The MAK Collection for Occupational Health and Safety

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N-Butyl-1,2-benzisothiazolin-3-one

MAK Value Documentation – Translation of the German version from 2021

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Keywords

N-butyl-1,2-benzisothiazolin-3-one; skin sensitization; irritation; metabolism; toxicokinetic; toxicity

Abstract

The German Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission) summarized and evaluated the data for *N*-butyl-1,2-benzisothiazolin-3-one [4299-07-4] to derive an occupational exposure limit value (maximum concentration at the workplace, MAK value) considering all toxicological end points. The critical effect is the corrosive action of *N*-butyl-1,2-benzisothiazolin-3-one on the skin of rabbits. This strong irritant effect was also observed in oral repeated dose studies in rats (stomach ulcers) and dogs (vomiting). As a repeated inhalation study is not available, a MAK value cannot be derived. *N*-Butyl-1,2-benzisothiazolin-3-one did not lead to developmental toxicity in rats up to an oral dose of 300 mg/kg body weight and day; this dose was toxic to the dams (stomach ulcers). Germ cell mutagenicity tests are not available. An in vitro mouse lymphoma test with *N*-butyl-1,2-benzisothiazolin-3-one was negative. A positive chromosomal aberration test in human lymphocytes was not confirmed in vivo by the results of a mouse micronucleus test. A carcinogenicity study has not been performed with *N*-butyl-1,2-benzisothiazolin-3-one. Skin contact is not expected to contribute significantly to systemic toxicity. Animal studies on sensitizing effects yielded positive and negative results. As the limited human data show clinically relevant skin sensitization, *N*-butyl-1,2-benzisothiazolin-3-one is designated with “Sh”. There are no data for sensitizing effects on the respiratory tract.

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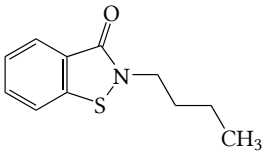
MAK value	**not yet established, see Section II b of the List of MAK and BAT Values**
Peak limitation	–
Absorption through the skin	–
Sensitization (2020)	**Sh**
Carcinogenicity	–
Prenatal toxicity	–
Germ cell mutagenicity	–
BAT value	–
Synonyms	2-butyl-1,2-benzisothiazol-3(2H)-one 2-*n*-butylbenzo[d]isothiazol-3-one 2-butyl-2,3-dihydro-1,2-benzothiazol-3-on
Chemical name (IUPAC)	2-butyl-1,2-benzisothiazolin-3-one
CAS number	4299-07-4
Structural formula	
Molecular formula	$C_{11}H_{13}NOS$
Molar mass	207.29 g/mol
Melting point	no data; liquid at 20 °C (ECHA 2020 a)
Boiling point at 1013 hPa	> 300 °C (ECHA 2020 a)
Vapour pressure at 25 °C	0.00015 hPa (ECHA 2020 a)
log K_{OW}	2.86 at 25 °C (ECHA 2020 a); 2.32 (no other data; US EPA 2016)
Solubility	< 0.5 mg/l water at 25 °C, pH: 6.7 (ECHA 2020 a)
1 ml/m³ (ppm) ≙ 8.601 mg/m³	**1 mg/m³ ≙ 0.116 ml/m³ (ppm)**
Stability	no data
Production	no data
Purity	no data
Impurities	no data
Use	as microbiocidal, microbiostatic, fungicidal and fungistatic additive in metal-working fluids (only in closed systems), plastics, polymers and building materials (US EPA 2016)

N-butyl-1,2-benzisothiazolin-3-one is currently undergoing the biocide approval process at the European Chemicals Agency (ECHA). The data have not yet been published (ECHA 2020 b).

The documentation is based mainly on publicly available registration data from the U.S. Environmental Protection Agency (US EPA 2016).

The concentrations for the use of *N*-butyl-1,2-benzisothiazolin-3-one in metal-working fluids are given as follows (US EPA 2016): initial use 200 µl/l, maintenance concentration 30 µl/l.

1 Toxic Effects and Mode of Action

N-Butyl-1,2-benzisothiazolin-3-one is corrosive to the skin of rabbits and has sensitizing effects on the skin of humans and guinea pigs.

