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TOXICOLOGICAL EVALUATIONS

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TOXICOLOGICAL EVALUATION

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2-Butyne-1,4-diol

No. 117

CAS No. 110-65-6

BG Chemie
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2-Butyne-1,4-diol

1 Summary and assessment

Various studies investigating the distribution, metabolism and excretion of 2-butyne-1,4-diol have been carried out in which nonradiolabelled 2-butyne-1,4-diol or radiolabelled (^{14}C)-2-butyne-1,4-diol was administered to groups of 5 or 6 adult male Fischer-344 rats, Sprague-Dawley rats or B6C3F1 mice as a single dermal, intravenous or oral dose. According to these studies, 2-butyne-1,4-diol is eliminated from the body mainly via urine, irrespective of the route of administration. 2-Butyne-1,4-diol absorption after dermal administration is minimal (≤ 10%). By 72 hours, approx. 20% of the absorbed fraction is excreted via the kidney into the urine, approx. 10% is exhaled as CO_2 and approx. 9% undergoes faecal excretion via the intestine. Following intravenous administration, elimination takes place very rapidly, with more than 70% being recovered by 24 hours after dosing, approx. 51% and approx. 22% being excreted via the kidney into the urine and as exhaled CO_2, respectively, and approx. 16% undergoing faecal excretion via the intestine by 72 hours after dosing. The half-life in the blood is < 30 minutes. In the process, about 60% of the 2-butyne-1,4-diol is excreted into bile, but a large fraction then undergoes enterohepatic recirculation and is subsequently excreted mainly in urine and CO_2. Two biliary metabolites of 2-butyne-1,4-diol have been identified as 4,4-bis(S-glutathionyl)-2-hydroxytetrahydrofuran and 3-(S-glutathionyl)-2(5H)-furanone. Metabolically, 2-butyne-1,4-diol apparently is rendered toxic by liver alcohol dehydrogenase, i.e. converted to a toxic metabolite. Thus, intraperitoneal administration of high doses of 2-butyne-1,4-diol to Wistar rats resulted in dose-related mortality and marked toxicity, which could be prevented by pretreatment with pyrazole, a competitive inhibitor of liver alcohol dehydrogenase. It has also been demonstrated in rat liver extract that 2-butyne-1,4-diol is a substrate for alcohol dehydrogenase and that pyrazole competitively inhibits the oxidation and hence the metabolism of 2-butyne-1,4-diol. These studies permit the conclusion that it is the products of oxidative metabolism which are responsible for the toxicity of 2-butyne-1,4-diol.

Based on the available acute toxicity studies, 2-butyne-1,4-diol is toxic following oral administration (LD_{50} rat oral mostly about 100 mg/kg body

weight), toxic by inhalation (LC_{50}, 4 hours, approx. 690 mg/m³ in the rat) and harmful by dermal absorption of aqueous solutions (LD_{50} rat dermal 659 mg/kg body weight). The main clinical signs of toxicity are apathy, disturbances of balance, convulsions and diarrhoea, the predominant necropsy findings being congestion of the internal organs, pulmonary oedema and haemorrhage and toxic fatty degeneration of the liver. The results of exposure in the inhalation hazard test are dependent on the temperature at which the atmosphere is generated and the duration of exposure. Thus, all 12 rats that underwent 8-hour exposure to atmosphere enriched or saturated with 2-butyne-1,4-diol at 20 °C survived, while atmosphere generated at 70 °C was tolerated for 2 hours but was fatal to all 6 rats after exposure for 8 hours. In mice exposed for 2 hours to 2-butyne-1,4-diol vapours generated at 35 to 40 °C, signs of mucous membrane irritation occurred, as well as motor excitation with subsequent depression and fatalities. Necropsy and examination by light microscopy revealed congestion of the internal organs and the brain, haemorrhages and bronchitis in the lungs and tubular degeneration in the kidneys.

Oral administration of 2-butyne-1,4-diol to rats at dose levels of 5, 10 or 20 mg/kg body weight by oral gavage for 5 days caused no observable treatment-related effects, except for a dose-related increase in cholesterol level in males, an effect which attained statistical significance only at the top dose level. Neurofunctional tests also showed no pathological effects. Following 2-week week oral administration of 2-butyne-1,4-diol to rats at dose levels of 1, 10 or 100 mg/kg body weight, the *no observed adverse effect level* (NOAEL) was also 10 mg/kg body weight, while it was 1 mg/kg body weight after 4 weeks of oral dosing, with congestion of the internal organs, pulmonary oedema, marked tubular degeneration in the kidney and diffuse parenchymal necrosis and fatty degeneration of the liver occurring from 10 mg/kg body weight in the 4-week study. In addition, haematology changes were reported as signs of anaemia. A range-finding study in accordance with OECD guideline for testing No. 412 and Directive 92/69/EEC was conducted in groups of 5 male and 5 female Wistar rats that underwent head-nose exposure to liquid aerosols of aqueous solutions of 2-butyne-1,4-diol (99.5% pure) at concentration levels of 0 (controls), 25, 100 or 300 mg/m³ for 6 hours per day for 5 consecutive days. In summary, exposure of rats to 2-butyne-1,4-diol at a concentration of 300 mg/m³ for 5 days resulted in systemic toxicity characterised by functional and morphological

impairment of the liver including increases in urinary urobilinogen and delayed body weight development in males and females. In addition, this concentration produced local inflammation and/or epithelial changes in the nasal cavity and the larynx. The intermediate concentration of 100 mg/m³ was associated with increased urinary urobilinogen, local effects in the nose and larynx and even the low concentration group (25 mg/m³) showed toxic damage to the hepatic parenchyma as evidenced by increases in urinary urobilinogen levels and signs of inflammation and/or epithelial changes in the larynx, and therefore a *no observed adverse effect concentration* (NOAEC) was not found in the 5-day study. The subsequent subacute inhalation neurotoxicity study was conducted in accordance with OECD guidelines for testing nos. 412 and 413, Directive 92/69/EEC and US EPA Health Effects Testing Guidelines 40 CFR §§ 798.6059, 798.6200 and 798.6400. In this study, groups of 16 male and 16 female Wistar rats underwent head-nose exposure to liquid aerosols of aqueous solutions of 2-butyne-1,4-diol (99.5% pure) at concentration levels of 0 (controls), 0.5, 5 or 25 mg/m³ for 6 hours per day. In order to investigate the concentration-time-response relationship, half of the animals served as a concurrent satellite group for 15 study days (10 exposures), while the other half of the animals were maintained for 30 study days (20 exposures). In summary, none of the concentrations tested produced systemic toxicity. Neither the functional observational battery assessments and motor activity measurements nor the neurohistopathological examinations identified any statistically or biologically relevant neurotoxicological changes. Moreover, no treatment-related clinical, clinico-chemical, haematological, gross pathological or micropathological changes were observed. However, the high and intermediate concentration (25 and 5 mg/m³) produced local irritant effects in the upper respiratory tract both after 10 and 20 exposures. There was some indication of an increase in inflammatory incidence in the upper respiratory tract with prolongation of exposure time and increase in concentration, but no increase in severity of histopathological changes was observed (all histopathological findings were graded minimal to slight). When the results from the 5-day range-finding study and the satellite groups given 10 exposures were taken into account, there was no indication of cumulative systemic toxicity when the duration of exposure was extended to 20 exposures at concentration levels of up to 25 mg/m³. It was considered that the effects noted in the larynx and trachea should be interpreted as unspecific responses to local irritation due to the deposition of

2-butyne-1,4-diol aerosol in the aerodynamic traps presented by the larynx and tracheal bifurcation. The NOAEC for systemic toxicity was 25 mg/m³ (highest concentration tested), while that for local toxicity to the upper respiratory tract was 0.5 mg/m³.

Numerous skin and eye irritation studies of 2-butyne-1,4-diol have been carried out in rabbits. The chemical has been tested in various formulations and at different dilutions, giving differing results. At concentration levels of up to 20%, 2-butyne-1,4-diol in aqueous solution was nonirritating to the skin, but higher concentrations caused irritation and corrosion. Pure solid 2-butyne-1,4-diol, caused corrosion to the skin and eye when applied as an undiluted powder.

