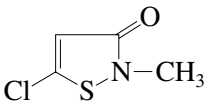
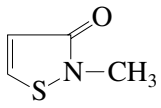
5-Chloro-2-methyl-2,3-dihydroisothiazol-3-one and 2-Methyl-2,3-dihydroisothiazol-3-one* S

Classification/MAK value: 0.05 mg/m^3

MAK value dates from: 1991

Synonyms:	5-chloro-2-methyl-3-isothiazolone	2-methyl-3-isothiazolone
	5-chloro-2-methyl-4-isothiazolin-3(2*H*)-one	2-methyl-4-isothiazolin-3-one 2-methyl-4-isothiazolin-3(2*H*)-one
Chemical name (CAS):	5-chloro-2-methyl-4-isothiazolin-3-one	2-methyl-3(2*H*)-isothiazolone
CAS number:	26172-55-4	2682-20-4
mixture:	55965-84-9	
Structural formula:		
Molecular formula:	C_4H_4ClNOS	C_4H_5NOS
Molecular weight:	149.59	115.15
Impurities:	Kathon® biocide is commercially available under the names Kathon® MW (metalworking), Kathon® WT (water-treating), Kathon® LC (latex), Kathon® CG (cosmetic grade) [1,2].	
Composition:	The commercial product Kathon® (MW) contains 13.9% active isothiazolones [3]: active isothiazolones 13.9% K1 (5-chloro-2-methyl-2,3-dihydroisothiazol-3-one) 10.1% K2 (2-methyl-2,3-dihydroisothiazol-3-one) 3.8%	

* 5-Chloro-2-methyl-2,3-dihydroisothiazol-3-one (K1) and 2-methyl-2,3-dihydroisothiazol-3-one (K2) arise during the industrial production process as a mixture in the ratio 3:1 and are available commercially in this form under the names Kathon® and Kathon® biocide. Since most of the studies described in the present review were carried out with this product, the abbreviation KB is used in the text for this mixture of isothiazolones.

inert materials:	
magnesium chloride	9.0%
magnesium nitrate	15.0%
copper nitrate	–
water	60.8%
organic impurities	1.3%
physical state	liquid
specific gravity	1.32
pH	2–4
solubility in water	100%

Stability: The formulations are stable for at least one year at room temperature and for $^1/_2$ year at 50 °C

Production: The two isothiazolones are obtained as a mixture in the reaction of dithio-N,N'-dimethyldipropionamide in ethylene dichloride with an excess of sulfuryl chloride [4].

Uses: Biocides containing isothiazolones are used as
- broad spectrum biocides for metal-working fluids, glues and waxes and for applications in the leather and textile industries
- microbiocides in cooling water systems
- preservatives in latex emulsions, cosmetics and other products subject to the Cosmetics Act as well as products which are voluntarily produced to meet the requirements of the Act, e.g. washing up liquids, etc [5].

Note

The producer recommends that total active isothiazolone concentrations between 3.4 and 14.6 ppm be used in practice in metal-working fluids [6] and 15 ppm in cosmetics [5].

There is no information available as to decomposition products or chemical reactions of the isothiazolones with other components of these mixtures and emulsions, whether used once or repeatedly.

1 Toxic Effects and Modes of Action

Kathon® biocide (KB) contains two active isothiazolones, 5-chloro-2-methyl-2,3-dihydroisothiazol-3-one (K1) which has predominantly bactericidal action and 2-methyl-2,3-dihydroisothiazol-3-one (K2) which is predominantly fungicidal. Since most studies have been carried out with the commercial product and not with the pure isothiazolones, the following assessment deals with the toxicology of the commercial product which contains the above two components in the ratio 3:1.

The acute toxicity of KB has been investigated after oral administration to rats (LD_{50} *c* 60 mg active isothiazolones/kg), dermal application to rabbits (LD_{50} *c* 80 mg active

isothiazolones/kg) and inhalation by rats (LC_{50} *c* 200–300 mg active isothiazolones/m^3). The biokinetics and metabolism have been studied in the rat; the metabolism has not been elucidated completely. N-Methylmalonamic acid, malonamic acid and malonic acid have been detected as excretion products.

Solutions of the microbiocide which contain more than 0.5% (5000 ppm) active isothiazolones produce severe irritation of human skin and can cause corrosion of mucous membranes and the cornea. Solutions containing > 100 ppm active isothiazolones can irritate the skin; even concentrations as low as 25 ppm can still cause sensitization.

There are no studies of systemic effects in man.

