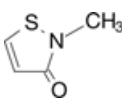
2-Methyl-4-isothiazolin-3-one

MAK value	**see Section II b of the List of MAK and BAT values**
Peak limitation	**–**
Absorption through the skin	**–**
Sensitization (2012)	**Sh**
Carcinogenicity	**–**
Prenatal toxicity	**–**
Germ cell mutagenicity	**–**
BAT value	**–**
Synonyms	2-methyl-3-isothiazolone 2-methyl-4-isothiazolin-3-one 2-methyl-4-isothiazolin-3(*2H*)-one
Chemical name (CAS)	2-methyl-3(*2H*)-isothiazolone
CAS number	2682-20-4
Structural formula	
Molecular formula	C_4H_5NOS
Molecular weight	115.2 (Burnett et al. 2010)
Melting point	no data available (Burnett et al. 2010)
Boiling point	100°C (Burnett et al. 2010)
Density at 25°C	1.02 g/cm^3 (Burnett et al. 2010)
Vapour pressure at 25°C	0.026 hPa (Burnett et al. 2010)
log K_{OW}[1)]	–0.486 (Burnett et al. 2010)

1) octanol/water partition coefficient

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Solubility	completely soluble in water, readily soluble in acetonitrile, methanol and hexane, poorly soluble in xylene (Burnett et al. 2010)
1 ml/m³ (ppm) ≙ 4.780 mg/m³	**1 mg/m³ ≙ 0.209 ml/m³ (ppm)**
Stability	2-methyl-4-isothiazolin-3-one reacts oxidatively with thiols such as glutathione to form disulfides. The reaction depends on the pH. Cystine and mercaptoacrylamide are released (Burnett et al. 2010)
Production	no data available
Purity	96.8% (technical grade)
Impurities	5-chloro-2-methyl-4-isothiazolin-3-one (0.1%), 4,5-dichloro-2-methyl-4-isothiazolin-3-one (0.1%), *N*,*N*′-dimethyl-3,3′-dithiodipropionamide (0.2%), *N*,*N*′-dimethyl-3,3′-trithiodipropionamide (0.5%), *N*-methyl-3-chloropropionamide (0.1%), ammonium chloride (0.3%), H_2O (0.2%), ethyl acetate (0.1%), acetic acid (0.1%), unknown (1.5%) (Burnett et al. 2010)
Uses	preservative in cosmetics and other body-care products (up to 0.01%), in cleaning agents, glues, coating agents, fuels, metal-working fluids, resin emulsions and diagnostic reagents; bactericide for the production of paper and cellulose, in cooling-water systems, oilfield plants, air purifying systems and as a wood preservative (Burnett et al. 2010)

2-Methyl-4-isothiazol-3-one was formerly used with 5-chloro-2-methyl-4-isothiazolin-3-one, mostly in the form of a 1:3 mixture (see supplement "5-Chloro-2-methyl-2,3-dihydroisothiazol-3-one and 2-Methyl-2,3-dihydroisothiazol-3-one" 2007). Now, 2-methyl-4-isothiazolin-3-one is often used on its own in the same fields as the mixture.

Much of this documentation is based on the evaluation of 2-methyl-4-isothiazolin-3-one by the Cosmetic Ingredient Review Expert Panel (Burnett et al. 2010).

1 Toxic Effects and Mode of Action

2-Methyl-4-isothiazolin-3-one can cause sensitization of the skin in humans and animals.

Undiluted 2-methyl-4-isothiazolin-3-one is corrosive to the skin of rabbits, while 0.1% 2-methyl-4-isothiazolin-3-one was not found to be irritating to the skin. A 0.01% 2-methyl-4-isothiazolin-3-one solution was not irritating in the rabbit eye.

The 4-hour LC_{50} was found to be 110 mg/m^3. Mild to pronounced reddening of the pulmonary lobes and sporadic red, pin-sized foci were observed in the lungs of exposed rats at 46 mg/m^3 and above. After 10 minutes, the RD_{50} in the mouse was found to be greater than 157 mg/m^3 (44% respiratory depression).

The administration of 2-methyl-4-isothiazolin-3-one with the drinking water (66 to 94 mg/kg body weight and day) or with the diet (41 mg/kg body weight and day) for three months did not lead to adverse effects in rats or dogs.

In a 2-generation study with 2-methyl-4-isothiazolin-3-one in rats, neither impaired fertility nor effects on the offspring were found. Developmental toxicity studies in rats and rabbits yielded no evidence of a foetotoxic or teratogenic potential at maternally toxic doses.

2-Methyl-4-isothiazolin-3-one was not found to be mutagenic in bacteria or in HPRT tests in CHO cells (a cell line derived from Chinese hamster ovary). An in vitro chromosomal aberration test yielded positive results only at cytotoxic concentrations. A UDS (**u**nscheduled **D**NA **s**ynthesis) test in rat hepatocytes and a mouse micronucleus test provided no evidence of genotoxic effects in vivo. There are no studies available for the carcinogenicity of 2-methyl-4-isothiazolin-3-one. An study with oral administration in the rat and another with dermal application in the mouse using a mixture of 2-methyl-4-isothiazolin-3-one (3.6%–3.8%) and 5-chloro-2-methyl-4-isothiazolin-3-one yielded no evidence of a tumorigenic potential.

2 Mechanism of Action

2-Methyl-4-isothiazolin-3-one and 5-chloro-2-methyl-4-isothiazolin-3-one (see documentation "5-Chloro-2-methyl-2,3-dihydroisothiazol-3-one and 2-Methyl-2,3-dihydroisothiazol-3-one" 1993) have two electrophilic centres where reactions with nucleophilic groups can occur (at the double bond in position 5 and at the sulfur atom) (Aptula et al. 2005). Studies with model peptides showed that 2-methyl-4-isothiazolin-3-one and 5-chloro-2-methyl-4-isothiazolin-3-one are able to react with glutathione or with free SH groups of cysteine residues with simultaneous opening of the isothiazolinone ring. Under specific conditions (excess of haptens, alkaline environment), 5-chloro-2-methyl-4-isothiazolin-3-one, but not 2-methyl-4-isothiazolin-3-one, also reacts with free amino groups of lysine or histidine residues (Alvarez-Sánchez et al. 2003, 2004 a, b; Gerberick et al. 2007; Mutschler et al. 2009). It appears that 2-methyl-4-isothiazolin-3-one does not undergo a reaction of

the Michael addition type. The initially rapid reaction with the peptide SH groups is followed by a regeneration of free SH groups and other reactions (Roberts and Natsch 2009). 2-Methyl-4-isothiazolin-3-one should therefore, as also assumed for 5-chloro-2-methyl-4-isothiazolin-3-one (Roberts et al. 2007), participate in a nucleophilic substitution reaction of the second order with electrophilic sulfur.

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

Absorption

The absorption of the test substance was determined using rat skin mounted on diffusion cells. Twenty-four hours after the application of a 25, 75 or 150 µg/ml solution of 2-methyl-4-isothiazolin-3-one radioactively labelled on the fourth and fifth carbon atom of the isothiazolinone ring, 29.2% to 46.4% (depending on the concentration) of the radioactivity was recovered in the epidermal layer, 3.8% to 10.4% in the stratum corneum and 0.2% to 0.9% in the dermis of the rat skin. The amounts absorbed after 24-hour incubation with a solution of 25, 75 or 150 µg/ml were 0.0059, 0.0277 and 0.0841 µg/cm^2, respectively. The amount of absorbed radioactivity was 21.4%, 33.7% and 51.2%, respectively (Burnett et al. 2010).