2-Butyne-1,4-diol showed no skin-sensitising effect in two valid studies in the guinea pig. In a third valid study, 5 out of 20 guinea pigs showed positive responses at both readings, with clear negative responses in the control group. 2-Butyne-1,4-diol therefore had a weak sensitising effect. In a guinea pig study with intradermal injection of a 10% aqueous solution of 2-butyne-1,4-diol and two challenge treatments at 14 days and another 3 weeks later, positive responses after the first challenge were unequivocal in 5/11 guinea pigs and equivocal in a sixth guinea pig, and positive responses after the second challenge were unequivocal in 6/11 and equivocal in an additional 2 guinea pigs. The same animals showed no positive response when challenged with a 10% solution of formaldehyde. Hence, 2-butyne-1,4-diol was the sensitising agent. The study was not conducted in accordance with current guidelines, but it is nonetheless also indicative of the sensitising effect of 2-butyne-1,4-diol. This is supported by an older rabbit study which was also positive, and therefore 2-butyne-1,4-diol must be considered a weak sensitiser of animal skin.

Longer-term oral administration of 0.04 and 0.2 mg 2-butyne-1,4-diol/kg body weight/day for 6 months caused no changes in behaviour, body weight gain or blood parameters in the rat. At 2 mg/kg body weight, the predominant findings were delayed development of conditioned reflexes, patchy congestion of organs and toxic liver changes. The *no observed effect level* (NOEL) in this study was 0.2 mg/kg body weight. The inhalation by rats of 2-butyne-1,4-diol at approx. 0.008 to 0.01 mg/l air (equivalent to approx. 8 to 10 mg/m³) as an aerosol resulted in marginal local and systemic effects that were reversible after one month. Both studies were not

conducted in accordance with current guidelines and, due to inadequate reporting, are suitable for evaluation purposes only to a limited extent.

2-Butyne-1,4-diol was nonmutagenic in Salmonella/microsome tests on *Salmonella typhimurium* strains TA 97, TA 98, TA 100, TA 1535, TA 1537 and TA 1538 with and without metabolic activation and caused no chromosome aberrations in Chinese hamster V79 cells. In vivo, 2-butyne-1,4-diol was devoid of clastogenic activity in a micronucleus assay in which mice received a single intraperitoneal dose of up to 70 mg/kg body weight. In summary, there is no indication that 2-butyne-1,4-diol possesses any genotoxic potential.

2-Butyne-1,4-diol showed no tumour-initiating effect in an early dermal initiation/promotion study in mice after application of 2-butyne-1,4-diol once a week for 10 weeks and croton oil once a week for 18 weeks. However, the short duration of the study, 19 weeks in total, prevent a definite conclusion regarding the tumour-initiating effect or carcinogenicity of 2-butyne-1,4-diol.

In a preliminary study to an embryotoxicity/teratogenicity study, 2-butyne-1,4-diol produced dose-related maternal toxicity when administered to pregnant Wistar rats at dose levels of 20, 40 and 60 mg/kg body weight by oral gavage from day 6 to 15 post coitum. Body weight and food consumption were initially decreased in a statistically significant manner in the high dose group and dose-dependently impaired hepatic function was observed in all three dose groups. Based on the results, dose levels of 10, 40 and 80 mg 2-butyne-1,4-diol/kg body weight/day were chosen for the main study. In the high dose group in the main study conducted at dose levels of 10, 40 and 80 mg 2-butyne-1,4-diol/kg body weight/day, signs of maternal toxicity included significant reduction in food intake, and body weight loss, and there was one death with apathy, poor general state and vaginal haemorrhages, with necropsy of the deceased animal revealing grossly mottled liver and marginal emphysema of the lungs. An increased number of foetuses per litter with accessory 14th ribs noted in the high dose group was evaluated as a marginal sign of developmental toxicity in the embryos, representing a manifestation of nonspecific stress on the dams, not a teratogenic effect. The NOAEL for the dams and foetuses was given as 40 mg/kg body weight/day. 2-Butyne-1,4-diol was administered to groups of 25 male and 25 female Wistar rats (F_0 parental generation) in their drinking water at concentrations of 0 (controls), 10, 80 or 500 ppm (equivalent to 0 (con-

trols), approx. 1, 7.6 or 40 mg/kg body weight/day) in an extended one-generation reproductive toxicity study in accordance with OECD guideline No. 415. The scope of the study was expanded to include the following parameters as required by OECD guideline No. 416 and US EPA guideline OPPTS 870.3800: oestrus cycle, sperm parameters, determination of organ weights in selected offspring parental animals, extensive histology and signs of sexual maturation. The F_0 generation animals were allowed to mate no earlier than 76 days after the beginning of treatment. The resulting offspring (F_1 generation) were reared until day 21 after parturition. The parental F_0 animals and their offspring were then killed, with the exception of one male and one female pup from each litter of the F_1 generation. The latter were raised to sexual maturity and then sacrificed. Animals received drinking water containing 2-butyne-1,4-diol throughout the entire study. Based on the results of the clinical observations and the gross and histopathological examinations, 2-butyne-1,4-diol was devoid of adverse effects on the reproductive performance and fertility of the parental F_0 animals at all three dose levels. Signs of general, systemic toxicity in the parental F_0 animals were confined to the 80 and 500 ppm groups. In the 500 ppm group, reduced water consumption was noted for the parental F_0 animals during the premating phase and for the F_0 females during gestation and lactation. Impairment of body weight gain with concurrent lowered food consumption was observed in F_0 females during premating, gestation and lactation. In addition, the 500 ppm animals were found to have substance-related, statistically significant increases in absolute and relative kidney (males and females) and liver weights (females) and statistically significant decreases in absolute and relative adrenal and thymus weights (females). In addition, the 80 ppm group exhibited impaired water consumption during premating (males and females) and gestation (females) and statistically significant increases in absolute and relative kidney (males and females) and liver weights (females). Signs of systemic toxicity observed in the 500 ppm group of F_1 offspring reared to sexual maturation included reduced water consumption (males and females), impaired body weights/body weight gain with concurrent reductions in food consumption (males) as well as reduced body weights (females). Substance-related signs of developmental toxicity were observed in the progeny of the parental F_0 males and females only after administration of 500 ppm; this resulted in impairments of pup body weight gain and causally related decreases in organ weights. In addition, a sign of general delay in physical development noted in the

reared F_1 males and females was a delay in preputial separation and vaginal opening, respectively. The 10 and 80 ppm levels did not cause developmental toxicity in the progeny. The investigators evaluated the highest test concentration of 500 ppm (approx. 40 mg/kg body weight/day) as the NOAEL for reproductive performance and fertility of the parental F_0 animals, whilst the NOAEL for systemic toxicity was 10 ppm (approx. 1 mg/kg body weight/day). The NOAEL for developmental toxicity (growth and physical development of the offspring) was given as 80 ppm (approx. 7.6 mg/kg body weight/day) for the F_1 progeny. Signs of developmental toxicity thus occurred only at a dose level which was also systemically toxic to the parental males and females. No impairment of reproduction (fertility) was observed.

2-Butyne-1,4-diol can lead to a reduction in body temperature in rats, possibly caused by peripheral vasodilation, which has also been observed in rats.

In humans, dermatitis caused by 2-butyne-1,4-diol has been described and there are 9 reported cases of contact allergy that were unequivocally identified by testing.

The United States National Toxicology Program (NTP) is planning to conduct a carcinogenicity study of 2-butyne-1,4-diol.

In accordance with Annex I to Directive 67/548/EEC, the European Commission has classified 2-butyne-1,4-diol as sensitising by skin contact (R 43). The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area ("MAK-Kommission") of the Deutsche Forschungsgemeinschaft has designated 2-butyne-1,4-diol with "Sh" for skin-sensitising substances and listed it in the "Yellow Pages" ("Substances being Examined for the Establishment of MAK Values and BAT Values") of the List of MAK and BAT Values 2004 on the suggestion of BG Chemie in order that a MAK value be established for the chemical.