In a 90-day study in rats, body weight gains and food consumption were reduced in males and females in the high dose groups given *N*-butyl-1,2-benzisothiazolin-3-one doses of 149.2 mg/kg body weight and day and 162.4 mg/kg body weight and day, respectively. Histopathology revealed irritation of the forestomach. In a 90-day study in male and female beagles, vomiting, diarrhoea, soft or mucoid faeces, and a dose-dependent decrease in serum albumin (males) and total protein (females) were observed at 75 mg/kg body weight and day and above.

In a 2-generation study in Sprague Dawley rats, decreased body weights and decreased food consumption were observed in the parent animals at the highest *N*-butyl-1,2-benzisothiazolin-3-one doses tested (141 and 157 mg/kg body weight and day in males and females, respectively). Decreased body weights, body weight gains and spleen weights (F2 generation) were observed in the offspring at the same dose. No effects on fertility were observed up to the highest dose tested. In a developmental toxicity study in rats, gavage doses of 100 mg/kg body weight and day and above led to ulceration of the stomach. Developmental toxicity was not observed in the foetuses up to the highest dose tested of 300 mg/kg body weight and day.

A $TK^{+/-}$ mutation assay with L5178Y mouse lymphoma cells yielded negative results. In a chromosomal aberration test with human lymphocytes, *N*-butyl-1,2-benzisothiazolin-3-one was clastogenic. This was not confirmed in vivo in a micronucleus test with erythrocytes from the bone marrow of CD mice. In addition, in an in vivo/in vitro assay, DNA repair synthesis (UDS) in rat hepatocytes was not induced.

There are no studies of the carcinogenicity of *N*-butyl-1,2-benzisothiazolin-3-one.

2 Mechanism of Action

There are no data available.

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

A single gavage dose of [^{14}C]-*N*-butyl-1,2-benzisothiazolin-3-one (labelled at the benzene ring, > 98% radiochemical purity) was given to male Han-Wistar rats (2 animals per dose for the study in expired air, 20 animals per dose for the distribution-in-tissue study) at doses of 5 or 300 mg/kg body weight. The highest levels of radioactivity were measured in the blood 1 hour and 4 hours, respectively, following the administration of 5 and 300 mg/kg body weight; these declined rapidly and were no longer detectable after 48 hours. In the bladder and gastrointestinal tract, the maximum radioactivity was observed 1 and 4 hours after administration of the substance, respectively, and likewise decreased rapidly within 48 hours. Three days after substance administration, 0.3% of the administered dose remained in the lymph nodes, abdominal fat, pancreas, skin, residual body, liver and kidneys. Most of the administered radioactivity was

excreted with the urine and faeces. The amounts in the expired air were negligible. The distribution of the radioactivity in tissue and the elimination profiles were similar at both doses (US EPA 2016).

In a study of the distribution in tissue over time, gavage doses of [^{14}C]-*N*-butyl-1,2-benzisothiazolin-3-one (labelled at the benzene ring, > 98% radiochemical purity) were administered to 30 male Han-Wistar rats at a level of 5 mg/kg body weight and day for 14 days. Selected tissues were examined for radioactivity up to 60 days following the last dose. Most of the administered radioactivity was excreted in the urine and faeces. About 87% of the 1st dose and 92% of the 14th dose were eliminated in the 24-hour urine after substance administration. The highest concentrations of radioactivity were found in the gastrointestinal tract (including its contents), followed by the skin, bladder, residual carcass, liver and kidneys. The high concentrations in the gastrointestinal tract can be explained by excretion, liver and kidneys being the main organs of metabolism. Those found in the skin and residual carcass (0.440 and 0.201 µg equivalent/g tissue, respectively) can be explained by lipophilic deposits in these tissues. After termination of dosing, the residual radioactivity declined rapidly in most tissues and reached background levels after 7 days. In the liver, kidneys, skin and residual carcass, the decline was slower, and radioactivity was still detectable in the skin and residual carcass after 60 days. The half-lives of *N*-butyl-1,2-benzisothiazolin-3-one in the skin and residual carcass were 29.6 and 27.4 days, respectively (US EPA 2016).