In another chamber system, human epidermis was treated with ^{14}C-labelled 2-methyl-4-isothiazolin-3-one concentrations of 52, 104 and 313 µg/ml in aqueous solution (20 µl/cm^2 skin) or 100 µg/ml in formulations with shampoo, body lotion and face cream (20 mg/cm^2 skin). After application for 24 hours, 11% to 13% of the applied radioactivity was still found in the aqueous solution, 7% to 15% was washed from the skin, 2% to 4% was found in the stratum corneum and 11% to 36% in the remaining epidermis. The total amount absorbed was 29.8%, 38.0% and 54.7%, after incubation with 52, 104 and 313 µg/ml, respectively. The absorption rate of the medium concentration was 0.037 µg/cm^2 and hour. In the formulations, 4% to 9% of the applied radioactivity was found in the donor chamber, 30% to 69% was washed from the skin, 2% to 4% of the dose was recovered in the stratum corneum and 17% to 20% in the epidermis. The total amount absorbed was 29.5%, 8.98% and 19.6% in the shampoo, body lotion and face cream, respectively. The rates of absorption were in the range from 0.007 to 0.026 µg/cm^2/hour (Burnett et al. 2010). The rate of absorption for the highest concentration of the aqueous solutions used was 0.143 µg/cm$^{2)}$/hour calculated from the available data. Taking this as a basis, 286 µg 2-methyl-4-isothiazolin-3-one is absorbed after skin contact of both hands and forearms (area 2000 cm^2) with a saturated aqueous solution for 1 hour.

Distribution

After 3 male and 3 female CD-1 mice were given single gavage doses of ^{14}C-labelled

2-methyl-4-isothiazolin-3-one of 100 mg/kg body weight, the blood, plasma, bone marrow, upper thigh bones and liver of the animals were examined after 1, 3, 6, 24 and 48 hours. At the early readings, the radioactivity in all tissues was high, the highest being in the liver, the lowest in the bones. After 24 hours, the radioactivity in the tissues decreased significantly, after 48 hours the blood had the highest tissue/plasma ratio. In the bone marrow, the concentrations were 1.2 to 39.4 µg/ml in the male mice and 1.1 to 30.4 µg/ml in the females (Burnett et al. 2010).

Elimination

After single oral gavage doses of ^{14}C-labelled 2-methyl-4-isothiazolin-3-one of 5 or 50 mg/kg body weight in 4 male and 4 female Sprague Dawley rats, 80% to 87% of the radioactivity was eliminated within 24 hours. Of this, 53% to 70% was found in the urine and 21% to 37% in the faeces. After 96 hours, only 1.9% to 3.6% was still present in the rest of the body, and this mainly in the blood. The total recovery was 92% to 96%. The initial elimination half-time from plasma was 3 to 6 hours and was not dose dependent. No difference between males and females was observed (Burnett et al. 2010).

Another study was carried out using 4 female bile duct-cannulated rats given single oral doses of ^{14}C-labelled 2-methyl-4-isothiazolin-3-one of 50 mg/kg body weight and day. Bile, urine and faeces were collected from the animals for the subsequent 24 hours. More than 88% of the administered dose was recovered in the 24-hour period, of which 29.1% was found in the bile, 52.9% in the urine and 6.1% in the faeces (Burnett et al. 2010).

3.2 Metabolism

In the study described in Section 3.1 with single oral doses of ^{14}C-labelled 2-methyl-4-isothiazolin-3-one of 5 or 50 mg/kg body weight in 4 male and 4 female Sprague Dawley rats, 23 metabolites were determined by means of HPLC (high performance liquid chromatography) in the urine and faeces. The test substance itself was not detected in either the urine or faeces. The main metabolites in urine were *N*-methyl malonaminic acid, the 3-mercapturic acid conjugate of 3-thiomethyl-*N*-methyl propionamide and *N*-methyl-3-hydroxyl propionamide in amounts of 21% to 23%, 10% to 23% and 4% to 5% of the administered dose, respectively (Burnett et al. 2010).

In one of the experiments with 4 bile duct-cannulated rats (see Section 3.1) given single oral doses of ^{14}C-labelled 2-methyl-4-isothiazolin-3-one of 50 mg/kg body weight and day, 31 metabolites, but not the test substance itself, were identified in the bile, urine and faeces with liquid chromatography/mass spectrometry (LC/MS) and tandem mass spectroscopy (LC/MS/MS). The main metabolites were *N*-methyl malonaminic acid and the 3-mercapturic acid conjugate of 3-thiomethyl-*N*-methyl propionamide (Burnett et al. 2010).

4 Effects in Humans

4.1 Single exposures

There are no data available.

4.2 Repeated exposure

There are no data available.

4.3 Local effects on skin and mucous membranes

Skin irritation was investigated in 40 volunteers after the application to their backs of 15 µl of a 2-methyl-4-isothiazolin-3-one solution in concentrations of 0.01%, 0.03% or 0.06% for 24 hours (method of application not specified). The irritation index, combining the readings after 1 and 24 hours, was 6.3, 1.3 and 6.3 for the concentrations 0.01%, 0.03% and 0.06%, respectively. The irritation index of the negative controls (water) was 5.0. Under these test conditions, 2-methyl-4-isothiazolin-3-one was not irritating (Burnett et al. 2010).

In similar investigations in groups of 40 volunteers with 0.01% 2-methyl-4-isothiazolin-3-one in shampoo, body lotion or sun cream, the test substance was not found to be irritating to the skin (Burnett et al. 2010).

4.4 Allergenic effects

4.4.1 Sensitization of the skin

Patch tests

In patch tests carried out in Germany, 2-methyl-4-isothiazolin-3-one is tested in the form of a 0.05% (500 ppm) preparation in water. The 0.01% aqueous preparation of the 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one mixture contains 0.0025% (25 ppm) 2-methyl-4-isothiazolin-3-one. The reaction index[2)] for the 0.05% 2-methyl-4-isothiazolin-3-one preparation of 0.43 and the po-

2) The reaction index is defined as the ratio: (a – d – i) / (a + d + i); with: a = the number of allergic reactions, d = the number of questionable reactions, i = the number of irritative reactions (Brasch and Henseler 1992).

sitivity ratio[3)] of 61.4% indicate that the test preparation is well suited for use in patch tests (Schnuch et al. 2011 a).

Eleven patients sensitized to 2-methyl-4-isothiazolin-3-one were patch tested with 2-methyl-4-isothiazolin-3-one preparations (in water/ethanol 9:1) in serial dilutions of approximately 0.2%, 0.1%, 0.05%, 0.029%, 0.015%, 0.01%, 0.005%, 0.0015%, 0.0007%, 0.0005%, 0.00035% and 0.000035% (corresponding to 2-methyl-4-isothiazolin-3-one concentrations of about 60, 30, 15, 8.8, 4.4, 2.9, 1.5, 0.4, 0.2, 0.15, 0.105 and 0.0105 μg/cm^2). Ten of the patients reacted to 60, 30 and 15 μg/cm^2, and 6 of them to 1.5 μg/cm^2. There were no reactions to lower concentrations although 7 patients reacted to 0.2 and 0.105 μg/cm^2 and 2 patients to 0.0105 μg/cm^2 in the repeated open application test (ROAT) (Lundov et al. 2011 b).