2 Name of substance

2.1	Usual name	2-Butyne-1,4-diol
2.2	IUPAC name	2-Butyne-1,4-diol
2.3	CAS No.	110-65-6
2.4	EINECS No.	203-788-6

3 Synonyms, common and trade names

Agrisynth B3D
Bis(hydroxymethyl)acetylene
trans-2-Butin-1,4-diol
Butindiol
Butin-2-diol-1,4
But-2-in-1,4-diol
2-Butin-1,4-diol rein krist.
Butynediol
1,4-Butynediol
2-Butynediol
2-Butynediol-1,4
2-Butyne-1,4-diol
But-2-yne-1,4-diol
Butynediol pure cryst.
1,4-Dihydroxy-butin-2
1,4-Dihydroxy-2-butyne
Golpanol
Golpanol BOZ liquid
Golpanol pure solid
Korantin BH
Korantin BH flüssig
Korantin BH liquid
Korantin BH solid

4 Structural and molecular formulae

4.1 Structural formula $HO–CH_2–C\equiv C–CH_2–OH$

4.2 Molecular formula $C_4H_6O_2$

5 Physical and chemical properties

5.1 Molecular mass, g/mol 86.09

5.2 Melting point, °C

50	(Lide and Frederikse, 1997)
54–55	(Riedel-de Haën, 1996)
54–56	(BASF, 1992 c, 1997 a)
55–57	(BASF, 2001 a)
57	(Sax, 1999)
58	(Gräfje et al., 2002)

5.3	Boiling point, °C	88.3 (at 0.59 hPa) 95.1 (at 0.93 hPa) 101 (at 1.33 hPa) 106.5 (at 1.87 hPa) (EC, 2000) 125–127 (at 3 hPa; decomposition starts at 150 °C) (BASF, 2001 a) 141 (at 13.3 hPa) (EC, 2000) 150 (at 18 hPa; slow decomposition between 160 and 200 °C, rapid decomposition above 200 °C) (Gräfje et al., 2002) 194 (at 133 hPa) (EC, 2000) 238 (at 1011 hPa) (Fliege et al., 1975; BASF, 1992 c, 1997 a) 238 (at 1013 hPa; decomposition from 140 °C) (Riedel-de Haën, 1996) 248 (at 1013 hPa) (EC, 2000)
5.4	Vapour pressure, hPa	0.0017 (at 20 °C) (SIDS, 1996) 2 (at 100 °C) (EC, 2000) 1.33 (at 102 °C) (BASF, 2001 a) 2.3 (at 120 °C) (Fliege et al., 1975) 21.33 (at 150 °C) (EC, 2000) 242.38 (at 208.5 °C) (EC, 2000)
5.5	Density, g/cm³	1.05 (at 20 °C) (Riedel-de Haën, 1996) 1.17 (at 20 °C) (Falbe and Regitz, 1996) 1.114 (at 60 °C) (EC, 2000)
5.6	Solubility in water	20 g/l (at 0 °C) (EC, 2000) Completely miscible (at 20 °C) (BASF, 2001 a) ca. 750 g/l water (at 20 °C) (EU, 2002) Very readily soluble: 374 g/100 g water (equivalent to 3740 g/l; at 25 °C) (Fliege et al., 1975; Gräfje et al., 2002)
5.7	Solubility in organic solvents	Readily soluble in alcohols and acetone; poorly soluble in ether and hydrocarbons; almost insoluble in benzene (Fliege et al., 1975; Gräfje et al., 2002) Soluble in polar solvents (BASF, 2001 a) Very readily soluble in alcohols and esters, poorly soluble in ethers, ketones and chlorinated hydrocarbons, very poorly soluble in aromatic hydrocarbons and practically insoluble in aliphatic hydrocarbons (BASF, 1997 a)

5.8	Solubility in fat	No information available
5.9	pH value	ca. 3 (at 100 g/l water and 20 °C) (BASF, 1987 a; Riedel-de Haën, 1996) ca. 4 (at 20 g/l and 20 °C) 6.4 (at 100 g/l and 20 °C) (BASF, 2001 a)
5.10	Conversion factor	1 ml/m³ (ppm) ≙ 3.51 mg/m³ 1 mg/m³ ≙ 0.28 ml/m³ (ppm) (at 1013 hPa and 25 °C)

6 Uses

As a brightening agent in electroplating, corrosion inhibitors, stabiliser for halogenated hydrocarbons; intermediate in the manufacture of 2-butene-1,4-diol, 1,4-butanediol, insecticides, herbicides, corrosion inhibitors and flame retardants; additive for electroplating baths (nickel and copper) as brightening agent (Gräfje et al., 2002).

Intermediate used in the chemical industry, for instance for the manufacture of pharmaceuticals, plant protection agents and pesticides, textile auxiliaries corrosion inhibitors, plasticisers, synthetic resins and stabilisers; additive for gloss nickel plating baths (BASF, 1980).

Used as an intermediate in electroplating as a brightening agent in nickel and copper baths, as a corrosion-inhibiting additive in mineral acid pickling baths and in the production and processing of mineral oil and natural gas, for the removal of scale deposits and paints, as a chain-lengthening agent for polyurethane prepolymers, in the manufacture of defoliating agents and in the manufacture of pharmaceuticals (BASF, 1997 a).

Approximately 98% of all 2-butyne-1,4-diol is further processed into butanediol and butenediol. Approximately 2% of the solid substance is used in the form of flakes and aqueous solutions (32 to 34%) in the production of further chemicals (polyols, auxiliaries for the paint industry, flameproofing agents), as an additive to electroplating baths, in cleaning solutions for the removal of scale deposits using acids, in acid pickles and in organic solvent paint removers. 2-Butyne-1,4-diol serves as an ingredient in the following types of consumer products: cleaning products for sanitary installations (concentration < 2%), car cleansing products (< 1%), building façade cleansers (< 3%), disinfectants for sanitary installations (0.33 to 2%), pipe

descaling agents (0.15 to 1%), descaling agents (0.2 to 5%; SIDS, 1996; EU, 2002).

7 Experimental results

7.1 Toxicokinetics and metabolism

Various studies investigating the distribution, metabolism and excretion of 2-butyne-1,4-diol were carried out in which 99% nonradiolabelled 2-butyne-1,4-diol or 94% radiolabelled (^{14}C)-2-butyne-1,4-diol was administered to groups of 5 or 6 adult male Fischer-344 rats, Sprague-Dawley rats or B6C3F1 mice as a single dermal, intravenous or oral dose. Irrespective of the route of administration, 2-butyne-1,4-diol was eliminated from the body mainly via urine. Following dermal administration, absorption of 30 and 0.3% aqueous solutions of 2-butyne-1,4-diol was minimal (≤ 10%). Of the dermally absorbed radioactivity, approx. 20% was recovered in urine, approx. 10% in exhaled CO_2, 9% in faeces and approx. 1% in exhaled volatile organics by 72 hours after dose administration. Following intravenous administration of 2-butyne-1,4-diol to rats or mice at 0.5 mg/kg body weight, elimination of the delivered dose of radioactivity was very rapid in both species, with 70 to 84% recovered 24 hours after dosing and approx. 51% excreted via the kidney into the urine, approx. 22% as exhaled CO_2 and approx. 16% by the intestines into the faeces by 72 hours after dosing. 2-Butyne-1,4-diol was removed from the blood very rapidly with a half-life of < 30 minutes, as little as approx. 1% of the administered radioactivity remaining in the blood at 4 hours after dosing. Only approx. 10% of the administered dose of radioactivity was recovered in tissues after 24 hours. With approx. 16% of the substance being eliminated in faeces, intravenous studies showed that 2-butyne-1,4-diol was excreted in bile and then reabsorbed further down the intestinal tract upon administration of 5 mg/kg (^{14}C)-2-butyne-1,4-diol to male Sprague-Dawley rats, which excreted 63% of the delivered dose in bile by 24 hours, and to male Fischer-344 rats, which excreted approx. 59% of the delivered dose by 4 hours after dosing. NMR and mass spectrometry were used to identify two biliary metabolites, 4,4-bis(S-glutathionyl)-2-hydroxytetrahydrofuran and 3-(S-glutathionyl)-2(5H)-furanone. Together the results suggest a high degree of enterohepatic recirculation and further metabolism, with ultimate excretion via the kidneys

into the urine or via the lungs as CO_2. A similar excretory pattern was demonstrated in rats and mice after oral administration of 50 mg (^{14}C)-2-butyne-1,4-diol/kg body weight. The majority of the administered radioactivity was excreted in the urine and faeces and as exhaled CO_2, the respective percentages being approx. 54%, approx. 20% and 5 to 9% (RTI, 2002).