The chronic toxicity of KB has been investigated in several studies with rats and dogs and in one study with rabbits. After administration of KB in the diet to rats and dogs for 90 days, neither deaths, substance-related changes in blood, urine or biochemical parameters nor histopathological changes in any of the principal organs were found. Long-term dermal application of KB to rabbits led to deaths from pulmonary oedema. Apart from dose-dependent erythema with slight oedema and small changes in organ weights, there were no histopathological changes in any of the principal organs in the survivors of this study. No toxic effects, apart from irritation of the upper airways and rhinitis, were seen in rats exposed in a 90-day inhalation study.

In a carcinogenicity study with mice, long-term dermal application of KB did not increase the incidence of skin tumours above that in the control group treated with water. Reproduction and teratogenicity studies in the rat did not yield pathological findings. In another study in rabbits, maternally toxic doses of KB caused increased foetal absorption but no malformations; KB is therefore embryotoxic but not teratogenic.

KB is mutagenic in the Ames test in *Salmonella typhimurium* with and without metabolic activation; KB is also mutagenic in mouse lymphoma cells *in vitro.* In other *in vitro* test systems (cell transformation, UDS) and *in vivo (Drosophila melanogaster,* cytogenetic studies in rat and mouse, micronucleus test) KB could not be shown to have mutagenic activity.

1.1 Pharmacokinetics

The biokinetics and skin absorption of KB have been investigated in several studies with ^{14}C-labelled 5-chloro-2-methyl-2,3-dihydroisothiazol-3-one (K1) and/or ^{14}C-labelled 2-methyl-2,3-dihydroisothiazol-3-one (K2) [1]. After intravenous administration to rats of a single KB dose containing 0.8 mg active isothiazolone per kg body weight with the ^{14}C-label on the chlorinated component, the radioactivity was eliminated rapidly from the plasma; 96 hours after the injection, the ratio of the total ^{14}C-radioactivity in blood (29% of the applied dose) to that in plasma was 10:1. After 24 hours, more than 50 % of the administered radioactivity had been excreted in the urine and faeces, after 96 hours about 70% (faeces 35%, urine 31 %, CO_2 4%). The highest ^{14}C-concentrations (calculated as µg K1-equivalents per g fresh weight) were found in the kidneys (0.45–1.1 ppm) and liver (0.25–0.58 ppm); the level in the testes was only 0.03–0.05 ppm. The half-life of K1 measured as ^{14}C-radioactivity was 300 hours in blood, 38 hours in plasma and between 80 and 120 hours in liver, kidneys and testes [1].

Rats treated dermally (24 hours, occlusive) with 0.2 ml of an aqueous solution of KB containing 2000 ppm active isothiazolones labelled either on K1 or K2 absorbed ^{14}C-labelled K1 more rapidly than ^{14}C-labelled K2 (94% and 82% in 24 hours). The systemic dose was considerably smaller than the absorbed dose because, even 24 hours after the application, about 50% of the absorbed radioactivity remained in the skin at the application site. The percutaneous absorption of KB with ^{14}C-labelled K1 was about 89–94% of the applied dose and was independent of concentration in the range 500–4000 ppm active isothiazolones. The skin at the application site contained more ^{14}C-radioactivity than did any other of the tissues investigated. Since the amount of ^{14}C retained in the skin remained constant with increasing dose while the amount excreted in faeces and urine increased, the systemic dose must be assumed to be greater after dermal application of larger amounts of KB [1].

In a 28-day study with rats (Sprague-Dawley, 36 ♂♂, 4 animals killed on each sacrifice date), 0.2 ml doses of KB containing 2000 ppm active isothiazolones (^{14}C-labelled on K1) were applied occlusively to the skin of the animals for 24 hours (6 hours for the 0.25 day value). The animals were killed 0.25, 1, 2, 3, 4, 7, 14 and 28 days after application of the substance, and ^{14}C-levels determined in tissues and organs. Six hours after the application, 57% of the dose was recovered in the skin. After 28 days this fraction was reduced to 21 %. The highest ^{14}C-levels were found in the blood after 6 hours and in plasma and testes between 6 and 48 hours. The half-lives of the radioactivity in blood, plasma and testes were 11.4, 4 and 8.6 days, respectively. ^{14}C-radioactivity was still detectable in blood, plasma and testes after 28 days. The concentration (calculated as µg K1-equivalents per g fresh weight) was below 0.1 ppm in blood and below 2 ppb in plasma and testes [1]. Unlike elimination after intravenous injection, during the 96 hours after dermal application only 4 % of the ^{14}C-dose was eliminated in the faeces; most of the substance (about 10–18 % of the dose) was excreted in the urine.

N-Methylmalonamic acid was detected as the main metabolite of KB in the urine of rats given oral doses of either of the two isothiazolones. Malonamic acid and malonic acid were also identified as metabolites. According to the authors [7], this is evidence that oxidation, ring-opening and desulfuration play a part in the rapid degradation of the isothiazolones after intravenous injection. N-Methylmalonamic acid and malonamic acid were also found in the faeces where, however, the main metabolite could not be identified.