For a saturated aqueous solution, the models of Fiserova-Bergerova et al. (1990) and IH SkinPerm (Tibaldi et al. 2014) yielded fluxes of 0.134 and 0.006 µg/cm^2 and hour, respectively. Assuming the exposure of 2000 cm^2 of skin (area of hands and forearms) for 1 hour, this would correspond to absorbed amounts of 0.268 and 0.012 mg, respectively.

3.2 Metabolism

In a metabolism study, gavage doses of [^{14}C]-*N*-butyl-1,2-benzisothiazolin-3-one (labelled at the benzene ring, > 98% radiochemical purity) were administered to male Han-Wistar rats (4 animals per dose for the pharmacokinetic study, 2 per dose for the expired air study, 4 per dose for the excretion study, and 20 per dose for the tissue distribution study). A single dose of 5 or 300 mg/kg body weight was used, and [^{14}C]-*N*-butyl-1,2-benzisothiazolin-3-one doses of 5 mg/kg body weight and day were administered for 14 days. The metabolism after the administration of 5 or 300 mg of [^{14}C]-*N*-butyl-1,2-benzisothiazolin-3-one was similar and largely complete. Less than 0.5% of the administered dose was recovered from the urine and faeces in unmetabolized form. Only trace amounts of metabolites were found in the expired air. The main metabolites in the urine were a methyl sulfoxide derivative (26% to 27% of the administered dose), an *S*-glucuronide metabolite (23% to 24%), and a methyl sulfoxide metabolite with a carbonyl group (11% to 18%). These metabolites were also detected in the faeces, although at lower and more variable concentrations. Based on the metabolites, two metabolic pathways are postulated. One pathway involves the opening of the isothiazoline ring followed by conjugation of the sulfur atom with glucuronic acid. The second pathway involves direct oxidation and methylation of the sulfur atom in the isothiazoline ring to form the methyl sulfoxide, which is then further oxidized and/or *N*-demethylated at the *N*-alkyl side chain. The administration of 5 mg/kg body weight and day for 14 days to rats yielded a qualitatively similar metabolite profile (US EPA 2016).

4 Effects in Humans

There are no data available for the end points single exposures, repeated exposure, sensitizing effects on the airways, reproductive toxicity, genotoxicity and carcinogenicity.

There are only two publications on occupational sensitization to *N*-butyl-1,2-benzisothiazolinone. The substance is not available as a commercially available test preparation.

One case involved a metal worker who worked at a CNC (Computerized Numerical Control) machine with recurrent eczema on 2 fingers of each hand that had been present for 5 months at the workplace. In the patch test he produced weak reactions to the metal-working fluid used. Further patch tests were carried out using 0.005% and 0.05% *N*-butyl-1,2-

benzisothiazolin-3-one in ethanol and petrolatum, respectively, to which the employee produced a 1+, a 2+ and a questionably 1+ reaction after 3 days. In graded tests, he reacted also to 0.05% (2+), 0.0158% (2+), 0.005% (2+), 0.00158% (1+) and 0.0005% (1+) *N*-butyl-1,2-benzisothiazolin-3-one in ethanol (Dahlin and Isaksson 2015).

In another metal worker with scaling and rhagadiform eczema on the 3 middle fingers of both hands, which had been present for 6 months, the 10% metal-working fluid tested led to a 2+ reaction on day 4. The employee produced 2+ reactions to 0.1% and 0.2% *N*-butyl-1,2-benzisothiazolin-3-one in ethanol, but not to 0.05% *N*-butyl-1,2-benzisothiazolin-3-one in petrolatum (Foti et al. 2019).

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

The LC_{50} in male and female rats following inhalation exposure to *N*-butyl-1,2-benzisothiazolin-3-one (purity 45.2% w/w; no data as to whether a formulation was used) was greater than 733 mg/m³ (males) or in the range between 197 and 733 mg/m³ (females) (no data for exposure duration, strain, methods and recovery period) (US EPA 2016).

5.1.2 Oral administration

The oral LD_{50} for *N*-butyl-1,2-benzisothiazolin-3-one (purity 95.5% w/w) was 4267 mg/kg body weight in male and 4732 mg/kg body weight in female rats (no data for strain, methods and recovery period) (US EPA 2016).