2-Butyne-1,4-diol was oxidised by alcohol dehydrogenase to as yet undefined degradation products in the rat. The value for the Michaelis constant (K_m) for the reaction (under in-vitro conditions) was 8.2×10^{-4} M (compared with ethanol: 7.9×10^{-4} M). With the results of the animal studies described below, Taberner and Pearce (1974) succeeded in demonstrating that 2-butyne-1,4-diol is rendered toxic by liver alcohol dehydrogenase in vivo, i.e. is converted to a toxic metabolite. Groups of 6 male and 6 female Wistar rats (weighing 320 to 360 g) were either administered a single intraperitoneal dose of 2-butyne-1,4-diol at levels of 0.558, 0.614, 0.675, 0.743, 0.817 or 1.116 mmol/kg body weight or they additionally received pyrazole at 2.9 mmol/kg body weight 10 minutes prior to dosing with 2-butyne-1,4-diol at 1.116 mmol/kg body weight. Following administration of 2-butyne-1,4-diol alone, the respective mortalities determined in the above-mentioned dose groups after 18 hours were 1/6, 2/6, 5/6, 6/6 and 6/6 animals, from which the LD_{50} was calculated as 0.609 to 0.635 mmol/kg body weight (equivalent to approx. 52.4 to 54.7 mg/kg body weight; see also Section 7.2). No deaths occurred in the group treated with pyrazole, a competitive inhibitor of liver alcohol dehydrogenase, prior to receiving a dose of 2-butyne-1,4-diol in excess of the LD_{100}. Whilst 2-butyne-1,4-diol alone additionally caused marked toxic effects, which persisted for 6 to 8 hours (e.g. sedation, coughing, diarrhoea, decreased motor activity, decrease in body temperature), administration of pyrazole prevented the occurrence of toxic effects following the subsequent administration of 2-butyne-1,4-diol at the high dose level. The in-vitro study with rat liver extract also showed that pyrazole competitively inhibited the oxidation of 2-butyne-1,4-diol by rat liver alcohol dehydrogenase. These studies permit the conclusion that it is the products of oxidative metabolism which are responsible for the toxicity of 2-butyne-1,4-diol (Taberner and Pearce, 1974).

7.2 Acute and subacute toxicity

Acute toxicity

The oral LD_{50} values for the rat, mouse, guinea pig, rabbit and cat are summarised in Tables 1, 2 and 3 below. Oral LD_{50} values for the rat (see Table 1) were found to be about 100 mg/kg body weight in most studies. Therefore, 2-butyne-1,4-diol was toxic following acute oral administration. After 4-hour inhalation exposure the combined LC_{50} for both sexes was approx. 690 mg/m³ (> 690 < 1030 mg/m³ and 600 mg/m³ for males and females, respectively). Therefore, the substance was also toxic by inhalation. The chemical was harmful upon dermal exposure, with LD_{50} values ranging between 424 and 1240 mg/kg body weight. The LD_{50} for intraperitoneal administration was between 52 and 55 mg/kg body weight. The LD_{50} values for the mouse (see Table 2) were found to be 100 and 105 mg/kg body weight after oral administration, between 15 and 100 mg/kg body weight after intraperitoneal injection and 63 mg/kg body weight after subcutaneous administration. In the rabbit and the guinea pig (see Table 3), the respective oral LD_{50} values were 150 mg/kg body weight and 130 mg/kg body weight. Clinical signs of toxicity on acute exposure included sedation, analgesia, disturbances of balance, lying on the side, tonic-clonic convulsions, ruffled fur, accelerated breathing, bradycardia, apathy, salivation, and, in rats, coughing (according to the investigators; Taberner and Pearce, 1974), hyperaemia of the visible skin, reductions in body temperature and diarrhoea. Necropsy revealed pulmonary haemorrhages, petechiae of the mucous membrane of the gastrointestinal tract, oedema and congestion of the internal organs and signs of toxic fatty degeneration of the liver and nephrosis. The substance should therefore be evaluated as toxic on oral and inhalation exposure and as harmful on dermal application.

Table 1. Acute toxicity of 2-butyne-1,4-diol in the rat

Species	No. of animals/dose	Sex	Route of administration	Observation period	LD_0 (mg/kg body weight)	LD_{100} (mg/kg body weight)	LD_{50} (mg/kg body weight)/LC_{50}	Reference
Rat	–	–	oral (2-butyne-1,4-diol pure, solid)	7 days	–	–	136	BASF, 1959 a
Rat	–	–	oral (2-butyne-1,4-diol ca. 30%)	7 days	–	–	53	BASF, 1959 b
Rat	10	–	oral	14 days	50	200	100 (150 LD_{70})	Stasenkova and Kochetkova, 1965; Izmerov et al., 1982
Rat	–	–	oral	–	–	–	104.50	Knyshova, 1968
Rat	–	–	oral	7 days	–	–	0.13 ml/kg b. w. (≙ 135 mg/kg b. w.)	BASF, 1987 b
Rat (Sprague-Dawley)	10	males, females	oral (aqueous solution of ca. 34% 2-butyne-1,4-diol and 4% hexamethylenetetramine)	14 days	males 147 females 215	males 215 females 464	ca. 240	BASF, 1981 a
Rat	–	–	oral (2-butyne-1,4-diol technical grade, solid)	7 days	–	–	ca. 100	BASF, 1973 a
Rat	–	–	oral (aqueous solution of 94% 2-butyne-1,4-diol, pure, solid, and 6% hexamethylenetetramine)	7 days	–	–	ca. 110	BASF, 1973 b
Rat (Wistar)	10	males, females	oral	14 days	–	–	males 132 females 176	Jedrychowski et al., 1992 a
Rat	–	–	oral	–	–	–	125	General Aniline and Film Corporation, 1962
Rat (ChR-CD)	–	–	oral (as a 10% and a 2% solution)	–	–	–	ca. 300[1]	Haskell Laboratory, 1966
Rat (Wistar)	10	males, females	oral (2-butyne-1,4-diol 50% in double-distilled water)	14 days	–	males 501 females > 794	492[2]	Hüls, 1985 d

Table 1. Acute toxicity of 2-butyne-1,4-diol in the rat								
Species	No. of animals/dose	Sex	Route of administration	Observation period	LD_0 (mg/kg body weight)	LD_{100} (mg/kg body weight)	LD_{50} (mg/kg body weight)/LC_{50}	Reference
Rat	–	–	inhalation (2 hours)	–	–	–	150–280 mg/m³[3]	Stasenkova and Kochetkova, 1965; Izmerov et al., 1982
Rat (Wistar)	10	males, females	inhalation (4 hours; aqueous solution, 99.5% pure)	14 days	males 690 mg/m³ females 320 mg/m³	1030 mg/m³	690 mg/m³ (males > 690 < 1030 mg/m³, females 600 mg/m³)	BASF, 1996
Rat (Sprague-Dawley)	20	males, females	inhalation (4 hours; aqueous solution of ca. 34% 2-butyne-1,4-diol and 4% hexamethylenetetramine)	14 days	males 252 mg/m³ females 1990 mg/m³	males 3280 mg/m³ females > 3280 mg/m³	2500 mg/m³ (males 2200 mg/m³, females 2900 mg/m³)	BASF, 1980 b
Rat	5	–	dermal (2-butyne-1,4-diol pure, as a 30% aqueous solution)	–	after exposure for more than 2.5 hours	–	–	BASF, 1959 a
Rat	5	–	oral (undiluted "technical grade" 2-butyne-1,4-diol approx. 30%)	48 hours	after exposure for more than 1 hour	after exposure for more than 4 hours	–	BASF, 1959 b
Rat (Wistar)	10	males, females	dermal (ca. 99% pure, made into a paste with NaCl solution)	14 days	–	2000 (in females)	1250[4]	Hoechst, 1988
Rat	5–10	males, females	dermal (ca. 99% pure, made into a paste with NaCl solution)	14 days	males 50 females 400	females 2000[5]	659 (males 424, females 983)	Hoechst, 1990
Rat (Wistar)	11	females	dermal (ca. 99% pure, solid, made into a paste with water)	14 days	5000	–	–	Jedrychowski et al., 1992 a

Table 1. Acute toxicity of 2-butyne-1,4-diol in the rat

Species	No. of animals/dose	Sex	Route of administration	Observation period	LD_0 (mg/kg body weight)	LD_{100} (mg/kg body weight)	LD_{50} (mg/kg body weight)/LC_{50}	Reference
Rat (Wistar)	16	females	dermal (ca. 99% pure, as a 40% aqueous solution)	14 days	–	–	5000[6]	Jedrychowski et al., 1992 a
Rat (Wistar)	6	–	intraperitoneal	24 hours	0.2 mmol/kg b. w. (≙ 17.2 mg/kg b. w.)	0.817 mmol/kg b. w. (≙ 70.38 mg/kg b. w.)	0.609–0.635 mmol/kg b. w. (≙ 52.4–54.7 mg/kg b. w.)	Taberner and Pearce, 1974[7]