Case reports of work-related sensitization

Dermatitis-like changes occurred in a painter after repeated contact of the hands, forehead and forearms with pastes and glues containing 2-methyl-4-isothiazolin-3-one and 1,2-benzisothiazol-3(2H)-one as preservatives. A worker employed in the production of starch for the paper industry had accidental contact with an undiluted preservative containing 2-methyl-4-isothiazolin-3-one and 1,2-benzisothiazol-3(2H)-one. His hands and face were decontaminated immediately, but not his feet or shoes. About 1 hour later the man reported a burning sensation in his feet and dermatitis-like changes, after which his feet were decontaminated and socks and shoes changed. One week later, a vesicular skin reaction developed on his hands, which healed within 2 weeks. In patch tests, both patients reacted to 0.02% 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one in water. In further tests with serial dilutions, both patients reacted also to 2-methyl-4-isothiazolin-3-one and to a biocide containing 2-methyl-4-isothiazolin-3-one down to concentrations of the active substance of about 0.003% (30 ppm; 1+ and 2+ reactions) and to 5-chloro-2-methyl-4-isothiazolin-3-one in concentrations of 0.0075% and above (2+ and 3+ reactions), but not to 0.00375% 5-chloro-2-methyl-4-isothiazolin-3-one (Isaksson et al. 2004).

After introducing a biocide containing 7% to 10% 2-methyl-4-isothiazolin-3-one and 1% to 2.5% 2-octyl-4-isothiazolin-3-one for product preservation, skin changes, especially on the hands, and also on the neck and forearms, occurred in 4 of 14 workers employed in the production of paint. In patch tests, all 4 workers produced a 2+ reaction to 0.105% 2-methyl-4-isothiazolin-3-one (diluted in water, biocide containing commercially available 2-methyl-4-isothiazolin-3-one) and one a 1+ reaction, one a 2+ reaction and 2 workers a questionable reaction to 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one. In the paper production industry, another product containing 2-methyl-4-isothiazolin-3-one and 1,2-benzisothiazol-3(2H)-one was used, and likewise manually processed in an open system, whereas the previously used biocide containing 5-chloro-2-methyl-4-iso-

3) The positivity ratio is defined as the percentage of single positive reactions among the total positive reactions (Geier et al. 2003).

thiazolin-3-one/2-methyl-4-isothiazolin-3-one in the meantime was processed mainly in a closed dosing system (Thyssen et al. 2006).

Other case reports

In one case, the dermatitis-like changes in the armpits were attributed to 2-methyl-4-isothiazolin-3-one in a deodorant. In patch tests, the female patient produced a 2+ reaction to 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one, to the deodorant and, after 2 days, to 0.02% 2-methyl-4-isothiazolin-3-one in petrolatum. Further information as to whether the deodorant actually contained only 2-methyl-4-isothiazolin-3-one as the preservative was not given, however (Amaro et al. 2011).

A contact gel for a slimming belt that contained 2-methyl-4-isothiazolin-3-one and 5-chloro-2-methyl-4-isothiazolin-3-one in unknown concentrations induced an erythematous reaction over the area of contact in one man. The patient had additionally been suffering from perianal dermatitis for some time, and reported having used three different types of moist toilet paper. In patch tests, he produced a questionable reaction to 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one and a 2+ reaction to 2-methyl-4-isothiazolin-3-one after 72 hours (Uter et al. 2012).

Five of 6 patients with an allergic reaction to moist toilet paper containing 2-methyl-4-isothiazolin-3-one and 1 female patient with dermatitis of the eyelids after using a make-up remover containing 2-methyl-4-isothiazolin-3-one were patch tested with 0.1% 2-methyl-4-isothiazolin-3-one among other substances, with a positive result in all cases (1+ to 3+ reactions on day 4). There was either no reaction or it was less pronounced in 4 of the patients when tested with 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one. In 2 cases, also a 1+ reaction to 0.001% (10 ppm) 2-methyl-4-isothiazolin-3-one was recorded, the remaining patients were tested with the high concentration of 2-methyl-4-isothiazolin-3-one only (García-Gavín et al. 2010).

In further patch tests, reactions to 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one were observed in 3 other cases with dermatitis after using moist toilet paper containing 2-methyl-4-isothiazolin-3-one. 2-Methyl-4-isothiazolin-3-one was, however, not tested separately (Gardner et al. 2010).

Two months after starting work at a restaurant in which the walls had been freshly painted, a female employee contracted work-related periorbital oedema and later vesicular facial dermatitis. In patch tests, she produced a 2+ reaction to both 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one and 0.2% 2-methyl-4-isothiazolin-3-one in water. Exposure to a face cleaning cosmetic preserved with 2-methyl-4-isothiazolin-3-one immediately produced an exacerbation of her facial dermatitis (Kaae et al. 2012).

Data from clinical epidemiological studies

Between May 2006 and February 2010, 2-methyl-4-isothiazolin-3-one was routinely patch tested in the form of a 0.2% aqueous preparation in 2536 patients at the

university clinic of Copenhagen. There was a reaction in 37 patients (1.5%), 15 of whom produced a simultaneous reaction to 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one (Lundov et al. 2010).

In 8 Finnish clinics, 10 821 patients were also patch tested with 0.1% and 0.03% 2-methyl-4-isothiazolin-3-one in water (dilution of a commercial biocide containing 9.8% 2-methyl-4-isothiazolin-3-one) between 2006 and 2008; 147 (1.4%) of the patients reacted to the higher concentration and 69 (0.6%) to the lower concentration. The 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one mixture produced a reaction in 194 patients, and there was no reaction to 2-methyl-4-isothiazolin-3-one in 97 cases. Simultaneous reactions to 0.1% 2-methyl-4-isothiazolin-3-one and 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one were observed in 97 patients; 50 patients reacted to 0.1% 2-methyl-4-isothiazolin-3-one, but not to the mixture. A ROAT carried out with a lotion containing 0.01% 2methyl-4-isothiazolin-3-one in 33 patients who had reacted to 2-methyl-4-isothiazolin-3-one yielded positive results in 10 patients (with 1+ to 3+ reactions to 0.1% 2-methyl-4-isothiazolin-3-one and simultaneous 1+ or 2+ reactions to 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one) after a maximum of 2 weeks (Ackermann et al. 2011).

At the clinics belonging to the Information Network of Departments of Dermatology (IVDK), 2-methyl-4-isothiazolin-3-one was tested in 13 433 patients, first of all in 2005 in the form of an appendix to the standard series ("Monitor block"), then in 2006 and 2007 more or less sporadically, and in 2008 and 2009 as a component of the "preservatives" and "industrial biocides" test series. Altogether, 215 reactions (1.5%) were recorded. Of 199 patients who reacted to 2-methyl-4-isothiazolin-3-one, 134 (67.3%) also produced a reaction to 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one (Schnuch et al. 2011 a).

In 2009, the percentage of reactions in 6789 tested patients was 1.9% (men 2.6%, women 1.6%). This had increased by 2011 to 4.4% of 7292 tested patients (4.0% in men and 4.6% in women). Parallel to this, also the number of reactions to 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one increased from 2.3% to 3.9% (in men from 2.5% to 3.8% and in women from 2.2% to 3.9%). As the number of simultaneous reactions to 2-methyl-4-isothiazolin-3-one also increased from 43.3% to 58.9 % of the patients who reacted to 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one, it can be assumed that the increase in the number of reactions to the chlorinated mixture can be attributed to cross-reactions as the result of increasing sensitization to 2-methyl-4-isothiazolin-3-one, which has been used more frequently over recent years. This interpretation is supported by the fact that the increase in these cross-reactions is particularly noticeable in the group of younger patients (from 27.3% to 60.7%), in whom intolerance to cosmetics was suspected, which can probably be explained by the fact that 2-methyl-4-isothiazolin-3-one, but not the mixture with the chlorinated compound, has been used more frequently in cosmetics in recent years (Geier et al. 2012).

On the basis of 3541 leave-on products and the relative frequency of use of 2-methyl-4-isothiazolin-3-one in cosmetics, a sensitization risk has been estimated

for 2-methyl-4-isothiazolin-3-one which corresponds approximately to that for the formaldehyde releasers imidazolidinyl urea and diazolidinyl urea (Schnuch et al. 2011 b).

