assessment. *Toxicol Lett.* 2011;205(2):97-104.

12. European Union. Regulation (EC) No. 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products. 2009. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:en:PDF. Accessed 11/9/2017.

13. Scientific Committee on Consumer Safety. Opinion on Methylisothiazolinone (P94) - Submission II, 12 December 2013. 2014. SCCS/1521/13. https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_145.pdf. Accessed 12/18/2019.

14. US Environmental Protection Agency (EPA). Registration Review Draft Risk Assessment for 2-methyl-4-isothiazolin-3-one (MIT) and 5-chloro-2-methyl-4-isothiazolin-3-one (CMIT). Washington, DC April 6, 2020. Pages 94. (Accessed May 27, 2020.)

15. Anonymous. 2019. 13-Week inhalation toxicity study in rats (1984): Study summary. Unpublished data submitted on the mixture MCI/MI by the Personal Care Products Council on November 22, 2019.

16. DeKoven JG, Warshaw EM, Belsito DV, et al. North American Contact Dermatitis Group patch test results 2013-2014. *Dermatitis.* 2017;28(1):33-46.

17. Warshaw EM, Aschenbeck KA, Zug KA, et al. Wet wipe allergens: Retrospective analysis from the North American Contact Dermatitis Group 2011-2014. *Dermatitis.* 2017;28(1):64-69.

18. Salman A. Methylchloroisothiazolinone and methylisothiazolinone contact allergy: A retrospective cohort study from a tertiary dermatology clinic in Turkey. *Contact Dermatitis.* 2019;80(3):193-194.

19. Assier H, Tetart F, Avenel-Audran M, et al. Is a specific eyelid patch test series useful? Results of a French prospective study. *Contact Dermatitis.* 2018;79(3):157-161.

20. Ljubojevic Hadzavdic S, Uter W, Ilijanic Samoscanec M, Johansen JD. Methylisothiazolinone contact allergy in Croatia: Epidemiology and course of disease following patch testing. *Contact Dermatitis.* 2018;79(3):162-167.

21. Flury U, Palmer A, Nixon R. The methylisothiazolinone contact allergy epidemic in Australia. *Contact Dermatitis.* 2018;79(3):189-191.

22. Jacob SE, McGowan M, Silverberg NB, et al. Pediatric contact dermatitis registry data on contact allergy in children with atopic dermatitis. *JAMA Dermatol.* 2017;153(8):765-770.

23. Bouschon P, Waton J, Pereira B, Schmutz J-L, Le Bouedec M-CF, D'Incan M. Methylisothiazolinone allergic contact dermatitis: Assessment of relapses in 139 patients after avoidance advice. *Contact Dermatitis.* 2018;80(5):304-310.

24. Gallo R, Signori A, Gervasio S, Riva S, Parodi A. Methylisothiazolinone contact allergy - are rinse-off cosmetics and household products relevant sources of exposure? *Contact Dermatitis.* 2016;75(5):319-321.

25. Foley CC, White S, Merry S, et al. Understanding the role of cutaneous allergy testing in anogenital dermatoses: A retrospective evaluation of contact senstization in anogenital dermatoses. *Int J Dermatol.* 2019;58(7):806-810.

26. Uter W, Aalto-Korte K, Agner T, et al. The epidemic of methylisothiazolinone contact allergy in Europe: Follow-up on changing exposures. *J Eur Acad Dermatol Venereol.* 2020;34:333-339.

27. Deschamps T, Nosbaum A, Delcroix F, Vocanson M, Berard F, J-F N. Long-lasting allergic contact dermatitis to methylisothiazolinone misdiagnosed as atopic dermatitis. *Eur J Dermatol.* 2019;29(1):100-101.

28. Gilmore R, Shan G, Katta R. Allergic contact dermatitis to methylisothiazolinone in hair care products: Report of a case. *Dermatol Online J.* 2017;23(8):1-4.

29. Mamodaly M, Dereure O, Raison-Peyron N. A new source for isothiazolinone contact allergy in traditional, non-digital photography. *Contact Dermatitis.* 2019;81(1):76-77.

30. Martinez-Mera C, Gonzalez MA, Hospital M, Turrion-Merion L. Isothiazolinones in paint as a cause of airborne contact dermatitis in a patient with psoriasis receiving anti-interleukin-17 therapy. *Contact Dermatitis.* 2019;80(5):328-329.

31. Herry J, Esquirol Y, Giordano-Labadie F. An intriguing occupational atypical dermatitis with respiratory symptoms. *Contact Dermatitis.* 2016;75(5):322-323.

32. Dahlin J, Hindsen M, Persson C, Isaksson M. What lash stylists and dermatologists should know! *Contact Dermatitis.* 2016;75(5):317-319.

33. Bennike NH, Johansen JD, Zachariae C. Please, label the label: A case report of occupational allergic contact dermatitis caused by methylisothiazolinone in adhesive labels. *Contact Dermatitis.* 2016;75(5):314-315.

34. El-Houri RB, Christensen LP, Persson C, Bruze M, Andersen KE. Methylisothiazolinone in a designer spectacle frame - a surprising finding. *Contact Dermatitis.* 2016;75(5):310-312.

35. Madsen JT, Andersen KE, Nielsen DT, Hvid L, El-Houri RB, Christensen LP. Undisclosed presence of methylisothiazolinone in '100% natural' Konjac sponge. *Contact Dermatitis.* 2016;75(5):308-309.

36. Dendooven E, Foubert K, Naessens T, Pieters L, Lambert J, Aerts O. Periocular contact allergy from spectacle frames cleaned with methylisothiazolinone-containing household detergents. *J Eur Acad Dermatol Venereol.* 2019;33:e412-e413.