– no data
b. w. body weight
[1] approximate lethal dose (ALD)
[2] presumably based on the formulation containing 50% 2-butyne-1,4-diol; under this assumption, the LD_{50} for pure 2-butyne-1,4-diol would be 246 mg/kg body weight
[3] no precise data on the number of animals that died; 2-butyne-1,4-diol was heated to 35 to 40 °C
[4] in females; males were tested only at 2000 mg/kg body weight (= LD_{80} in males), whereas females were tested at 1250, 1600 or 2000 mg/kg body weight
[5] in males 2000 mg/kg body weight = LD_{80}
[6] only dose tested; 8/16 animals died within 48 hours
[7] see also Section 7.1

Table 2. Acute toxicity of 2-butyne-1,4-diol in the mouse

Species	No. of animals/dose	Sex	Route of administration	Observation period	LD_0 (mg/kg body weight)	LD_{100} (mg/kg body weight)	LD_{50} (mg/kg body weight)/ LC_{50}	Reference
Mouse	10	–	oral	14 days	50	200	100 (150 LD_{70})	Stasenkova and Kochetkova, 1965; Izmerov et al., 1982
Mouse	–	–	oral	–	–	–	104.75	Knyshova, 1968
Mouse	20	–	inhalation (2 hours)	–	–	–	150–280 mg/m³[1]	Stasenkova and Kochetkova, 1965; Izmerov et al., 1982
Mouse	–	–	intraperitoneal	–	–	–	15	Carlson and Morgan, 1954
Mouse	–	–	intraperitoneal[2]	7 days	–	–	84	BASF, 1959 a
Mouse	–	–	intraperitoneal[3]	7 days	–	–	42	BASF, 1959 b
Mouse	–	–	intraperitoneal	7 days	–	–	ca. 100	BASF, 1973 a
Mouse	–	–	intraperitoneal (aqueous solution of 94% 2-butyne-1,4-diol and 6% hexamethylenetetramine)	7 days	–	–	ca. 100	BASF, 1973 b
Mouse	10	males, females	intraperitoneal (aqueous solution of ca. 34% 2-butyne-1,4-diol and 4% hexamethylenetetramine)	18 days	–	–	ca. 200	BASF, 1981 a
Mouse	–	–	subcutaneous[2]	7 days	–	–	63	BASF, 1959 a
Mouse	–	–	subcutaneous[3]	7 days	–	–	53	BASF, 1959 b

– no data
[1] no precise data on the number of animals that died; 2-butyne-1,4-diol was heated to 35 to 40 °C
[2] 2-butyne-1,4-diol, pure
[3] 2-butyne-1,4-diol, approx. 30%

Table 3. Acute toxicity of 2-butyne-1,4-diol in the guinea pig, rabbit and cat								
Species	No. of animals/dose	Sex	Route of administration	Observation period	LD_0 (mg/kg body weight)	LD_{100} (mg/kg body weight)	LD_{50} (mg/kg body weight)/ LC_{50}	Reference
Guinea pig	–	–	oral	–	–	–	130	Knyshova, 1968
Guinea pig	–	–	oral	–	–	–	125	General Aniline and Film Corporation, 1962
Rabbit	2	–	oral[1]	–	50	100	–	BASF, 1959 a
Rabbit	2	–	oral[2]	–	150	300	–	BASF, 1959 b
Rabbit	–	–	oral	–	–	–	150	Knyshova, 1968
Rabbit	3	–	dermal[3]	4 weeks	ca. 65 after exposure for 24 hours[4]	–	–	BASF, 1959 b
Rabbit	–	–	dermal[5]	7 days	–	–	> 2000	BASF, 1981 a
Cat	2	–	oral[1]	–	–	50	–	BASF, 1959 a
Cat	2	–	oral[2]	–	–	150	–	BASF, 1959 b

– no data
[1] 2-butyne-1,4-diol, pure
[2] 2-butyne-1,4-diol, 30%
[3] undiluted “technical grade” 2-butyne-1,4-diol, approx. 30%
[4] no deaths after 20-hour exposure of both ears; dose converted to pure 2-butyne-1,4-diol equivalents
[5] aqueous solution of ca. 34% 2-butyne-1,4-diol and 4% hexamethylenetetramine

Several studies are discussed below in detail as examples.

An acute oral toxicity study was conducted in groups of 5 male and 5 female Wistar rats (322 ± 43 g and 209 ± 21 g, respectively) given a single dose of 2-butyne-1,4-diol (approx. 99% pure) at 100, 150, 180, 200 or 250 mg/kg body weight as a 10% aqueous solution by gavage. The observation period was 14 days. The calculated LD_{50} values for male and female rats were 132 (89 to 158) mg/kg body weight and 176 (118 to 270) mg/kg body weight, respectively. The animals died within 48 hours of dosing. No clinical signs of toxicity were described. Macroscopic effects in the rats that died included diarrhoea and congestion of the internal organs, while the main histological effects were liver and kidney damage. These were also observed in the rats sacrificed at the end of the observation period (Jedrychowski et al., 1992 a).

When a single oral dose of 2-butyne-1,4-diol was administered to ChR-CD rats as a 2 or 10% aqueous solution, 300 mg/kg body weight was observed as an approximate lethal dose (no further details; Haskell Laboratory, 1966).

The acute inhalation toxicity was investigated in accordance with OECD guideline No. 403. Groups of 5 male and 5 female Wistar rats underwent a single exposure to concentration levels of 0.26, 0.32, 0.69 or 1.03 mg/l (equivalent to 260, 320, 690 or 1030 mg/m³) for 4 hours. For this purpose, a liquid aerosol was generated from an aqueous solution of 99.5% pure 2-butyne-1,4-diol. Whereas there were no fatalities at 0.26 and 0.32 mg/l, 4/5 females but no male died at 0.69 mg/l and all animals died at 1.03 mg/l. The female rats therefore were slightly more susceptible to the acute actions of 2-butyne-1,4-diol. The mass median aerodynamic diameter of the aerosol particles was 0.5 to 1.0 µm and thus within the respirable range. Clinical observations revealed some signs of respiratory tract irritation and general toxicity; however animals died without showing any specific signs. During the 14-day observation period, male body weights were unaffected at the three lower dose levels (all top-dose males died) whilst female body weights were slightly decreased. Necropsy of the high-dose animals that died during the study revealed red discoloration of the lungs and light brown discoloration of the liver, whereas animals from the second highest concentration group showed erosion/ulceration of the glandular stomach or general congestion. Animals sacrificed at the end of the study showed no macroscopic changes. The results of the study led the investigators to con-

clude that the highest concentration in the subsequent 5-day inhalation study should not exceed 0.4 mg/l (equivalent to 400 mg/m³). The LC_{50} values were calculated as approx. 0.69 mg/l (equivalent to 690 mg/m³) for both sexes combined and as > 0.69 to < 1.03 mg/l for male rats and 0.6 mg/l for female rats (BASF, 1996).

In an inhalation hazard test, rats were exposed to atmosphere enriched with the components of an aqueous solution of approx. 34% 2-butyne-1,4-diol and 4% hexamethylenetetramine that were volatile at 20 °C. None of the 12 exposed rats died after 3-hour exposure, whereas 2 out of 12 died after 7-hour exposure. Eyelid closure, rough coat and intermittent and accelerated breathing were observed during exposure. The deceased animals were found to have toxic hepatocellular steatosis with peripheral necrosis at necropsy (BASF, 1981 a).

An inhalation hazard test was carried out using atmospheres enriched or saturated at 20 °C with 2-butyne-1,4-diol or a product containing 94% 2-butyne-1,4-diol and 6% hexamethylenetetramine. In both studies, all 12 rats per 8-hour exposure group survived without signs of toxicity (BASF, 1973 a, b).

No mortality was observed after 2 hours in an inhalation hazard test in which 6 rats were exposed to atmosphere enriched or saturated with 2-butyne-1,4-diol (pure) at 70 °C. Exposure for 8 hours was lethal to 6/6 rats after 4.5 to 24 hours. When the same test was performed using atmosphere saturated with 2-butyne-1,4-diol (approx. 30% pure) at 20 °C, none of 6 exposed animals died after 2-hour exposure whilst one out of 6 rats died 2 days after 8-hour exposure. The rat that died showed jaundice as a sign of liver damage (BASF, 1959 a, b).