2 Effects in Man

KB solutions containing more than 0.5 % (5000 ppm) active isothiazolones are highly irritating to human skin and can cause corrosion of mucous membranes and of the cornea [8].

Eight 0.3 ml aliquots of aqueous KB solutions at various concentrations (6.25, 12.5, 25, 50, 100, 200, 400, 800 ppm active isothiazolones) were applied to the backs of each of 12 test persons (1 ♂, 11♀) in a Lauman-Maibach patch test (occlusive patch test, 23 hours daily, 5 days). Severe irritation was seen at 400 and 800 ppm, slight irritation at

200 ppm and no irritation at 100 ppm or at lower concentrations. In a subsequent test for sensitization in the same persons, 6/12 produced a positive reaction to provocation with 100 ppm active isothiazolones; the induction concentration could not be determined with this experimental protocol [9].

Ten test persons were exposed to an aqueous KB solution containing 56 ppm active isothiazolones in a combined skin test (occlusive patch test 24 hours daily,

5 days per week for 4 weeks and simultaneously an arm dip test in which the same persons dipped their arms into the test solution twice daily for 15 minutes, 5 days per week for 4 weeks; after a rest period of two weeks challenge with the same solution for 24 hours). Neither irritation nor sensitization was observed [10].

In another occlusive patch test with 18 test persons, 0.3 ml of an aqueous KB solution containing 25 ppm active isothiazolones was applied for 24 hours daily, 3 days per week for 3 weeks; after a rest period of 2 weeks and challenge with 25 ppm active isothiazolones, a sensitization reaction was seen in one of the test persons [10].

The sensitizing potential of KB in cosmetic formulations (lotions and ointments containing between 6 and 56 ppm active isothiazolones) was also studied. At 56 ppm active isothiazolones, sensitization was observed in 6 of 60 test persons; negative results were obtained with 28 ppm active isothiazolones tested on 20 persons and
6 ppm on 105 persons [10].

A 90-day study of 248 test persons who washed their hair daily with shampoo containing 9 ppm active isothiazolones and were tested with challenge concentrations of 12.5 and 27 ppm active isothiazolones at the end of the study period revealed sensitization in one person [10].

Cumulative irritation assays (see Table 1) carried out with aqueous KB solutions (13 persons tested with 1, 10, 15, 25, 50 ppm and 12 persons with 100, 200, 300 ppm active isothiazolones) revealed slight irritation in only 3 of the persons treated with concentrations up to 100 ppm for 21 days (0.2 ml, 5 days per week, 21 applications). Only at concentrations of 200 and 300 ppm active isothiazolones was more severe irritation seen in 4 test persons. Sensitization testing with 100 ppm active isothiazolones after a 2-week treatment-free interval produced positive reactions in 4/12 persons [11].

In the Draize sensitization test (see Table 1), no sensitization was observed in 96 persons treated with induction and challenge concentrations of 50 ppm active isothiazolones (0.2 ml applied occlusively 3 times weekly for 3 weeks) [11]. When 52 of these persons were treated again with a challenge concentration of 100 ppm active isothiazolones, one person was shown to have been sensitized.

In a second study with 104 test persons and induction and challenge carried out with an aqueous solution containing 100 ppm active isothiazolones, two persons were shown to be sensitized to KB. A comparable study with 89 persons treated with 100 ppm active isothiazolones in KB in vaseline yielded negative results [11].

The effects of KB in cosmetic formulations (c 5 ppm active isothiazolones) was tested on 18 persons shown to react with contact allergy to challenge with 100 ppm KB.

Table 1. Clinical studies with Kathon® biocide (KB) (concentrations in ppm active isothiazolones)

Conc. ppm	Solvent	Test	Test persons	Number of persons with reactions			Ref.
				A	**I**	**S**	
1000	H_2O	patch test (ICDRG)	36	8	0	0	[12]
1000*			40*	0	10	5	
+ 300*				0	0	5	
300			976	43 (4.4%)	0	8	
250			170	10 (5.9%)	0	2	
100			210	4 (1.9%)	0	0	
7			2006	0	0	0	
100			34**	17			
30			34**	8			
10			34**	2			
20	H_2O	patch test (Draize: applied 9 times in 3 weeks)	45			2	[13]
15			200			0	
12.5			84			1	
10			602			0	
6			103			0	
5			416			0	
			1450* **			**3*** **	
150 (1 % CG)	vaseline	patch test	179	6 (3.4%)			[14]
100 (0.67% CG)	vaseline	patch test	501	7 (1.4%)			[15]
300	H_2O	patch test (ICDRG)	645	6 (0.9%)	15		[16]
200			526	3 (0.6%)	3		
150			534	4 (0.7%)	3		
100			124	0	1		
100	H_2O	patch test	167	0			[17]
			260	3 (1.2%)			
			292	2 (0.7%)			
			151	1 (0.7%)			
			306	13 (4.2%)			
			285	14 (4.9%)			
			1461	**33** (2.3%)			
100	H_2O	patch test	1511	13 (0.8%)			[18]
15	H_2O	patch test	300	0			[19]
50	H_2O	cumulative irritation during 21 d: 5 d/week, 21 applications of 0.2 ml	13	neither irritating nor sensitizing with 50 ppm challenge concentration			[11]
25							
15							
10							
1							