Up to the end of 2010, at the university clinics of Lisbon and Louvain (Leuven), 2-methyl-4-isothiazolin-3-one was assumed in 23 cases to be the ingredient in cosmetic products that causes sensitization. Between November 2005 and April 2006, 2-methyl-4-isothiazolin-3-one was first tested in concentrations of 0.1% and then of 0.15% and 0.2% and then again of 0.1% and, starting in May 2010, in concentrations of 0.05%. However, there are no data available for the number of tested persons and the intensity of reactions (Travassos et al. 2011).

At the centres of the Danish Contact Dermatitis Group, 219 painters were patch tested with 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one as part of the standard series from 2001 to 2010. The results were positive in 22 of these persons. Of those investigated, 41 were tested also with 2-methyl-4-isothiazolin-3-one (no details of test concentration), in 11 cases with a positive result (Mose et al. 2012).

Reports of patch test reactions to methyl-4-isothiazolin-3-one in patients with primary sensitization to the mixture 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one

Of 22 patients with existing sensitization to the 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one mixture and initial patch test reactions to 0.03% or 0.025% of the mixture in water, 2 patients also reacted in patch tests to 0.0075% 2-methyl-4-isothiazolin-3-one in methanol/water (no other details), corresponding to the 2-methyl-4-isothiazolin-3-one content in the 0.03% 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one preparation. No reactions to the 2-methyl-4-isothiazolin-3-one test preparation were seen in 6 patients, who had presumably been sensitized to the mixture through the patch test. A further 18 patients with existing sensitization to 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one (4 of these with sensitization through patch testing) were tested with 0.0225% 2-methyl-4-isothiazolin-3-one with positive results in 4 cases (Bruze et al. 1987 a).

In another study, 3 of 12 patients with existing sensitization to 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one from patch tests produced questionable reactions to 0.0115% 2-methyl-4-isothiazolin-3-one in ethanol and one of them also to 0.00575% 2-methyl-4-isothiazolin-3-one (Bruze et al. 1989).

According to an unpublished study by the clinics of the IVDK, 27 of 85 patients with existing sensitization to 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one- produced a 1+ or 2+ reaction also to 0.05% or 0.1% (irritation threshold) 2-methyl-4-isothiazolin-3-one in water (Burnett et al. 2010).

Two workers from the metal industry with sensitization to 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one contained in a metal-working

fluid, reacted in patch tests with the same intensity (2+ and 3+ reaction) to 0.03% 5-chloro-2-methyl-4-isothiazolin-3-one and 0.03% 2-methyl-4-isothiazolin-3-one, which had been prepared in petrolatum after chromatographic separation. Also one of the authors of the publication, who had become sensitized by the chromatographic separation of the components, produced a 2+ reaction to both test preparations (Pilger et al. 1986).

After working for around 24 years with regular contact with diesel oil, to which three years previously a 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one mixture had been added for preservation, a car mechanic developed dermatitis on the hands, which was exacerbated after using moist toilet paper preserved with 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one. In patch tests, he developed a 2+ reaction to 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one and to 0.02% 2-methyl-4-isothiazolin-3-one in water (Bruynzeel and Verburgh 1996).

Of 3 workers sensitized to 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one (2 of them as the result of accidental corrosive burns) from a plant producing binders for paints and glues, who had produced 3+ reactions to 0.02% 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one and to 0.015% 5-chloro-2-methyl-4-isothiazolin-3-one in patch tests, only one produced a 1+ reaction to 0.1% 2-methyl-4-isothiazolin-3-one (vehicle not specified), but not to a technical-grade aqueous preparation containing 0.095% 2-methyl-4-isothiazolin-3-one (Isaksson et al. 2008).

In a patient with known sensitization to several preservatives, including 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one, erythema developed on the face and respiratory symptoms occurred during the course of applying wall paint containing, among other substances, small amounts of 5-chloro-2-methyl-4-isothiazolin-3-one in addition to 1,2-benzisothiazol-3(2H)-one and 2-methyl-4-isothiazolin-3-one. The symptoms persisted for no longer than one day after systemic corticoid and antihistamine treatment. After a 3-week absence and the discontinuation of his medication, the man returned to the house. Three days later, the skin and respiratory symptoms recurred. Reactions occurred also on the areas of skin where the man had been exposed 2 months previously in patch tests (Lundov et al. 2011 b; see "Patch tests") to serial dilutions of 2-methyl-4-isothiazolin-3-one. In a second case, skin reactions were likewise attributed to wall paint containing 2-methyl-4-isothiazolin-3-one. In patch tests, however, the affected patient produced only a questionable reaction to 0.2% 2-methyl-4-isothiazolin-3-one in water (Lundov et al. 2011 a).

Repeated insult patch tests

In a repeated insult patch test (RIPT), 2-methyl-4-isothiazolin-3-one (purity 98%) was applied occlusively in concentrations of 0.0050%, 0.01%, 0.025% (groups 1 to 3), 0.05% (group 4) or 0.1% (group 5) to 80 volunteers per group for 23 hours a day on 21 consecutive days. After an exposure-free interval of 10 to 14 days, challenge

treatment with the concentrations used for induction was carried out in groups 1 to 3. In groups 4 and 5, 0.01%, 0.025% and 0.05% or 0.025%, 0.05% and 0.1% 2-methyl-4-isothiazolin-3-one were used in the challenge. During the induction treatment, reactions evaluated as transient, slight and irritative were observed in all groups. Only 2 volunteers in group 5 reacted to the challenge treatment (no other details). This was regarded as evidence of sensitization (Burnett et al. 2010).

A group of 98 volunteers, who had not reacted to 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one in patch tests, were subjected in a RIPT to a 23-hour occlusive application of 150 µl of a 0.01% 2-methyl-4-isothiazolin-3-one preparation on 4 days a week for 3 weeks. After a 1-week interval, challenge treatment was carried out in the same way, but with application for around 24 hours. With the exception of 1 volunteer, who in retrospect was considered to have already been sensitized, none of the volunteers produced allergic reactions to the substance (Burnett et al. 2010).

In a series of 5 RIPTs (24-hour occlusive application of 200 µl three times a week for 3 weeks), the following 2-methyl-4-isothiazolin-3-one-concentrations (dilutions of an industrial preservative concentrate containing 50% 2-methyl-4-isothiazolin-3-one) were used: 0.02% (group 1, 100 volunteers), 0.03% (group 2, 98 volunteers), 0.04% (group 3, 116 volunteers), 0.05% (group 4, 210 volunteers) and 0.06% (group 5, 214 volunteers). No irritation or allergic reactions were observed during the induction phase. Challenge treatment with the concentrations used for induction was carried out in groups 1 and 2 after a 1-week interval, and in groups 3 to 5 after an interval of 10 to 14 days. Only one volunteer each in groups 3 and 4 produced an erythematous reaction (Burnett et al. 2010).

4.4.2 Photosensitization

In a 2-week RIPT (repeated insult patch test), in which a 0.02% 2-methyl-4-isothiazolin-3-one preparation (20 µl in the first and 6 µl in the following applications) was applied a total of 6 times for 24 hours, additional irradiation with twice the MED (minimal erythemal dose) of UVA/UVB (ultraviolet A and B) was also carried out. After an interval of 9 to 14 days, 24-hour occlusive application (5 µl/cm^2) with a subsequent application of 2 µl/cm^2 and irradiation with 10 J/cm^2 UVA and 0.5 MED UVA/UVB was carried out. According to the readings obtained, there was no reaction in any of the 32 volunteers after 24 and 48 hours (Burnett et al. 2010).