The acute dermal toxicity was investigated in male and female Wistar rats of the Hoe:WISKf(SPF71) strain after a single 24-hour application and with a 14-day observation period in accordance with OECD guideline for testing No. 402. The purity of the substance tested in two separate substudies was 98.9 or 99.5% and the doses ranged from 50 to 2000 mg/kg body weight 2-butyne-1,4-diol (made into a paste with 0.9% aqueous saline). Signs of toxicity observed included difficulty in breathing, motor disturbances, narrow palpebral fissures, bloody encrustation of the nose and margins of the eyelids and loss of righting and paw-pinch reflexes. Body weight gain was

depressed in the first week of the study. The lethally poisoned rats died within 3 days. Necropsy revealed accumulation of blood in the colon and urinary bladder, hepatic lobular markings and discoloration of the liver and spleen. The LD_{50} value in male rats was 424 mg/kg body weight while that in female rats was 983 mg/kg body weight. A combined LD_{50} of 659 mg/kg body weight was calculated for both sexes together. Male rats were thus more susceptible than female rats (Hoechst, 1988, 1990).

The acute dermal toxicity of 2-butyne-1,4-diol was investigated in a further study. Wistar rats (213 ± 17 g) had a single 5000 mg/kg dose of 2-butyne-1,4-diol (approx. 99% pure) applied occlusively to the clipped dorsal skin either in the solid form (moistened with water; 11 rats) or as a 40% aqueous solution (16 rats). The exposure lasted for 24 hours and the post-exposure observation period was 14 days. There were no deaths after application of the moist paste of solid 2-butyne-1,4-diol, whilst administration of the 40% aqueous solution resulted in the death of 8/16 rats within 48 hours. Necropsy revealed liver and kidney lesions. Rats killed after the 14-day observation period also had liver lesions. The same results were obtained in rats exposed to undiluted 2-butyne-1,4-diol (Jedrychowski et al., 1992 a).

A group of 20 mice underwent a single 2-hour exposure to the volatile vapours of 2-butyne-1,4-diol at 35 to 40 °C (“dense white vapours”). The concentration in air ranged from 0.15 to 0.28 mg/l. Signs of mucous membrane irritation were observed (closed eyes, rubbing of the snout) as well as motor excitation with subsequent depression. Fatalities occurred both during and after exposure (no further details). The surviving animals recovered only slowly. Autopsy of the mice that died revealed congestion of the internal organs and the brain. The lungs showed uneven congestion and small haemorrhages as well as marked catarrhal-desquamative bronchitis. Histopathological effects noted after the 14-day observation period included congestion and oedema of the internal organs, an increase in polynuclear leucocytes in the lungs and spleen and moderate albuminous degeneration of the convoluted tubules of the kidney (Stasenkova and Kochetkova, 1965).

The lowest published lethal concentration (LCLo) for a 2-hour inhalation exposure to 2-butyne-1,4-diol was 150 mg/m³ (equivalent to 42 ppm) in rats and mice (no further details; Stasenkova and Kochetkova, 1965; Izmerov et al., 1982).

In an exploratory study, a single oral administration of 50 mg/kg body weight was lethal to cats, whilst two rabbits both survived two administrations at the same dose level (50 mg/kg body weight) after showing accelerated breathing or diarrhoea. In the rabbit, a single dose of 100 mg/kg body weight resulted in death. Gross pathology identified scattered haemorrhages in the gastrointestinal tract, fatty degeneration of the liver and pulmonary oedema (BASF, 1959 a, b, 1987 b, 1992 c).

Subacute toxicity

In a 5-day study, groups of 5 male and 5 female Wistar rats received 2-butyne-1,4-diol (98.9% pure, dissolved in double-distilled water) at 5, 10 or 20 mg/kg body weight/day by oral gavage. All animals were tested for neurological function (for a detailed account, see Section 7.10) before the first dose, 24 hours after the first dose and 24 hours after the fifth dose, and kept under close observation. The clinical chemistry and haematology parameters were studied in accordance with OECD guideline No. 407. In addition cholinesterase activity was determined in the serum and erythrocytes. Organ weights were determined for the liver, kidneys, adrenal glands and testes. Analytical verification of the administered concentrations revealed that respective actual exposure levels for the top, intermediate and low dose groups were 98.5% (equivalent to 19.7 mg/kg body weight), 87% (equivalent to 8.7 mg/kg body weight) and 80% (equivalent to 4 mg/kg body weight) of the target concentrations. Apart from a dose-dependent increase in cholesterol in males, which attained statistical significance in the high dose group only, there were no treatment-related changes. The *no adverse effect level* (NOAEL) in this 5-day study was thus 10 mg/kg body weight (see also Section 7.10; BASF, 1992 a).

In order to study the subacute toxicity of 2-butyne-1,4-diol (97 to 99% pure), groups of 10 male and 10 female Sprague-Dawley rats (200 to 300 g) were given doses of 1, 10, 100 or 10 mg/kg body weight as an aqueous solution by oral gavage on 14 consecutive days prior to being killed on day 15. After 8 doses, one male rat in the top dose group and one female rat in the low dose group died, although these fatalities were judged not to be treatment-related (probably gavaging errors). Body weight gain was significantly ($p \leq 0.05$) decreased relative to the control group in the male but not the female rats in the highest dose group (weight gain 51 ± 13 g in top-dose males, 86 ± 16 g in controls). Some rats at the top dose level had

blood-tinged nasal discharges, piloerection and diarrhoea. A significant ($p \leq 0.05$) increase in serum cholesterol occurred in both sexes in the top dose group (males 77.1 mg/100 ml, controls 60.0 mg/100 ml; females 112.1 mg/ml, controls 65.3 mg/ml), with an increase in serum calcium found in female rats in this dose group (11.2 mg/ml, controls 10.6 mg/ml) as well as a decrease in aspartate aminotransferase (glutamic-oxaloacetic transaminase) activity (106.5 mU/ml, controls 144.8 mU/ml) and in glucose level (154.6 mg/100 ml, controls 177.5 mg/100 ml). Female rats in the top dose group also showed a slight increase in aminopyrine demethylase activity (14.8 nmol HCHO/hour/mg protein, controls 11.0 nmol HCHO/hour/mg protein). Haematology revealed decreased red cell counts (6.8×10^6/µl, controls 7.5×10^6/µl) and haemoglobin content (13.1 g/100 ml, controls 14.4 g/100 ml) in females. Male rats exhibited no significant change in red blood cell count. At necropsy on day 15, relative liver weights were significantly increased ($p \leq 0.05$) in animals from the top dose group (males 5.5 ± 0.5%, controls 3.6 ± 0.3%; females 5.3 ± 0.5%, controls 3.5 ± 0.4%). Histopathological examination (28 organs) revealed no treatment-related effects. The NOAEL for 14-day oral administration was thus 10 mg/kg body weight (Komsta et al., 1989).

In a 4-week study, groups of 8 male and 8 female Wistar rats (mean initial weights 188 ± 20 g and 145 ± 15 g, respectively) were given 2-butyne-1,4-diol at dose levels of 0 (controls), 1, 10 or 50 mg/kg body weight as an aqueous solution once daily by gavage. In the top dose group, 3 male and 3 female rats died after 7 to 28 days, most after 26 to 28 days. Body weight gain was significantly ($p < 0.05$) lower in male rats, while that of the females was unaffected. Haematological effects at the end of treatment included significant reductions in erythrocyte count ($p \leq 0.01$), haemoglobin concentration ($p \leq 0.05$) and haematocrit value ($p \leq 0.01$) in top-dose female rats and a significant increase in reticulocyte count in both sexes in the top dose group ($p \leq 0.01$). White blood cell counts were significantly ($p \leq 0.01$) increased in both sexes at the top dose level, with neutrophils ($p \leq 0.05$ and ≤ 0.01) and lymphocytes ($p \leq 0.05$) affected. Clinical chemistry determinations at the end of the study showed significant increases in sorbitol dehydrogenase activity for both sexes at the top dose level ($p \leq 0.01$). Additionally, significant ($p \leq 0.05$) increases were observed in the total serum protein concentration in top-dose females and the glucose level in top-dose males. At the end of the study, there were increases in

the absolute and relative liver weights ($p \leq 0.01$) and in the absolute and relative kidney weights ($p \leq 0.05$ to ≤ 0.01) in both sexes at the top dose level. The relative liver weight was also increased in female rats at the intermediate dose level ($p \leq 0.05$). Histopathological examination of rats treated at the intermediate and high dose levels revealed congestion of the internal organs, pulmonary oedema, marked tubular degeneration and interstitial mononuclear cell infiltrations in the kidney and severe diffuse hepatic parenchymal necrosis (mainly centrilobular) accompanied by fatty degeneration in the rest of the parenchyma, effects which were absent in the low dose group. The *no effect level* in this study was thus 1 mg/kg body weight (Jedrychowski et al., 1992 b).