Table 1 (continued)

Conc. ppm	Solvent	Test	Test persons	Number of persons with reactions			Ref.
				A	**I**	**S**	
300 200 100	H_2O	cumulative irritation during 21 d	12	minimal irritation in 3 persons at 100 ppm, irritating for 4 persons from 200 ppm			[11]
100 50 25	vaseline	cumulative irritation during 21 d	14	neither irritating nor sensitizing			[11]
50		sensitization according to Draize	96 52	no sensitization with 50 ppm challenge concentration, positive result in one person (1.9%) when test repeated with 100 ppm			[11]
100	H_2O	(0.2 ml, occlusive)	104	2 positive (1.0%) with 100 ppm challenge concentration			[11]
100	vaseline	(3 ml/week for 3 weeks)	89	negative			
15		13-week use test with lotion	209	neither irritating nor sensitizing with 100 ppm challenge concentration			[20]
1.5 (0.01 % CG)	H_2O	patch test	98	6 allergic (6.1 %)			[21]
Patients with proven contact allergy to Kathon® biocide (KB)							
				allergic reaction			
12		non-occlusive application of a skin ointment or lotion, 2 × /day	5	3 (60%)			[2]
7			10	5(50%)			[17]
15			2	1 (50%)			[17]
15–19			12	6(50%)			[22]
15			13	7(54%)			[12]
7.7–8.6			11	–			[18]

A allergic reaction, **I** irritant reaction, **S** patch test sensitization

* 40 persons patch tested with 1000 ppm and 300 ppm simultaneously

** 34 persons who had given positive reactions to 300 ppm or 250 ppm in a former test series

*** this result was confirmed in a repeat study with a challenge concentration of 100 ppm

CG cosmetic grade (Kathon® biocide)

The cosmetics were used by all test persons regularly for 3 to 6 weeks without producing any allergic reactions. When the threshold concentration was determined subsequently in 9 of the 18 persons, no allergic reaction was seen at concentrations up to 15 ppm active isothiazolones in water. Allergic reactions could be induced in one person with 25 ppm active isothiazolones and in another 6 with 50 ppm [23]. Irritative, allergenic and sensitizing effects of KB in persons coming into contact with the biocide at work [24–

27] or in cosmetics [28–30] have been described in a few case studies. The results of several studies involving large numbers of test persons (mostly patients with suspected contact dermatitis) are shown in Table 1.

The reactions of more than 3000 test persons to concentrations between 1000 and 7 ppm active isothiazolones were examined in routine ICDRG patch tests (Table 1 [12]). Whereas concentrations of 1000 ppm caused skin irritation, at concentrations of 300 ppm or less no irritation was seen. Allergic reactions were seen in 4.4 % of the persons tested with 300 ppm, 5.9% with 250 ppm and 1.9% with 100 ppm; sensitization developed at concentrations between 250 ppm and 1000 ppm.

A total of 1450 persons were subjected to a patch test carried out according to the method of Draize with active isothiazolone concentrations between 5 and 20 ppm; sensitization reactions were seen in two persons at 20 ppm and one at 12.5 ppm [13].

In another patch test, allergic reactions were seen in 3.4% of 179 persons tested with 150 ppm active isothiazolones and in 1.4% of 501 persons with 100 ppm [14,15].

When 1800 persons were subjected to an ICDRG patch test with active isothiazolone concentrations of 100–300 ppm, skin irritation was seen at all the concentrations tested including 100 ppm; the incidence of irritation increased with the dose. Allergic reactions to KB were seen in 0.9 % of persons at 300 ppm, 0.6 % at 200 ppm, 0.7% at 150 ppm and 0% at 100 ppm [16].

Allergic reactions were also seen in 0.8 % of 1511 patients with suspected contact dermatitis after a patch test with 100 ppm active isothiazolones [18].

Negative results were obtained in all 300 persons examined after a patch test with 15 ppm active isothiazolones [19].

In two double blind studies in which a total of 209 test persons without contact allergy to KB (at 100 ppm active isothiazolones) took part in a 13 week use test (15 ppm active isothiazolones in a body lotion), KB was classified as neither irritating nor sensitizing (challenge concentration 100 ppm) [20].