4.4.3 Sensitizing effects on the airways

A patient with known sensitization to several preservatives, including a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one, incurred symptoms in the airways in addition to facial erythema during the application of wall paint containing small amounts of 5-chloro-2-methyl-4-isothiazolin-3-one in addition to 1,2-benzisothiazol-3(2H)-one

and 2-methyl-4-isothiazolin-3-one; the symptoms persisted for no longer than a day under systemic treatment with corticoids and antihistamines. After three weeks absence and the discontinuation of his medication, the man returned to the house, whereupon 3 days later symptoms recurred both on the skin and in the airways. Despite inhaling a β-adrenergic agonist, the patient's pulmonary function was markedly decreased (forced expiratory volume during the first second (FEV_1): 1.4 l (39% of the expected value); forced vital capacity (FVC): 2.2 l (47%)). Taking local and systemic corticoid medication, the man returned to the house, where he discontinued medication after 2 weeks. During this time, the rooms had been sufficiently ventilated, so that no recurrence of his symptoms took place (FEV_1: 3.0 l; FVC: 4.0 l). Provocation tests or other immunological studies were not carried out (Lundov et al. 2011 a).

4.5 Reproductive and developmental toxicity

There are no data available.

4.6 Genotoxicity

There are no data available.

4.7 Carcinogenicity

There are no data available.

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

In an inhalation study, 6 male and 6 female Sprague Dawley rats were exposed nose only to 2-methyl-4-isothiazolin-3-one (purity 97.8%) concentrations of 46, 129, 150, 1070 or 2090 mg/m^3 for 4 hours. The two high concentrations were lethal to all animals. Gross-pathological examination revealed that all (no other details) animals had slight to marked reddening of the pulmonary lobes, sporadic red, pinhead-sized foci in the lungs, and stomachs filled with gas. The LC_{50} in this study was 110 mg/m^3 (Burnett et al. 2010).

In male Swiss Webster mice, the RD_{50} was greater than 157 mg/m^3 after exposure for 10 minutes. Groups of male animals were exposed to 2-methyl-4-isothiazolin-3-one (purity 98.6%) concentrations of 3.12, 6.76, 10.5, 27.8, 64.6, 74.9, 90.7, 92.2 or 157 mg/m^3 (particle size not specified). Respiration rates were reduced by 25% at the low concentration and by 44% at the high concentration. The maximum respiratory depression was 47% at 74.9 mg/m^3 (Burnett et al. 2010).

5.1.2 Oral administration

Male and female Sprague Dawley rats were given single gavage doses of 2-methyl-4-isothiazolin-3-one (purity 97.5%) of 0, 75, 150, 180 or 225 mg/kg body weight. The male animals received an additional dose of 300 mg/kg body weight. The recovery period was 14 days. Signs of intoxication were observed in the female rats at the low dose and above, and in the males at 150 mg/kg body weight and above (no other details). In the surviving animals, these were reversible after 6 days. Gross-pathological examination revealed reddened stomachs and intestines with a reddish content in the deceased animals. The LD_{50} was 235 mg/kg body weight in the male rats and 183 mg/kg body weight in the females (Burnett et al. 2010).

Groups of 6 male and 6 female CD 1 mice were given gavage doses of 2-methyl-4-isothiazolin-3-one (purity 97.5%) of 0, 150, 200 or 250 mg/kg body weight. The recovery period was 14 days. The high dose was lethal to all animals. One hour after administration and thereafter, clinical symptoms of intoxication were observed in all groups. Gross-pathological examination revealed gastrointestinal changes in all deceased animals. The LD_{50} was 167 mg/kg body weight (no other details; Burnett et al. 2010).

The LD_{50} of the main metabolite N-methylmalonaminic acid is 3550 mg/kg body weight in male rats and 4100 mg/kg body weight in female rats (Burnett et al. 2010).

5.1.3 Dermal application

2-Methyl-4-isothiazolin-3-one (purity 97.5%) was applied occlusively to the skin of 6 male and 6 female Sprague Dawley rats in doses of 0, 100, 200, or 400 mg/kg body weight for 24 hours. An additional group of 6 male rats received applications of 300 mg/kg body weight. Clinical symptoms of intoxication (no other details) were recorded from the first day of treatment onwards. The surviving animals had recovered by day 5 of the 14-day recovery period. The body weight gains were reduced at 200 mg/kg body weight and above. On all areas of treated skin, bleaching, oedema, dark patches, crusts, eschar formation and desiccation were observed. Changes were found in the gastrointestinal tract of the animals in which treatment had been lethal. The dermal LD_{50} in this study was 242 mg/kg body weight (no other details; Burnett et al. 2010).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

There are no data available.

5.2.2 Oral administration

Rats

In a 3-month study, groups of 10 male and 10 female Sprague Dawley rats were given 2-methyl-4-isothiazolin-3-one (purity 97.5%) with the drinking water in concentrations of 0, 75, 250 or 1000 mg/l (doses of 0, 6.5–9.8, 19–25, 66–94 mg/kg body weight and day). After week 13, neurotoxicity tests (functional observational battery) and, during the final week of the study, a test for motor activity with an infrared motion activity cage system were carried out. An ophthalmological examination took place at the end of the study. The treatment was not lethal for any of the animals. Food consumption was decreased in the male rats of the high dose group and body weight gains were reduced in both sexes. The drinking water consumption was reduced in the females at 19 to 25 mg/kg body weight and day and in the males of all treatment groups. The authors attribute the reductions in body weight gains, food and water consumption to the unpleasant taste of the substance. No neurotoxic or other systemic substance-related effects occurred. Gross-pathological and microscopic examinations, ophthalmoscopy, haematology and clinical chemistry did not reveal any unusual findings. According to the authors, the NOAEL (no observed adverse effect level) was 1000 mg/l in the drinking water (66–94 mg/kg body weight and day) (Burnett et al. 2010).

Dogs

Groups of 4 male and 4 female beagles were given 2-methyl-4-isothiazolin-3-one with the diet in concentrations of 0, 100/130, 400 or 1500 mg/kg (purity 51.4%), corresponding to 0, 3, 10 or 41 mg/kg body weight and day for 3 months. “Unsatisfactory recovery” (no other details) in the low dose group led to the 100 mg/kg dose being increased to 130 mg/kg. Ophthalmological examinations were carried out at the end of the study. The haematological and clinico-chemical examinations took place prior to treatment, in week 7 and at the end of the study. There was no treatment-related mortality, clinical symptoms, effects on organ weights or histopathological findings. The body weight gains of the male and female animals of the high dose group were reduced during the first week. This effect was reversible in the third or fourth week. Food consumption in this dose group was reduced during the entire treatment period, but the reduction was not statistically significant. Also in this group, no significant changes in haematological parameters (no other details) were found. In the authors’ opinion, therefore, the NOAEL was 1500 mg/kg food (41 mg/kg body weight and day; Burnett et al. 2010).

5.2.3 Dermal application

There are no data available.

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

In a study of dermal irritation, 0.5 ml 2-methyl-4-isothiazolin-3-one (purity 97.8%) was applied to the shaved intact skin of New Zealand White rabbits, which was subsequently exposed semi-occlusively for 1 or 4 hours or non-occlusively for 3 minutes. The skin was examined 1 hour after treatment, after a further 24, 48 and 72 hours, and after 7 and 14 days. No signs of systemic toxicity were observed. One and 2 weeks after 1-hour and 4-hour exposure, pitted eschar appeared on the treated area. Three-minute exposure caused very mild to marked erythema, visible for up to 48 hours later, and very mild to moderate oedema, which was evident for up to 24 hours, and in one animal up to 72 hours. The authors concluded that semi-occlusive exposure of the skin to undiluted 2-methyl-4-isothiazolin-3-one for 1 hour is corrosive (Burnett et al. 2010).