5.1.3 Dermal application

The dermal LD_{50} for *N*-butyl-1,2-benzisothiazolin-3-one (purity 93.4% w/w) was greater than 2000 mg/kg body weight in male and female rats (no data for strain, methods and recovery period) (ECHA 2020 a; US EPA 2016).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

There are no data available.

5.2.2 Oral administration

In a 90-day study in Sprague Dawley rats (Wistar-derived, Alpk:APfSD), diets containing 0, 40, 200 or 2000 mg *N*-butyl-1,2-benzisothiazolin-3-one/kg (purity 95.5%) were administered to groups of 20 animals per sex and dose. The mean estimated compound intake in males was 0, 3.1, 15.3 and 149.2 mg/kg body weight and day and in females 0, 3.4, 16.6 and 162.4 mg/kg body weight and day. Treatment-related clinical signs were not observed. No treatment-related neurotoxic effects or effects on motor activity were observed in the Functional Observational Battery (FOB) and investigation of motor activity. Decreased body weight gains (–12%) and reduced food intake were observed in the high dose group. Ophthalmological, haematological and gross-pathological examinations did not yield substance-related findings and the organ weights were unchanged. The histopathological examination revealed irritant effects in the forestomach in 20% of the male and female animals of the high dose group. These were minimal to slight submucosal inflammation with or without epithelial hyperplasia and erosion. One male animal had a slight ulcer. The findings in the high dose group were considered treatment-related and adverse. Thus, the LOAEL (lowest observed adverse effect level) for the male animals was 149.2 mg/kg body weight and day and that for the female animals 162.4 mg/kg body weight and day.

The NOAELs (no observed adverse effect levels) for male and female animals were 15.3 mg/kg body weight and day and 16.6 mg/kg body weight and day, respectively (US EPA 2016).

In a 90-day study in beagle dogs, *N*-butyl-1,2-benzisothiazolin-3-one (purity 95.4%) was given to 4 animals per sex and group at doses of 0, 25, 75 or 250 mg/kg body weight and day. The substance was dissolved in corn oil and administered orally in gelatine capsules once daily. The high dose was reduced to 200 mg/kg body weight and day beginning on day 10 after one female at 250 mg/kg body weight and day was sacrificed in extremis. The animal exhibited abnormal excreta, dermal atonia, vomiting, excessive salivation, hypoactivity and thinness. No adverse treatment-related effects were observed in ophthalmoscopic examinations, urinalysis, organ weight determinations, or gross-pathological and microscopic examinations. Vomiting, diarrhoea, and soft or mucoid faeces were observed at doses of 75 mg/kg body weight and day and above in the male and female animals. In the high dose group, effects on body weights were observed in both sexes. The male animals lost weight in a statistically significant manner during the first 2 weeks. Cumulative body weight gains in the males at this dose remained decreased compared with the control values throughout the study, and attained statistical significance (details are not given). In the females, body weights were likewise decreased in the high dose group compared with those in the control group during the first 2 weeks of the study, but the difference was not statistically significant. Red blood cell counts, as well as haemoglobin and haematocrit levels, were decreased in the male and female animals. This effect was not statistically significant and not dose-dependent. However, the animals of the high dose group consistently exhibited the greatest decrease with a concomitant increase in the platelet count. In both sexes of the high dose group, serum albumin and total protein were decreased in a dose-dependent and statistically significant manner. In addition, in the middle dose group, serum albumin was decreased in the males and total protein was decreased in the females, both in a statistically significant fashion. Serum calcium, which is predominantly bound to albumin, was likewise decreased in a statistically significant manner in both sexes of the high dose group, but the effect in the females was observed only at week 7. The LOAEL was 75 mg/kg body weight and day based on the clinical findings in both sexes and the dose-dependent decrease in albumin (males) and total protein (females). The NOAEL was 25 mg/kg body weight and day (US EPA 2016).

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 in rabbits with semi-occlusive application, *N*-butyl-1,2-benzisothiazolin-3-one was corrosive to the skin (no other details; ECHA 2020 a; US EPA 2016).

5.3.2 Eyes

There are no data available.