In a recent review article [2], the incidence of sensitization revealed after challenge with an active isothiazolone concentration of 100 ppm in large collectives of patients with suspected contact dermatitis is given for various countries, for Sweden 1.9%, for Denmark 0.9%, Finland 2.9%, Holland 3.3% and West Germany 5.7%. For Switzerland, an average sensitization frequency of 3.5% (challenge concentration 100 ppm active isothiazolones) has been published [31]. It has been demonstrated with patch tests [31] and in tests with non-occlusive application of ointments containing KB [2] that the threshold concentrations required to produce a reaction in patients known to have contact allergy to KB are in the range between 7 and 25 ppm active isothiazolones (Table 1).

The results of one study, however, do not fit into this pattern. After application of 1.5 ppm active isothiazolones in patch tests to a total of 98 patients with facial contact dermatitis, there were 6 positive results [21].

After chromatographic separation of the components of KB, it was possible to demonstrate that the two isothiazolones do not contribute equally to the allergenic effects of the biocide. Whereas 28 patients known to have a contact allergy to KB all produced an allergic reaction to the chlorinated component K1, only two positive patch test results were obtained with K2 [32].

The systemic effects of KB in man have not been studied.

3 Effects on Animals

3.1 Acute toxicity

The oral LD_{50} for KB in the rat is 60 mg active isothiazolones per kg body weight, the dermal (24 hour) LD_{50} for the rabbit is 80 mg active isothiazolones per kg body weight. The symptoms of intoxication observed in the rat included lethargy, ptosis, diarrhoea, lacrimation and salivation, in the rabbit lethargy, erythema and oedema [33, 34].

In a 4-hour inhalation study in which rats were exposed to the saturated vapour of a solution containing about 14% active isothiazolones, no deaths were observed. The LC_{50} is given as > 650 mg/m^3 active isothiazolones.

In another 4-hour inhalation study, rats were exposed to aerosol concentrations of 0.7, 1.0, 1.5, 2.0 and 20.2 mg/l of a product containing about 14% active isothiazolones. The LC_{50} of the product was shown to be 1.2–2 mg/l, that is 200–300 mg/m^3 active isothiazolones. The observed toxicity symptoms included dyspnoea, salivation, pulmonary oedema and haemorrhage [35, 36].

Single doses of 0.5 ml of an aqueous solution of KB (560, 2800 or 5600 ppm active isothiazolones) were applied to the dorsal skin of rabbits; after 24 and 72 hours at 5600 ppm severe skin damage was seen, at 2800 ppm moderate damage and at 560 ppm no effect [37].

In a modified Draize test, undiluted KB (13.9 % active isothiazolones) caused very severe irritation when applied to rabbit skin in 0.5 ml doses [38].

Instillation of 0.1 ml of an aqueous solution of KB containing 560 ppm active isothiazolones into the rabbit eye did not produce irritation. Higher concentrations caused dose-dependent mild to severe irritation [39]. After instillation into the rabbit eye of a single dose of undiluted KB containing 13.9% active isothiazolones, clouding of the cornea, chemosis and swelling of the eyelids were observed [40].

Likewise, in the chicken embryo test, KB solutions containing 15 or 1.5% active isothiazolones proved to be highly irritating [41].

3.2 Subacute and subchronic toxicity

There are several studies of the subacute and subchronic toxicity of KB in the rat (4), dog (2) and rabbit (2) (Table 2).

In a feeding study, rats were given active isothiazolone doses of 0.8, 2.5, 8.2 or 24.4 mg/kg body weight and dogs 1.3, 4.4, 14.7 or 32.5 mg/kg body weight daily for two weeks. No substance-related deviation from the controls was seen in body weights or in blood, urine or biochemical parameters and no gross organ changes [43,44].

In a 90-day study with KB administered in the diet, rats were given daily active isothiazolone doses of 2.9, 9.5 or 29.1 mg/kg body weight and dogs daily doses of 2.7, 8.9 or 26.9 mg/kg body weight; no deaths were observed. Substance-related changes in blood, urine or biochemical parameters were not detected. Histological examination of all principal organs and tissues revealed no pathological changes [45, 46].