A range-finding study in accordance with OECD guideline for testing No. 412 and Directive 92/69/EEC was conducted in groups of 5 male and 5 female Wistar rats that underwent head-nose exposure to liquid aerosols of a 12.5% aqueous solution of 2-butyne-1,4-diol (99.5% pure) at concentration levels of 0 (controls), 25, 100 or 300 mg/m³ for 6 hours per day for 5 consecutive days. Animals were given clinical examinations before, during and after exposure on exposure days. Body weights were determined before the start of the preflow period and 3 times during the exposure period. Ophthalmoscopy was carried out prior to and at the end of the exposure period. At the end of the study, comprehensive clinicochemical and haematological examinations and urinalysis were performed. In addition, gross pathological and histopathological examinations were performed. The analytical concentrations determined by gas chromatography were 27.1 ± 6.5, 102.2, ± 30 and 305.2 ± 19.5 mg/m³ and essentially corresponded to the target concentrations. The mean particle size as measured by cascade impactor analysis was between 0.5 and 0.9 µm mass median aerodynamic diameter. One male and one female in the high concentration group (300 mg/m³) died during the exposure period. Clinical findings were confined to this concentration group and consisted in signs of upper respiratory tract irritation and reduction in general health. Body weight development was slightly delayed in both sexes. Both sexes also showed increased γ-glutamyltransferase activities and bilirubin and cholesterol levels and decreased urea in the serum as well as increased urobilinogen in the urine. Whereas no treatment-related gross pathological changes were observed at the high concentration, histopathology revealed single cell necrosis in the liver (4/5 females) and dystrophy of the liver (2/5 males, 1/5 females) –

which was identified as the cause of death in the two animals that died – as well as inflammation and/or epithelial changes in the nasal cavity (purulent rhinitis, focal disarrangement or atrophy of the olfactory epithelium) and the larynx (mixed cellular inflammation and hyperplasia and metaplasia of the transitional epithelium). The intermediate concentration group (100 mg/m³) showed toxic damage to the hepatic parenchyma as evidenced by increased urobilinogen levels in the urine and signs of inflammation and/or epithelial changes in the nasal cavity (focal disarrangement of the olfactory epithelium) and the larynx (mixed cellular inflammation and hyperplasia and metaplasia of the transitional epithelium). Inhalation of the low concentration of 25 mg/m³ caused a higher incidence of increased urobilinogen levels in the urine and inflammatory as well as epithelial changes in the larynx (mixed cellular inflammation and metaplasia of the transitional epithelium). In summary, inhalation exposure of rats to 2-butyne-1,4-diol at a concentration of 300 mg/m³ for 5 days resulted in systemic toxicity characterised by functional and morphological impairment of the liver including increases in urinary urobilinogen and delayed body weight development in males and females. In addition, this concentration produced local inflammation and/or epithelial changes in the nasal cavity and the larynx. Based on the findings that the intermediate concentration of 100 mg/m³ was associated with increased urinary urobilinogen, local effects in the nose and larynx and even the low concentration group (25 mg/m³) showed toxic damage to the hepatic parenchyma as evidenced by increases in urinary urobilinogen levels and signs of inflammation and/or epithelial changes in the larynx, it was decided that the subsequent main study should be conducted with concentrations below 50 mg/m³ (BASF, 1997 b).

The subsequent subacute inhalation neurotoxicity study was conducted in accordance with OECD guidelines for testing Nos. 412 and 413, Directive 92/69/EEC and US EPA Health Effects Testing Guidelines 40 CFR 798.6059, 798.6200 and 798.6400. In this study, groups of 16 male and 16 female Wistar rats underwent head-nose exposure to 2-butyne-1,4-diol at concentration levels of 0 (controls), 0.5, 5 or 25 mg/m³ for 6 hours per day. The aerosol was generated using a 2% aqueous solution of 99.5% pure test substance. At 0.5 mg/m³, it was present as a vapour, at 5 mg/m³ as a dry solid aerosol and at 25 mg/m³ either as a dry solid aerosol or as an aerosol consisting of droplets of highly concentrated aqueous solutions. In order to investigate the concentration-time-response relationship, half of

the animals served as a concurrent satellite group for 15 study days (10 exposures), while the other half of the animals were maintained for 30 study days (20 exposures). The examinations described below were carried out identically in the satellite and main study groups. Animals were given clinical examinations before, during and after exposure on exposure days. Body weights were determined weekly. Ophthalmoscopy was carried out prior to and at the end of the exposure period. Detailed neurotoxicological studies (functional observational battery) and motor activity measurements were performed in 5 animals per sex and group before the start of exposure, after 8 exposures (main and satellite groups) and after 18 exposures (main groups). At the end of the study, comprehensive clinicochemical and haematological examinations and urinalysis were performed in 5 animals per sex and group. In addition, gross pathological and histopathological examinations were performed in 5 animals per sex and group. Three animals per sex and group underwent perfusion fixation and subsequent neuropathological examination. The analytical concentrations determined by gas chromatography were 0.48 ± 0.05, 5.2, ± 0.51 and 25.6 ± 2.6 mg/m³ and essentially corresponded to the target concentrations (0.5, 5 and 25 mg/m³). The mean particle size as measured by cascade impactor analysis was between 0.81 and 0.99 µm mass median aerodynamic diameter for the intermediate and approx. 0.83 µm mass median aerodynamic diameter for the high concentration groups, whilst it was not measurable for the low concentration group. As shown in Table 4, the high and intermediate concentrations of 2-butyne-1,4-diol (25 and 5 mg/m³) produced local irritant effects in the upper respiratory tract both after 10 and 20 exposures (larynx, trachea). Squamous metaplasia and inflammation of the larynx occurred following 10 and 20 exposures to 25 mg/m³ and 20 exposures to 5 mg/m³. Focal inflammation at the tracheal bifurcation were observed only after 20 exposures to 25 mg/m³.

Table 4. Incidences of histopathological findings in rats from the intermediate and high concentration group after receiving 10 and 20 exposures in the 30-day inhalation study

Effects	Concentration (mg/m³)	10 exposures		20 exposures	
		male	female	male	female
Focal squamous metaplasia in section level I of the larynx	5	4/5	5/5	2/5	5/5
	25	4/5	5/5	4/5	5/5
Focal inflammation in section level I of the larynx	5	–	–	1/5	1/5
	25	4/5	4/5	2/5	2/5
Focal inflammation at the tracheal bifurcation	5	–	–	–	–
	25	–	–	2/5	2/5

There was some indication of an increase in inflammatory incidence in the upper respiratory tract with prolongation of exposure time and increase in concentration, but no increase in severity of histopathological changes was observed (all histopathological findings were graded minimal to slight). No treatment-related clinical, neurofunctional, clinico-chemical, haematological, gross pathological or micropathological changes were observed in the low concentration group (0.5 mg/m³). The increased urinary urobilinogen values observed as an indication of toxic damage to the hepatic parenchyma even at the low concentration of 25 mg/m³ (analytical concentration 27 mg/m³) in the 5-day range-finding study could not be verified after either 10 or 20 days of exposure in the 28-day study. This finding could be attributable to the slightly higher concentration in the 5-day study, where additionally concentrations were subject to greater day-to-day variations, or to functional adaptation during prolonged exposure. In summary, none of the concentrations tested caused systemic toxicity. However, the high and intermediate concentrations of 2-butyne-1,4-diol, 25 and 5 mg/m³, produced local irritant effects in the upper respiratory tract both after 10 and 20 exposures. When the results from the 5-day range-finding study and the satellite groups given 10 exposures were taken into account, there was no indication of cumulative systemic toxicity when the duration of exposure was extended to 20 exposures at concentration levels of up to 25 mg/m³. It was considered that the effects noted in the larynx and trachea should be interpreted as unspecific responses to local irritation due to the deposition of 2-butyne-1,4-diol aerosol in the aerodynamic traps presented by the larynx and tracheal bifurcation. The NOAEC for systemic toxicity was 25 mg/m³ (highest concentration tested), while that for local toxicity to the upper respiratory tract was 0.5 mg/m³ (see also Section 7.10; BASF, 1998).