5.4 Allergenic effects

5.4.1 Sensitizing effects on the skin

In a maximization test with groups of up to 5 or 10 female Hartley guinea pigs, a minimum required (intradermal) induction concentration of 0.005% was determined for *N*-butyl-1,2-benzisothiazolin-3-one. With 0.5% *N*-butyl-1,2-benzisothiazolin-3-one, 90% of the treated animals were sensitized. No animal could be sensitized by topical induction treatment only. The threshold concentration for the challenge was reported to be 0.0327% (corresponding to 21 µg *N*-butyl-1,2-benzisothiazolin-3-one/cm^2) (Noda et al. 2001; Yamano et al. 2005).

In another maximization test according to Magnusson and Kligman, none of the 19 Dunkin Hartley guinea pigs exhibited sensitization to *N*-butyl-1,2-benzisothiazolinone (using a preparation of 19.5% *N*-butyl-1,2-benzisothiazolin-3-one in propylene glycol). Propylene glycol or corn oil were the vehicles for intradermal induction, and petrolatum for topical induction and challenge treatment. Ten animals were used in each of the control groups, and alpha-hexylcinnamaldehyde served as the positive control. In a preliminary study, the lowest irritant concentration was 1% *N*-butyl-1,2-benzisothiazolin-3-one for intradermal and 3% for topical induction treatment. The maximum non-irritant concentration for challenge treatment was 0.5% *N*-butyl-1,2-benzisothiazolin-3-one. alpha-Hexylcinnamaldehyde had a moderate sensitizing potential in the positive control group, with positive reactions in 8 of 20 animals. The structurally analogous 1,2-benzisothiazolin-3-one (a preparation containing 20.2% 1,2-benzisothiazolin-3-one in an unidentified solvent), using the same induction concentrations and in a 1% preparation for challenge, likewise did not produce a reaction in 20 animals (Zissu 2002).

In a modified local lymph node assay in groups of 4 female BALB/c mice using 5-bromo-2′-deoxyuridine instead of ^{3}H-methylthymidine, a negative result was obtained with preparations of *N*-butyl-1,2-benzisothiazolin-3-one in dimethyl sulfoxide. The applied concentrations of 0.3%, 1% and 3% resulted in stimulation indices of 0.9, 1.5 and 2.0, respectively; the highest concentration was regarded as irritant by the authors. 1,2-Benzisothiazolin-3-one yielded a broadly similar result in this study at the same concentrations and using the same vehicle (stimulation indices: 0.7, 1.3 and 1.7, respectively) (Yamano et al. 2005).

5.4.2 Sensitizing effects on the airways

There are no data available.

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

In a 2-generation study from 2007, *N*-butyl-1,2-benzisothiazolin-3-one (purity 99.4%) was administered in the diet to 30 Sprague Dawley rats per sex and group at concentrations of 0, 300, 600 or 1700 mg/kg diet, corresponding to intakes of 0, 25, 49 and 141 mg/kg body weight and day, respectively, in males and 0, 27, 56 and 157 mg/kg body weight and day, respectively, in females. The F0 and F1 generations were exposed at least 70 days before mating. Parent animals selected from the first offspring (F1) for the F2 generation (30 per sex and dose group) received the same concentrations of *N*-butyl-1,2-benzisothiazolin-3-one in the diet as their parents from postnatal day 21 onwards. The F2 generation was maintained until weaning. No mortality occurred in the F0 generation. Of the adult F1 animals, 3 died, but not as a result of treatment. The body weight gains of the female F0 animals were decreased in a statistically significant manner ($p < 0.05$) before mating (week 0 to 10). Throughout the study period, neither body weights, body weight gains, absolute (g/animal and day) and relative feed intake (g/kg body weight and day), nor feeding efficiency were affected by the treatment. In the high dose F1 generation group, the body weights were reduced in a statistically significant manner before mating (weeks 18 to 28) in male ($p < 0.05$) and female ($p < 0.01$) animals. The body weight gains were not affected by the substance in either sex during the entire period before mating. The reduction in absolute feed intake was statistically significant during weeks 18–19, 22–23 and 25–26 in both males and females. The relative feed intake was not affected by the treatment. Feeding efficiency remained unchanged by the treatment in males, but was increased in the females during week 18 to 19 ($p < 0.05$) with statistical significance. During pregnancy, the body weights were reduced in the F1 parental generation in the high dose group, but not in a statistically significant manner and also without changes in body weight gains. Therefore, it is assumed that this is only a residual effect from the period before mating. Treatment during gestation had no effects on feed intake and feeding efficiency. No substance-related effects on body weights, feed intake and feeding efficiency were observed during the lactation period. No other findings occurred, so the LOAEL for parental toxicity was 1700 mg/kg feed (equivalent to 141 and 157 mg/kg body weight and day in male and female animals, respectively), based on decreased body weights and feed intake. The NOAEL was 600 mg/kg feed (equivalent to 49 and 56 mg/kg body weight and day in male and female animals, respectively).