Table 2. Subacute and subchronic toxicity of Kathon·biocide (KB)

Species (strain)	Number	Application route	Dose*	Duration	Observations	Ref.
rabbit (albino)	50	occlusive patch test	0.0056 and 0.03 mg/kg	5 d/week, 3 weeks	marked local skin irritation, no signs of systemic toxicity	[42]
rat (CD)	50	diet	0.82, 2.54, 8.21, 24.38 mg/kg/d	2 weeks	no deviations from control values in toxicologically relevant parameters; *autopsy:* no lesions	[43]
dog (beagle)	10	diet	1.3, 4.4, 14.7, 32.5 mg/kg/d	2 weeks	no deviations from control values in toxicologically relevant parameters; *autopsy:* no lesions	[44]
rat (CD)	120	diet	2.9, 9.5, 29.1 mg/kg/d	7 d/week, 90 d	no deaths, no substance-related changes, no histopathological changes in organs or tissues estimated NOEL >9.5 mg/kg/d	[45]
dog (beagle)	32	diet	2.7, 8.9, 26.9 mg/kg/d	7 d/week, 90 d	estimated NOEL >26.9 mg/kg/d	[46]
rabbit (New Zealand White)	48	dermal	100, 200, 400 ppm	1×/d, 5 d/week, 13 weeks	12 deaths, dose dependent: 0 (control)/3/5/4; minimal irritation, no conspicuous microscopic changes; estimated NOEL *c* 200 ppm	[47]
rat (COBS CD)	150	drinking water	2–3, 6.3–10.8, 16.3–24.7 mg/kg/d	7 d/week, 90 d	mild gastric irritation in 7/15 ♂ and 5/15 ♀ and slight changes in biochemical parameters in the highest dose group; NOEL 6.3–10.8 mg/kg/d	[48]
rat (Crl:CD BR)	128	aerosol	0.027, 0.23, 0.89 mg/m^3	6 h/d, 5 d/week, 13 weeks	no deaths; in the highest dose group: reduced body weight gain, reduced serum protein levels in ♀, spleen weight reduced in ♂, NOEL 0.027 mg/m^3	[49]

* dose of active isothiazolones

After administration of KB to groups of 75 male and 75 female rats in daily active isothiazolone doses of 2–3, 6.3–10.8 or 16.3–24.7 mg/kg in the drinking water, 7 days weekly for 90 days, histopathological examination revealed slight gastric irritation (7/15 ♂, 5/15 ♀) in the highest dose group but no other pathological findings in any other of the principal organs or tissues. In the animals of the highest dose group, increases in relative liver weights in males and relative kidney weights in females were recorded; in the males there were also quantitative changes in serum proteins and in the females the serum glutamate oxaloacetate transaminase activity was increased [48].

In a 90-day inhalation study, rats inhaled active isothiazolone concentrations of 0, 0.027, 0.23 or 0.89 mg/m^3 in the form of an aerosol, 6 hours daily, 5 days per week for 13 weeks. In the highest dose group, body weight gain was reduced relative to the controls in both sexes, serum protein levels were reduced in the females and spleen weights in the males. Histopathological examination revealed mild rhinitis in the 0.23 mg/m^3 group [49].

In an epicutaneous test, an aqueous solution of KB containing 0.056% active isothiazolones was applied occlusively to rabbits in daily doses of 0.0056 or 0.03 mg active isothiazolones per kg, 5 days per week for 3 weeks. KB produced marked local irritation. Systemic effects were not observed [42].

In a 90-day study with groups of 6 male and 6 female rabbits, an aqueous solution of 0, 100, 200 or 400 ppm active isothiazolones was applied to the shaved skin in single daily doses of 1 ml/kg body weight, 5 days weekly for 13 weeks. Deaths of 12/48 animals during the first 9 weeks, mostly from pulmonary oedema, were distributed almost equally among the dose groups (3 at 100 ppm, 5 at 200 ppm, 4 at 400 ppm). Although no deaths occurred in the control group, the authors of the study did not attribute the pulmonary oedema to direct systemic toxicity associated with KB but considered that it was related to endemic respiratory disease. In the survivors, apart from dose-dependent erythema with slight oedema and small changes in organ weights (reduced thyroid weight, increased heart and liver weights), there were no histopathological changes in any of the principal organs or tissues [47].

4 Allergenic Effects

To test KB for its potential to cause contact sensitization, guinea pigs were treated with 9 induction doses of solutions containing between 25 and 2000 ppm active isothiazolones on occlusive patches, 3 times weekly for 3 weeks. Provocation was carried out 12 to 15 days after the last induction treatment with concentrations between 20 and 2000 ppm active isothiazolones. The contact sensitization proved to be dependent on both induction and provocation doses. A concentration range in which contact sensitization did not occur or was not detectable was established and described by the maximal induction/provocation concentrations of 2000/20, 1000/ 50, 500/100, 50/100 and 25/200 ppm [50].

In a sensitization study carried out in guinea pigs according to the method of Magnusson and Kligman, the animals were given two intradermal injections of 0.1 ml KB containing 56 ppm active isothiazolones with Freund's adjuvant and then, 6 days

later, 0.5 ml of the same solution on an occlusive patch applied for 48 hours. Provocation with a challenge dose of 0.5 ml containing 56 ppm active isothiazolones applied occlusively demonstrated that the concentration of KB applied in this test was not sensitizing [51].