In further investigations with diluted 2-methyl-4-isothiazolin-3-one (0.01%), semi-occlusive exposure of the shaved intact skin of New Zealand White rabbits for 4 hours was not irritating. Neither did daily application of up to 0.1% 2-methyl-4-isothiazolin-3-one for 14 days produce any cumulative irritating effects (Burnett et al. 2010).

Phototoxicity

2-Methyl-4-isothiazolin-3-one was not phototoxic in female Hartley guinea pigs in concentrations of 0.02% in water. In this study, 20 µl of the preparation was applied topically and the application site subsequently irradiated with long-wave UVA light (300–400 nm; 10.0 to 11.9 J/cm^2). No skin reactions were observed 4, 24 and 48 hours later (Burnett et al. 2010).

5.3.2 Eyes

A 0.01% aqueous 2-methyl-4-isothiazolin-3-one solution, prepared from 9.69% 2-methyl-4-isothiazolin-3-one in Neolone 950, was instilled into the conjunctival sac of 6 rabbits (volume not specified). The eyes were rinsed after 24 hours and examined 1, 24, 48 and 72 hours after the instillation. The authors regarded 0.01% aqueous 2-methyl-4-isothiazolin-3-one solution as not irritating (Burnett et al. 2010).

In 6 rabbits, a Neolone 950 solution (prepared from 9.69% 2-methyl-4-isothiazolin-3-one in Neolone 950) diluted to 0.01% 2-methyl-4-isothiazolin-3-one in an

anionic body lotion was tested for its irritating effects on the eye. The instillation of 0.1 ml into the conjunctival sac did not produce any effects on the cornea, iris and conjunctiva. In a corresponding study with sun cream, there was also no irritation in the eyes (Burnett et al. 2010).

5.4 Allergenic effects

5.4.1 Sensitizing effects on the skin

Four of 24 female Dunkin Hartley guinea pigs reacted in a modified maximization test (intradermal induction with 0.076% 2-methyl-4-isothiazolin-3-one in propylene glycol; epicutaneous induction with 0.038% 2-methyl-4-isothiazolin-3-one after preceding topical treatment with sodium laurylsulfate in dimethylacetamide/acetone/ethanol (4:3:3); challenge treatment with 0.015% 2-methyl-4-isothiazolin-3-one in ethanol). In a second group, 11 of 24 animals produced a reaction, whereas no reactions occurred in 12 controls. Two days after the challenge treatment, the animals of the second group were given another intradermal injection of the 0.076% 2-methyl-4-isothiazolin-3-one preparation and were once more subjected to epicutaneous challenge treatment 5 days later: there were 8 reactions. Three animals of the second group pretreated with 2-methyl-4-isothiazolin-3-one produced a cross-reaction to 5-chloro-2-methyl-4-isothiazolin-3-one, but a reaction to 2-methyl-4-isothiazolin-3-one occurred in only 1 of 24 animals pretreated with 5-chloro-2-methyl-4-isothiazolin-3-one (Bruze et al. 1987 b).

In a similar maximization test in 24 female Dunkin Hartley guinea pigs pretreated with 4,5-dichloro-2-methyl-4-isothiazolin-3-one, no cross-reactions to 0.015% 2-methyl-4-isothiazolin-3-one in ethanol were observed (Bruze et al. 1987 c).

Aqueous 2-methyl-4-isothiazolin-3-one preparations (group 1: 0.1%; group 2: 0.5%; group 3: 1.5% and group 4: 3%; purity 99.8%) were used in a modified Buehler test for ten 6-hour occlusive induction treatments 3 times a week in groups of 10 Hartley guinea pigs. After a 2-week interval, the challenge treatment was carried out with 0.1%, 0.5% and 1.5% 2-methyl-4-isothiazolin-3-one in water. Only 1 animal in group 3 produced a reaction to the 0.1% preparation. Two, 1 and 2 animals reacted in groups 2, 3 and 4, respectively, to the challenge with 0.5% 2-methyl-4-isothiazolin-3-one. At the highest test concentration, reactions were induced in 1 of 10 (group 1), 6 of 10 (group 2), 3 of 10 (group 3) and 5 of 10 (group 4) animals (Burnett et al. 2010).

2-Methyl-4-isothiazolin-3-one (purity 99.7%) was used in a maximization test both for intradermal and epicutaneous induction and challenge treatment in groups of 20 female Hartley guinea pigs in concentrations of 0.055% and 0.08%. There were no reactions to the low concentration and only one to the higher concentration. After repeated challenge treatment with 0.1% 2-methyl-4-isothiazolin-3-one, there

was a slight erythematous reaction "in less than 30% of the animals" (Burnett et al. 2010).

In an open patch test, groups of 8 female HsdPoc:DH guinea pigs were given 20 applications of 100 µl of a 2-methyl-4-isothiazolin-3-one preparation in ethanol/water (group 1: 0.15%; group 2: 0.25%; group 3: 0.4%; group 4: 0.6%; group 5: 1.5%; group 6: 18%) for 4 consecutive weeks. Three days after the final induction treatment, a 6-hour open challenge application was carried out with 25 µl of the preparations. There were reactions in 1 of 8 (group 2), 3 of 8 (group 3), 1 of 8 (group 4), 1 of 8 (group 5) and 4 of 8 animals (group 6). Repeated challenge tests were performed in groups 1 to 4 with the parallel application of 0.4%, 0.6%, 1.5% and 18% 2-methyl-4-isothiazolin-3-one. The animals of group 5 were given 0.25%, 0.6%, 1.5% and 18% 2-methyl-4-isothiazolin-3-one and the animals of group 6 were given 0.15%, 0.6%, 1.5% and 18% 2-methyl-4-isothiazolin-3-one. There were reactions only in 2 of 8 animals in group 5 (1.5% challenge concentration), in 1 of 8 (1.5% challenge concentration) and 6 of 8 (18% challenge concentration) in group 6 (Burnett et al. 2010).

Local lymph node assay

The sensitizing properties of 2-methyl-4-isothiazolin-3-one were investigated also in several local lymph node assays (LLNA) in CBA/J mice.

After the application of 0.1%, 1% and 3% 2-methyl-4-isothiazolin-3-one (purity 99.8%) in acetone on 4 consecutive days, stimulation indices of < 1.0, 2.3 and 3.2 were determined in CBA/J mice corresponding to a concentration (EC_3 value) of about 2.5% necessary to triple lymphocyte proliferation (Potter and Hazelton 1995).

In another LLNA with application of the substance on 3 consecutive days, 2-methyl-4-isothiazolin-3-one (commercial product with a 2-methyl-4-isothiazolin-3-one content of 10.37%) concentrations of 0.15%, 0.45%, 0.76%, 1.35%, 1.57%, and 1.80% in acetone/olive oil (4:1) were used. The stimulation indices determined were about 2.1, 2.4, 2.2, 6.6, 4.7, and 6.6, respectively. The EC_3 value was calculated to be 0.86% (Burnett et al. 2010).

In an LLNA with about 0.05%, 0.1%, 0.2%, 0.5% and 1% 2-methyl-4-isothiazolin-3-one in acetone/olive oil (4:1) or 1%, 2%, 4.9% and 9.9% 2-methyl-4-isothiazolin-3-one in propylene glycol, three applications produced stimulation indices of 1.5, 1.5, 1.8, 3.8 and 2.5, and 1.9, 2.6, 7.0 and 7.6, respectively. From this, EC_3 values of 0.4% and 2.2%, respectively, were calculated (Basketter et al. 2003).