In the offspring of the F1 and F2 groups, no substance-related clinical findings occurred up to postnatal day 21. Litter parameters and mating were likewise not affected. No treatment-related effects were observed in the gross-pathological and histopathological examination. In the high dose group, the body weights on postnatal day 21 and body weight gains from postnatal day 1 to postnatal day 21 were reduced in the male and female F1 and F2 offspring ($p < 0.05$). The absolute and relative spleen weights of the F2 male and female offspring were decreased ($p < 0.05$) on postnatal day 21 as a result of the treatment.

There were no effects on mating, fertility, conception/copulation or pregnancy index. Likewise, no substance-related changes were observed in the period prior to mating or during gestation. The length of the oestrous cycle was not altered in either generation. There were no substance-related adverse effects on the spermatogenesis end points (mean testicular and epididymal sperm, sperm production, sperm motility, progressive motility and morphology) of the F0 and F1 generations. The NOAEL for fertility corresponded to the highest dose tested of 1700 mg/kg feed (equivalent to 141 and 157 mg/kg body weight and day in male and female animals, respectively) (US EPA 2016).

5.5.2 Developmental toxicity

In a developmental toxicity study from 1997 in Sprague Dawley rats, 24 pregnant animals per group were given gavage doses of *N*-butyl-1,2-benzisothiazolin-3-one (purity 95.5%) of 0, 30, 100 or 300 mg/kg body weight and day from days 7 to 16 of gestation. On gestation day 22, the animals were killed, underwent gross-pathological examination and the foetuses were examined for external, visceral and skeletal malformations. No substance-related mortality was observed, although deaths resulting from gavage errors did occur. Abnormal respiratory noise occurred in 4 animals of the high dose group from gestation days 8 to 19. The adjusted body weights of the high dose group were decreased in a statistically significant manner (96% to 99% of the control values) from gestation days 8 to 19. The body weight gain in these animals was 82% of that in the control animals, reflecting the slightly decreased (no other details) gravid uterine weights. Feed intake was reduced in a statistically significant manner in the high dose group to 83% to 86% of that in the control animals. Gross-pathological examination of the dams revealed gastric ulceration in 0, 1, 5 and 16 animals at 0, 30, 100 and 300 mg/kg body weight and day, respectively. Deceased animals in the middle dose group likewise exhibited gastric ulceration. The findings were attributed to the local irritant effect of the substance. No other substance-related findings occurred, so that the LOAEL for maternal toxicity was 100 mg/kg body weight and day and the NOAEL was 30 mg/kg body weight and day. No substance-related effects occurred with regard to the number of corpora lutea, implantation and resorption sites, preimplantation and postimplantation losses, foetal body weights, the number of foetuses per litter or the sex ratio. Likewise, no substance-related external, visceral or skeletal malformations or variations were observed in the foetuses. The NOAEL for developmental toxicity was 300 mg/kg body weight and day, the highest dose tested (US EPA 2016).