In another Magnusson and Kligman guinea pig maximization test the animals were given induction doses of 15 ppm active isothiazolones by intradermal injection and 3000 ppm on occlusive patches. Provocation with 300 ppm active isothiazolones produced skin reactions in 15/20 animals; 3/20 were classified as sensitized, 12/20 as not clearly sensitized; a second provocation with 150 ppm active isothiazolones produced negative results in all animals [52].

Other authors observed a sensitizing effect of KB after 24 hours in the Magnusson and Kligman guinea pig maximization test in 16/20 animals after induction with 45 ppm active isothiazolones and provocation with 100 ppm active isothiazolones and in the "Freund's complete adjuvant test" (induction 45 ppm, provocation 1000 ppm active isothiazolones) in 10/10 animals [53].

5 Reproductive and Developmental Toxicity

In a one generation reproduction study, groups of 10 COBS CD rats per sex and dose were given active isothiazolone doses of 0, 2–3, 6.3–10.8 or 16.3–24.7 mg/kg/ day, daily for 105 days in the drinking water, after which the reproductive phase of the study followed immediately. There were no detectable effects of KB on the fertility or health of the animals nor on the survival of the foetuses [48].

In a teratogenicity study, 4 groups of 25 Sprague-Dawley CR rats were given active isothiazolone doses of 1.4, 4.2 or 14 mg/kg/day or distilled water (control group) orally from day 6 to day 15 of gestation. In comparison with the control group, no treatment-related effects were seen either in the dams (body weights, number of pregnancies, implantations, resorptions, etc) or in the foetuses (organ or skeletal anomalies) [54].

In another teratogenicity study, 5 groups of 15 pregnant rabbits were given active isothiazolone doses of 1.5, 4.4 or 13.3 mg/kg/day or distilled water (1 ml/kg/day; control group I) or distilled water with added magnesium nitrate and chloride (1 ml/kg/day; control group II) orally from day 6 to day 18 of gestation. The medium and the high KB doses were lethal for more than 80% of the dams by about day 20. Even in the low dose group, the mortality of the dams was about 30%. Hypoactivity, ataxia, salivation and diarrhoea were observed preterminally in the dams. In most cases, autopsy revealed corrosion of the stomach mucosa and associated haemorrhage. In the low dose group, post-implantation losses were increased and there was a slight increase in foetal mortality. The number of implantations and *corpora lutea* were unchanged as were the foetal weights and the sex ratio. Malformations or other anomalies were not observed in the surviving foetuses so that KB may be considered to be embryotoxic but not teratogenic [55].

6 Genotoxicity

The mutagenicity studies with KB which were available in 1984 have been reviewed and assessed in the literature [56].

The mutagenicity of KB was tested in the *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 with and without a metabolizing system (S9 mix from Aroclor-induced rat liver). Active isothiazolone concentrations of 0.099–0.990 µg/ plate were used. Without the metabolic system, KB was extremely toxic to the bacteria and the toxicity increased markedly with the dose. With S9 mix the toxic effects were first seen at higher concentrations. KB was not mutagenic in the strains TA1535, TA1537 or TA98. In TA100 without a metabolic system at concentrations of at least 0.1–0.3 µg/plate a significant mutagenic effect was detected which was not seen in the presence of S9. An analogous study with K1 (purity 99.95%) and K2 (purity 99.99%) revealed no mutagenic effects of K2 in any of the four strains of bacteria with or without metabolic enzyme systems. The chlorinated compound K1 was mutagenic only in TA100 and only in the absence of S9 [57].

The absence of mutagenic activity of KB in the *S. typhimurium* strains TA1535, TA1537 and TA98 was confirmed in another study; at active isothiazolone concentrations of 0.134 µg/plate and more, markedly dose-dependent mutagenic effects were seen in strain TA100 without metabolic activation [58]. In contrast to the results mentioned above [57], dose-dependent mutagenicity was also detected in the presence of S9 mix, although the effects of the same concentrations of KB were less.

In agreement with these results, other groups [58, 59] found that the number of revertant colonies of the *S. typhimurium* strain TA100 was increased in a dose-dependent manner, without S9 mix at active isothiazolone concentrations of 0.03 µg/plate and more and with S9 mix from concentrations of 0.3 µg/plate.

Similarly, in *Escherichia coli* WP2uvrA(p), mutagenic effects were seen with active isothiazolone concentrations of 0.268 µg/plate and more with S9 mix from Aroclor-induced rat liver but were not detectable without S9 mix because of the toxic effects of the substance [58].

In a mutagenicity study with mouse lymphoma cells (L5178Y) *in vitro,* KB had mutagenic activity both with and without S9 mix from Aroclor-induced rat liver [57].