Photo-contact sensitization

In a modified split adjuvant test (intradermal injection of 100 µl Freund's complete adjuvant; stripping of the stratum corneum by adhesive tape; open application of 100 µl of a 0.02% 2-methyl-4-isothiazolin-3-one preparation in water), the treated surfaces were irradiated after 30 minutes with long-wave UVA (300–400 nm; 9.9 to 11.2 J/cm^2). Application of the substance and UVA irradiation were carried out on five consecutive days. Challenge treatment with 20 µl of the test preparation took

place 16 days after the first treatment, followed by UVA irradiation with 10.0 to 10.2 J/cm^2. After 24 and 48 hours no reactions were observed on the irradiated areas of skin or those not irradiated (Burnett et al. 2010).

Sensitizing effects on the airways

In a study of cytokine secretion induced by 2-methyl-4-isothiazolin-3-one, 50 µl of a 0.5% 2-methyl-4-isothiazolin-3-one preparation in acetone/olive oil (4:1) was applied to the shaved flanks of female BALB/c mice on days 1 and 6. Thereafter, 25 µl of the preparation was applied to both ears on days 12, 13 and 14. The auricular lymph nodes were removed and used to produce cell suspensions of 10^7 cells/ml and these were cultivated over six days with and without the T-cell mitogen concanavalin A. The cytokines IFN-γ, IL-10, IL-5 and IL-13 were determined in the supernatant fluid using ELISA (enzyme-linked immunosorbent assay) up to the time of maximum secretion after 96 to 120 hours, while the concanavalin A-induced IL-4 was determined after 24 hours. Although 2.5 ng/ml IFN-γ was found as evidence of a Th1 response in addition to 0.6 ng IL-10/ml, 0.9 ng IL-13/ml and 0.2 ng IL-5/ml, there was no measurable IL-4 as an indication of a Th2 response, unlike with trimellitic acid anhydride (1.3 ng IFN-γ/ml, 2.9 ng IL-13/ml, 9 ng IL-4/ml) which was tested in parallel (Basketter et al. 2003).

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

A 2-generation study was carried out with 30 male and 30 female Sprague Dawley rats per dose group. 2-Methyl-4-isothiazolin-3-one (purity 51.4%) was administered with the drinking water in concentrations of 0, 50, 200 or 1000 mg/l (corresponding to doses of 0, 4 to 7, 15 to 19, 69 to 86 mg/kg body weight and day in males and 0, 6 to 13, 22 to 26, 93 to 115 mg/kg body weight and day in females). The animals were exposed for a total of 70 days before and during mating, and during gestation and lactation. Thirty pups from each group from the F0 generation formed the F1 generation. The offspring (F1 and F2 generations) were examined on postnatal days 1, 4, 7, 14 and 21 and the anogenital distance was determined in the male animals. In the animals of the F0 and F1 generations, sperm analyses were carried out or the ovarian follicle counts recorded. The treatment was not lethal in any of the animals. The water consumption was decreased in all male F0 animals and in the female F0 and F1 animals during gestation and lactation at 200 ppm (15–19 mg/kg body weight and day) and above. The authors suspected that the taste or smell of the substance in the drinking water was the cause of this. In the high dose group, body weight gains and food consumption were decreased, and were regarded as secondary effects, presumably resulting from the decreased water consumption. Clinical symptoms of intoxication did not occur in any dose group. Examination of the

tissues and reproductive organs of the parent animals and examination of the offspring did not yield unusual findings. The NOAEL of this study was 69 to 86 mg/kg body weight and day in male rats and 93 to 115 mg/kg body weight and day in females, the highest dose investigated (Burnett et al. 2010).

5.5.2 Developmental toxicity

In a developmental toxicity study, groups of 25 Sprague Dawley rats were given gavage doses of 2-methyl-4-isothiazolin-3-one (purity 51.4%) of 0, 5, 20 or 60 mg/kg body weight and day on days 6 to 19 of gestation. The high dose was reduced to 40 mg/kg body weight and day after 3 days of treatment as a result of pronounced toxicity (no other details). Caesarean section was carried out on day 20 of gestation and the foetuses were examined. The high dose of 60 and then 40 mg/kg body weight and day was lethal for three dams between days 8 and 15 of gestation, 2 other moribund dams of this group were killed on days 8 and 9 of gestation. Necropsy revealed red areas in the glandular stomach and lungs of the animals in this dose group. In this group, body weight gains and food consumption were decreased from days 6 to 9 of gestation. This was reversible after reducing the dose to 40 mg/kg body weight and day. No effects on the parameters resorptions, live foetuses/litter, foetal body weights or foetal gender ratio were found in any of the dose groups. Neither were any substance-related effects found during external, visceral and skeletal examinations of the foetuses. The NOAEL for maternal toxicity was 20 mg/kg body weight and day, the NOAEL for developmental toxicity 40 mg/kg body weight and day (Burnett et al. 2010).

In another developmental toxicity study, groups of 25 New Zealand White rabbits were given gavage doses of 2-methyl-4-isothiazolin-3-one (purity 51.4%) of 0, 3, 10 or 30 mg/kg body weight and day from days 6 to 28 of gestation. Caesarean section was carried out on day 29 of gestation and the foetuses were examined. In the high dose group, maternal toxicity in the form of reduced defecation and dark red areas in the stomach was observed. One animal aborted on day 25 of gestation. External, visceral and skeletal examination of the foetuses did not reveal any substance-related effects in any of the dose groups. The NOAEL for maternal toxicity was 10 mg/kg body weight and day, the NOAEL for developmental toxicity 30 mg/kg body weight and day (Burnett et al. 2010).

In rats, in the 2-generation study with drinking water described in Section 5.5.1, neither maternal toxicity occurred nor developmental toxicity in the animals of the F1 or F2 generations. In this study the NOAEL in parents and offspring was 69 to 86 mg/kg body weight and day for the males and 93 to 115 mg/kg body weight and day for the females (Burnett et al. 2010).

5.6 Genotoxicity

5.6.1 In vitro

In the mutagenicity test with Salmonella typhimurium TA98, TA100, TA1535 and TA1537 in the presence and absence of a rat liver metabolic activation system (S9 mix), 2-methyl-4-isothiazolin-3-one (purity 99.9%) was not found to be mutagenic in concentrations of 0.0001 to 0.25 µg/plate (TA1535 and TA1537), 0.0001 to 1 µg/plate (TA98) or 0.0001 to 100 µg/plate (TA100). The positive controls yielded the expected results as evidence that the test system functioned properly. 2-Methyl-4-isothiazolin-3-one led to growth inhibition in strain TA100 at 25 µg/plate and above (Burnett et al. 2010).

In another mutagenicity test with Salmonella typhimurium TA98, TA100, TA102, TA1535 and TA1537, 2-methyl-4-isothiazolin-3-one (purity 97.5%) was likewise not found to be mutagenic in concentrations of 5 to 1000 µg/plate in either the presence or absence of a rat liver metabolic activation system (S9 mix). The high concentration was cytotoxic in all strains. The positive controls gave the expected results (Burnett et al. 2010).

The main metabolite, N-methylmalonaminic acid (purity 99.2%), was likewise not mutagenic in concentrations of 1.5 to 5000 µg/plate in tests with Salmonella typhimurium TA98, TA100, TA1535, TA1537 and Escherichia coli WP2 uvrA in the presence and absence of a rat liver metabolic activation system (S9 mix) (Burnett et al. 2010).

In a study in CHO cells, 2-methyl-4-isothiazolin-3-one (purity 97.5%) was tested in concentrations of up to 40 µg/ml with regard to the induction of chromosomal aberrations, both in the presence and absence of a rat liver metabolic activation system (S9 mix). A significant increase was observed only at cytotoxic concentrations (29% to 48% reduction, no other details). The authors of the study regarded 2-methyl-4-isothiazolin-3-one as not genotoxic, and attributed the chromosomal aberrations to the cytotoxicity of the substance (Burnett et al. 2010).