5.6 Genotoxicity

5.6.1 In vitro

Human lymphocytes from 2 subjects were exposed to *N*-butyl-1,2-benzisothiazolin-3-one (purity 95.5%) dissolved in dimethyl sulfoxide (DMSO) for 3 hours in a chromosomal aberration assay, and then cultivated for 17 or 41 hours. For evaluation after 17 hours, cells from donor 1 (female) were incubated with and without the addition of a metabolic activation system (S9 mix) at 2–20.25 µg/ml and cells from donor 2 (male) were incubated without S9 mix at 1–12.5 µg/ml and with S9 mix at 2–17.5 µg/ml. Cells from donor 2 were exposed to 5–12.5 µg/ml without S9 mix and to 10–17.5 µg/ml with S9 mix and evaluated after 41 hours. The S9 mix was obtained from liver cells of male Sprague Dawley rats treated with phenobarbital and β-naphthoflavone. Based on the determination of mitotic indices (no other details), the cells were examined for chromosomal aberrations at concentrations of up to 10 µg/ml without S9 mix and up to 15 µg/ml with S9 mix. The higher concentrations were cytotoxic. After 41 hours, a statistically significant increase in aberrations (primarily breaks and fragments; no other details) was observed in the presence and absence of metabolic activation (data for dose-dependency not given). *N*-butyl-1,2-benzisothiazolin-3-one was regarded as clastogenic in this study (US EPA 2016).

In a $TK^{+/-}$ mutation assay using L5178Y mouse lymphoma cells, *N*-butyl-1,2-benzisothiazolin-3-one (purity 95.5%) dissolved in DMSO was tested at concentrations of 0.2, 0.4, 0.8, 1.6 or 3.1 µg/ml in the absence of a metabolic activation system (S9 mix) or 1.6, 3.1, 6.3, 12.5 or 25.0 µg/ml with S9 mix. A second assay was carried out with concentrations of 0.1, 0.2, 0.4, 0.8 or 1.6 µg/ml in the absence of S9 mix and with 3.1, 6.3, 12.5, 25 or 50 µg/ml in the presence of S9 mix. In a third assay, concentrations of 0.4, 0.5, 0.6, 0.8, 1.1, 1.5 or 2.0 µg/ml were used in the absence of S9 mix. *N*-Butyl-1,2-benzisothiazolin-3-one was tested up to cytotoxic concentrations. S9 mix was obtained from liver cells of male Sprague Dawley rats treated with phenobarbital and β-naphthoflavone. The mutation frequency was not increased in a statistically significant manner compared with that in the controls. The positive controls indicated a functioning test system (US EPA 2016).

5.6.2 In vivo

DNA repair synthesis (UDS) in rat hepatocytes was determined in an in vivo/in vitro study in male Alpk:AP SD rats after gavage doses of *N*-butyl-1,2-benzisothiazolin-3-one of 0, 500 or 800 mg/kg body weight (purity 95.5%) in 2 to 3 animals. The hepatocytes were isolated 2 or 16 hours after treatment and cultured for the determination of ^{3}H-thymidine incorporation. The highest dose was selected based on histopathological changes in the liver. DNA repair synthesis was not increased in a statistically significant manner compared with that in the control group. The positive control indicated a functioning test system (US EPA 2016).

A micronucleus test in erythrocytes from the bone marrow of Crl:CD-1 mice was performed after gavage administration of *N*-butyl-1,2-benzisothiazolin-3-one (purity 95.5%) in corn oil. Ten male animals were given 1250 mg/kg body weight and 10 female animals 2000 mg/kg body weight. Half of the animals were examined after 24 hours, the rest after 48 hours. Five animals per sex and time point were exposed as negative controls to corn oil only. Cyclophosphamide served as a positive control (5 animals per sex, examined after 24 hours). The number of cells with micronuclei was not increased with statistical significance by the treatment with *N*-butyl-1,2-benzisothiazolin-3-one compared with that in the controls. The positive controls indicated a functioning test system (US EPA 2016). No data are available as to whether the target tissue was reached although the substance was tested up to the highest dose prescribed by the test guideline.

5.7 Carcinogenicity

There are no studies available.

6 Manifesto (MAK value/classification)

The critical effect is the corrosive effect of *N*-butyl-1,2-benzisothiazolin-3-one on the skin of rabbits. The strong local irritant effect was observed also in oral studies in rats (ulcers in the stomach) and dogs (vomiting). There are no studies in humans.

MAK value. *N*-Butyl-1,2-benzisothiazolin-3-one is corrosive to the skin of rabbits. Studies with repeated inhalation exposure are not available.