Other mutagenicity studies with KB yielded negative results. No cell transformation was observed in a cell transformation test with C3H10T$^{1}/_{2}$ mouse embryo fibroblasts with active isothiazolone concentrations of 0.0098–0.156 µg/ml [57].

Studies of gene mutations in *Drosophila melanogaster* revealed no mutagenicity under the chosen test conditions (feeding with a solution containing active isothiazolones at a concentration of 52 or 86 µg/ml or injection of 0.3 µl of an aqueous solution with 258 µg/ml) [57].

KB yielded negative results in cytogenetic studies of rat [60] and mouse [61] bone marrow; no chromosomal aberrations were observed after intraperitoneal injection of active isothiazolone doses of 0.28, 2.8 or 28 mg/kg per day for 5 days (rat) or oral doses of 1.5, 6 or 15 mg/kg per day for 5 days (mouse). Negative results with KB were also obtained in an *in vitro* UDS test in cultured rat hepatocytes [57].

In micronucleus tests, the two isothiazolones, K1 and K2, were administered separately to mice (C57B1/6J) by intraperitoneal injection in doses of 250 mg/kg on two

consecutive days. Bone marrow smears were prepared after 24 and 48 hours. For neither substance was the number of micronuclei increased relative to the control values [62].

A DNA binding study in mouse lymphoma cells *in vitro* was carried out with KB in which the chlorinated isothiazolone component K1 was ^{14}C-labelled (specific activity about 23000 dpm/µg). No covalent binding of ^{14}C to the DNA could be detected (detection limit about 1 in 1.6×10^5 nucleotides) after incubation of the cells and isolation of the DNA [63].

A single dose of 0.2 ml of an aqueous solution of KB containing 0.2% active isothiazolones (chlorinated isothiazolone component K1 ^{14}C-labelled, specific activity as above) was applied to the shaved dorsal skin of rats and, 24 hours later, DNA was isolated from the testes. No covalently bound ^{14}C could be detected on the testicular DNA (detection limit about 1 in 6.7×10^5 nucleotides) [63].

7 Carcinogenicity

In a dermal carcinogenesis study, 25 µl of an aqueous KB solution containing 400 ppm active isothiazolones (c 0.4 mg/kg body weight) was applied to the shaved dorsal skin of each of 40 male CD-1 mice, 3 times weekly for 30 months. Groups of 40 control animals were treated according to the same protocol with either 25 µl tap water or 25 µl of a solution of methylcholanthrene in acetone (*c* 1 mg/kg body weight). Whereas all the animals treated with methylcholanthrene developed squamous cell carcinomas at the application site, two of those treated with KB developed tumours, a haemangioma and a haemangiosarcoma of the skin at the application site. The authors considered that these tumours were not treatment-related because a haemangiosarcoma was also found in the liver in one of these animals and such skin tumours also occurred in the control group treated with tap water, in this case, however, not at the application site. In the animals treated with KB, epidermal necrosis, hyperplasia, hyperkeratosis and epidermal inflammation were observed. In the middle of the study, the percentage of survivors in the group treated with KB was slightly reduced relative to the water controls but after 30 months survival was comparable in the treated and control groups. The animals were subjected to detailed histological examination and the results indicate that KB applied dermally under the conditions of this study has neither dermal nor systemic carcinogenic potential [57, 64].

8 Manifesto (MAK value, classification)

At present, the main known effects of KB on health are skin irritation and allergenic effects. Case reports and animal studies demonstrated that solutions of the biocide containing more than 0.5% active isothiazolones cause corrosion of mucous membranes and the cornea.

The results of studies with large numbers of test persons indicate that active isothiazolone concentrations of 100 to 300 ppm and more must be expected to cause skin irritation. Dermatological studies have demonstrated that active isothiazolone concentrations below 20 ppm can cause sensitization to KB and that allergic reactions can be provoked in sensitized persons even with concentrations in the range of 7–15 ppm active isothiazolones.

KB is a biocide with bactericidal and fungicidal properties; at the concentrations generally used it is cytotoxic and directly mutagenic in *in vitro* mutagenicity tests in two species of bacteria and one mammalian cell line. Negative results were obtained in studies of the DNA-damaging potential of KB in mammalian cells *in vitro* and of cytogenetic effects and DNA-binding *in vivo.* KB had no carcinogenic effects when applied dermally in a long-term animal study.

There are no studies which permit the determination of the effects on man of known concentrations of KB in air. Therefore the results of the 90-day inhalation study with rats are used for the establishment of a MAK value. In this study, the most sensitive criterion for an effect of KB was found to be the diagnosis "rhinitis" which was found at an active isothiazolone concentration of 0.23 mg/m^3 but not at 0.027 mg/m^3. Thus the MAK value is established at 0.05 mg/m^3. Because of its high sensitization potency, the substance must be designated with an "S". KB is classified in pregnancy group D (with a tendency towards C).

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