In an HPRT test in CHO cells, 2-methyl-4-isothiazolin-3-one (purity 97.5%) was not genotoxic in the presence and absence of a rat liver metabolic activation system (S9 mix). In an initial test, the cells were exposed to concentrations of 0.5 to 25 µl/ml for 4 hours and cultivated for a further 9 days. In a second test, the cells were exposed to concentrations of 5 to 40 µl/ml for 4 hours and stored in culture for a further 8 days. The positive controls gave the expected results (Burnett et al. 2010).

5.6.2 In vivo

In a UDS test, groups of 4 male Sprague Dawley rats were given 2-methyl-4-isothiazolin-3-one (purity 51.1%) concentrations of 0, 100 or 200 mg/l and 6 animals concentrations of 300 mg/l (10 ml/kg body weight, administration route not specified, presumably gavage). The hepatocytes were isolated after 2 to 4 or 14 to 16 hours

and incubated with ^{3}H-thymidine for 4 hours. 2-Methyl-4-isothiazolin-3-one did not induce UDS (Burnett et al. 2010).

A micronucleus test with 2-methyl-4-isothiazolin-3-one (purity 97.5%) was carried out in the bone marrow of CD-1 mice. For this purpose, groups of 5 male and 5 female mice were given single oral doses of 0, 10, 50 or 100 mg/kg body weight. No increased incidences of polychromatic erythrocytes containing micronuclei were found either 24 or 48 hours after the administration of the substance (Burnett et al. 2010). Details of cytotoxicity are not available.

In another micronucleus test in the bone marrow of C57BI/6J mice, 9 to 10 animals were given intraperitoneal injections of 250 mg/kg body weight on 2 consecutive days. No increased incidences of micronuclei were found either 24 or 48 hours after the administration of the substance. Changes in the ratio of polychromatic to normochromatic erythrocytes were not treatment-related (Richardson et al. 1983).

5.7 Carcinogenicity

There are no studies available for the carcinogenicity of 2-methyl-4-isothiazolin-3-one.

In a valid drinking water study in rats with a microbiocide containing 2-methyl-4-isothiazolin-3-one (about 3.6%) and 5-chloro-2-methyl-4-isothiazolin-3-one, there was no indication of a tumorigenic potential (Burnett et al. 2010).

Also, a dermal carcinogenicity study in mice with a mixture of 2-methyl-4-isothiazolin-3-one (about 3.8%) and 5-chloro-2-methyl-4-isothiazolin-3-one did not provide evidence of an increase in the tumour incidence as a result of the treatment (see supplement "5-Chloro-2-methyl-2,3-dihydroisothiazol-3-one and 2-Methyl-2,3-dihydroisothiazol-3-one" 2007).

6 Manifesto (MAK value/classification)

The critical effects of 2-methyl-4-isothiazolin-3-one are its sensitizing effect and a corrosive effect on the skin, which also suggests it has marked irritative effects on the respiratory tract.

MAK value and peak limitation. In a 4-hour inhalation study, slight to marked reddening of the pulmonary lobes and sporadic, red, pin-head sized foci in the lungs were observed in the exposed rats at 46 mg/m^3 and above. The LC_{50} in this study was 110 mg/m^3. An RD_{50} after the 10-minute exposure of mice was greater than 157 mg/m^3 (44% respiratory depression).

The systemic toxicity after repeated oral administration of the substance to rats (drinking water) and dogs (diet) did not reveal any substance-specific target organs. The NOAEL in rats after administration for 3 months was 66 to 94 mg/kg body

weight and day, and in dogs 41 mg/kg body weight and day. After gavage administration in developmental toxicity studies, doses of 60 mg/kg body weight and day were lethal in rats, and resulted in reduced body weight gains and food consumption in rabbits at 40 mg/kg body weight and day. The higher toxicity after gavage administration is attributed to corrosive effects. As undiluted 2-methyl-4-isothiazolin-3-one is corrosive to the skin, and studies with repeated inhalation exposure in humans or animals are not available, no MAK value can be derived. 2-Methyl-4-isothiazolin-3-one is therefore listed in **Section II b** of the **List of MAK and BAT values**. In this case, peak limitation categories do not apply.

Prenatal toxicity. In developmental toxicity studies with rats and rabbits and in a 2-generation study with rats, no teratogenic or foetotoxic effects were observed up to doses toxic to the dams. The NOAEL in developmental toxicity studies corresponds to the highest dose tested of 40 mg/kg body weight and day in rats and 30 mg/kg body weight and day in rabbits. The NOAELs in the 2-generation study were 69 to 86 mg/kg body weight and day for the males and 93 to 115 mg/kg body weight and day for the females. As no MAK value can be established, classification in a pregnancy risk group does not apply.

Carcinogenicity / germ cell mutagenicity. 2-Methyl-4-isothiazolin-3-one was not found to be mutagenic in bacteria and in HPRT tests with CHO cells. An in vitro chromosomal aberration test yielded positive results only at cytotoxic concentrations. A UDS test in rat hepatocytes and a micronucleus test in mice provided no evidence of genotoxic effects in vivo. No adequate studies of the carcinogenicity of 2-methyl-4-isothiazolin-3-one are available for a conclusive assessment. A study with oral administration in the rat and another with dermal application in the mouse which used a mixture of 2-methyl-4-isothiazolin-3-one (3.6%–3.8%) and 5-chloro-2-methyl-4-isothiazolin-3-one provided no indication of a tumorigenic potential. Based on the available data, 2-methyl-4-isothiazolin-3-one is not suspected of having carcinogenic or germ cell mutagenic potential. 2-Methyl-4-isothiazolin-3-one is therefore not classified in one of the categories for germ cell mutagens or for carcinogens.

Absorption through the skin. Aqueous solutions of 2-methyl-4-isothiazolin-3-one can be expected to have local irritating or corrosive effects on the areas of skin affected at concentrations of 0.1% (corresponding to 1000 ppm) and above. Unnoticed skin contact with the substance is therefore only likely for solutions with concentrations below 0.1%. From in vitro tests with human skin, a flux of 143 µg/cm^2 and hour was obtained for a diluted aqueous solution of 2-methyl-4-isothiazolin-3-one (in a concentration of 313 µg/ml or ppm, corresponding to about 1/3 of a non-irritating concentration of 1000 ppm). Taking this flux as a basis, single 1-hour exposures of both hands and forearms would result in the absorption of 286 µg 2-methyl-4-isothiazolin-3-one, corresponding to 4.1 µg/kg body weight. This calculated absorbed amount is about 7000 times below the NOAEL determined in dogs

of 29 mg/kg body weight and day after toxicokinetic correction (1:1.4). Accordingly, absorption of toxicologically relevant amounts of 2-methyl-4-isothiazolin-3-one through the skin is not to be expected for solutions which are not irritating to the skin. The substance is therefore not designated with an "H" (for substances which are absorbed through the skin).

Sensitization. Numerous clinical reports are available for sensitization to 2-methyl-4-isothiazolin-3-one. Although it is not always evident whether this is to be attributed to exposure to the (formerly) frequently used mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one or to exposure to 2-methyl-4-isothiazolin-3-one alone, there are some results available which show that exposure to the non-chlorinated compound alone can induce sensitization. The results of experimental studies with guinea pigs and mice likewise confirm the contact sensitizing effects of 2-methyl-4-isothiazolin-3-one. Only one case report is available for its effects on the respiratory tract, which is not sufficient to demonstrate that the substance causes sensitization of the airways. 2-Methyl-4-isothiazolin-3-one is therefore designated with "Sh" (for substances which cause sensitization of the skin), but not with "Sa" (for substances which cause sensitization of the airways).

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