In a 90-day study with daily administration to beagle dogs, the NOAEL was 25 mg/kg body weight and day. A 90-day feeding study in Sprague Dawley rats revealed a NOAEL of about 16 mg/kg body weight and day. The following toxicokinetic data are taken into consideration for the extrapolation of the NOAELs to a concentration in workplace air: the daily exposure of the animals in comparison with the 5 days per week exposure at the workplace (7:5), the corresponding species-specific correction values for the dog and the rat (1:1.4 and 1:4, respectively), the assumed oral absorption (100%), the body weight (70 kg) and respiratory volume (10 m^3) of the person, and the assumed 100% absorption by inhalation. The concentrations calculated form this are 175 mg/m^3 (20.35 ml/m^3) from the dog study and about 39.2 mg/m^3 (4.56 ml/m^3) from the rat study. As the data are extrapolated from animal studies to humans (1:2) and taking into consideration the possible increase in effects with chronic exposure (1:2), the concentration from the rat study is

9.8 mg/m³ (1.1 ml/m³), which would correspond to a MAK value of 1 ml/m³ according to the preferred value approach. From the dog study, using a higher factor for time-extrapolation due to the longer lifetime (1:6), a similar value of 14.6 mg/m³ (1.7 ml/m³) would result.

However, as *N*-butyl-1,2-benzisothiazolin-3-one is corrosive and inhalation studies of local effects on the respiratory tract are not available, a MAK value cannot be derived. The US EPA used the structurally analogous 4,5-dichloro-2-octyl-2H-isothiazol-3-one (CAS No. 64359-81-5) to evaluate inhalation toxicity, with a NOAEC (no observed adverse effect concentration) of 0.02 mg/m³ from a 90-day study (US EPA 2016). This substance is also corrosive and sensitizing, but the extent of analogy is unclear due to the additional chlorine atoms on the ring. *N*-Butyl-1,2-benzisothiazolin-3-one is therefore assigned to Section IIb of the List of MAK and BAT Values. Peak limitation is not applicable.

Prenatal toxicity. The NOAEL for developmental toxicity in rats is 300 mg/kg body weight and day, the highest dose tested. At this dose, ulceration of the stomach was found in the dams.

Assignment to a pregnancy risk group is not applicable, since no MAK value has been derived.

Carcinogenicity and germ cell mutagenicity. There are no germ cell mutagenicity tests. A $TK^{+/-}$ mutation test using L5178Y mouse lymphoma cells yielded negative results, but in a chromosomal aberration test in human lymphocytes, *N*-butyl-1,2-benzisothiazolin-3-one was clastogenic, which was not confirmed in vivo in a micronucleus test in bone marrow erythrocytes of CD-1 mice. UDS in rat hepatocytes was not induced in an in vivo/in vitro study. The validity of this study is limited by the small number of animals used. Studies of the carcinogenicity of the substance are not available. The available data do not justify the classification of *N*-butyl-1,2-benzisothiazolin-3-one in one of the categories for germ cell mutagens or carcinogens.

Absorption through the skin. For humans, a maximum dermal absorption of 0.268 mg can be estimated from a model calculation (Section 3.1) for exposure to a saturated aqueous solution under standard conditions (2000 cm² of skin, exposure for 1 hour). The systemic NOAEC estimated for humans is 9.8 mg/m³ (see above), and the systemically tolerable amount at 100% absorption by inhalation and a respiratory volume of 10 m³ is 98 mg. Thus, dermal absorption is significantly less than 25% of the systemically tolerable amount. *N*-Butyl-1,2-benzisothiazolin-3-one has therefore not been designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. Only few clinical findings of the sensitizing effects of *N*-butyl-1,2-benzisothiazolin-3-one are available. Maximization tests in guinea pigs yielded both positive and negative results, and a tripling of lymphocyte stimulation was not achieved in the local lymph node assay up to a test concentration of 3%. Nevertheless, clinical findings indicate that *N*-butyl-1,2-benzisothiazolin-3-one can induce clinically relevant sensitization of the human skin. *N*-Butyl-1,2-benzisothiazolin-3-one has therefore been designated with "Sh" (for substances which cause sensitization of the skin). As there are no data for sensitizing effects on the airways, the substance has not been designated with "Sa" (for substances which cause sensitization of the airways).

Notes

Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (www.dfg.de/mak/conflicts_interest) ensure that the content and conclusions of the publication are strictly science-based.

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