A group of 20 mice (18 to 20 g) inhaled 2-butyne-1,4-diol vapours generated by heating the substance to 30 °C, for 2 hours daily, 6 times a week for one month. The concentration was between 0.09 and 0.12 mg/l air (equivalent to 90 and 120 mg/m³). Clear signs of irritation of the eyes and respiratory organs were observed. Body weight was retarded by 15 to 20% compared with the control group (10 animals) 14 days after the beginning of the study, an effect that continued during the last 2 weeks of the study. By the end of the second study week, the neuromuscular stimulation threshold was decreased to 5 mA compared with 8 to 9 mA in the control group. On day 18 of the study, 2 animals died during exposure. Necropsy

on mice killed immediately after the end of the last exposure revealed severe hyperaemia of the internal organs and the brain. Microscopic effects included congestion and mild oedema of the internal organs, moderately pronounced pulmonary emphysema, an increase in polynuclear leucocytes in the spleen and albuminous degeneration of the convoluted tubules of the kidney (Stasenkova and Kochetkova, 1965). The study was not conducted in accordance with current guidelines and, due to inadequate reporting, is suitable for evaluation purposes only to a limited extent.

7.3 Skin and mucous membrane effects

Skin irritation studies

In a primary skin irritation study of 2-butyne-1,4-diol (approx. 99% pure), groups of 4 rabbits (White Vienna; 3.8 to 4.2 kg) had either 0.3 g of the water-moistened solid or 20% or 40% aqueous solutions of the compound applied occlusively to the clipped intact or scarified skin of the flank (2 cm x 2 cm). The single exposure period was 24 hours. Reactions were scored according to the Draize method at 1, 24, 48 and 72 hours after treatment. The solid caused no irritation of the intact skin, whereas it was found to produce slight reddening of the abraded skin in 1 out of 4 rabbits. One rabbit out of 4 also showed slight reddening after treatment with the 40% solution, whereas no rabbit showed any signs of skin irritation after the 20% solution. Based on these findings, the investigators evaluated 2-butyne-1,4-diol as not irritating to the skin (Jedrychowski et al., 1992 a).

In a skin irritation study, 2-butyne-1,4-diol produced no irritation when a 30% aqueous solution of the chemical was applied to the dorsal skin of white rabbits for 1, 5 or 15 minutes, or to the ear for 20 hours. The application of approx. 30% undiluted 2-butyne-1,4-diol for 1, 5 or 15 minutes produced no irritant effects while 20-hour exposure caused oedema and mild reddening of the dorsal skin and the ear, effects which faded after several days (BASF, 1959 a, b).

When pure 2-butyne-1,4-diol was applied to a 25 cm² area of depilated rabbit skin (number of animals unspecified) for 2 hours, pain-sensitive severe reddening and hardening of the skin occurred after exposure. The effects subsided after 1 to 2 days, and after 3 to 5 days scabs formed, which

sloughed off after 5 to 7 days. Flaking of the skin was also observed. When a further animal was exposed to 0.5 ml of a 30% aqueous solution of 2-butyne-1,4-diol, marked reddening also occurred, which was completely reversible after 2 to 3 days (Stasenkova and Kochetkova, 1965).

When an aqueous solution of approx. 34% 2-butyne-1,4-diol and 4% unchanged hexamethylenetetramine was applied to the skin of 2, 2 and 4 rabbits for 3 minutes, 1 hour or 4 hours, respectively, it was found that 3-minute exposure caused no irritation during the 8-day observation period. Following 1-hour exposure, mild reddening and mild oedema were observed after 2 days and scaling and scab formation with mild reddening and very mild oedema were noted after 8 days. Following 4-hour exposure, mild reddening with severe oedema was observed after 4 hours; equivocal to mild reddening and mild to severe oedema extending beyond the area of exposure were seen after one day; mild reddening and mild oedema extending beyond the area of exposure were noted after 2 days; effects were no longer present in 2 animals after 8 days; equivocal reddening with scaling and very mild oedema were found in the third animal and very severe reddening with severe necrosis and mild oedema were present in the fourth. Thus, the 34% aqueous solution of 2-butyne-1,4-diol was corrosive in one animal, with severe necrosis in the animal sacrificed after 8 days macroscopically confirmed by the pathologist on incision of the skin (BASF, 1981 a).

Occlusive application of 0.5 ml of an aqueous solution of approx. 34% 2-butyne-1,4-diol and approx. 4% unchanged hexamethylenetetramine to a 2.5 cm x 2.5 cm area of intact or scarified skin of groups of 4 male and 2 female rabbits (White Vienna) for 24 hours was observed to produce mild reddening and mild oedema which both extended beyond the area of exposure in all animals at the end of the 24-hour exposure period. By 72 hours, 2 out of 6 animals with originally intact skin and 4 out of 6 animals with scarified skin developed necrosis with mottled skin. At the end of the 8-day observation period, 4 out of 6 rabbits with originally intact skin and 5 out of 6 rabbits with scarified skin exhibited severe necrosis and 2 out 6 and 1 out of 6 animals, respectively, showed superficial necrosis, findings which a pathologist confirmed macroscopically by incision of the skin after sacrifice of the animals. The study director calculated a primary irritation index of 5.2 according to the 1973 Federal Register and evaluated the substance as severely irritating in this study. No healing of the skin lesions

was observed (BASF, 1981 a). Thus, the 34% solution of 2-butyne-1,4-diol was corrosive to the skin following 24-hour occlusive exposure.

A series of 4 different experiments was conducted in which one drop (approx. 0.05 ml) of a 40% aqueous solution of solid 2-butyne-1,4-diol was lightly rubbed into the intact and/or abraded skin of male albino guinea pigs. In the first experiment, reactions were negative in all 11 animals, for both intact and abraded skin (reactions read after one day). At day 1 of the second study, reactions of the intact skin consisted in mild erythema in 4/10 and no reaction in 6/10 animals while reactions of the abraded skin consisted in mild erythema in 2/10 animals and no reaction in 8/10 animals. On the second day, reactions of the intact skin were present only in 2/10 animals, and the abraded skin no longer showed any signs of irritation. In the third study, one guinea pig reacted with moderate erythema, 5 animals with mild erythema and 4 with no erythema following application to the intact skin, while application to the abraded skin caused mild erythema in 6 guinea pigs and no erythema in 4 guinea pigs. In the fourth experiment, the intact skin of 2, 5 and 4 animals showed moderate, mild and no erythema, respectively, while the abraded skin exhibited mild erythema in 10 animals and no erythema in one animal. In a further study, decomposed solid 2-butyne-1,4-diol was applied in the same manner, which in the intact skin caused oedema with moderate erythema in 2 guinea pigs, mild erythema in 4 guinea pigs and no reaction in 4 guinea pigs. The abraded skin reacted to decomposed solid 2-butyne-1,4-diol with mild erythema in 5 animals and no erythema in 5 animals. The investigators judged the 40% aqueous solution of 2-butyne-1,4-diol as mildly to nonirritating to guinea pig skin (no further details; Haskell Laboratory, 1966).

An 80% aqueous preparation of 2-butyne-1,4-diol was applied to the dorsal skin of rabbits for 1, 5 or 15 minutes or 20 hours, or to the auricular skin of rabbits for 20 hours. The 20-hour exposure resulted in slight reddening and severe oedema of the dorsal skin and slight reddening of the auricular skin after 24 hours. After 8 days, auricular skin reactions were unchanged whereas the dorsal skin showed severe necrosis with leather-like changes and reddened skin. The substance was thus corrosive in this study (BASF, 1973 a).

An 80% aqueous preparation of 94% 2-butyne-1,4-diol and 6% hexamethylenetetramine was applied to the dorsal skin of rabbits for 1, 5 or 15

minutes or 20 hours, or to the auricular skin of rabbits for 20 hours. The 15-minute exposure resulted in severe oedema of the dorsal skin after 24 hours. The 20-hour exposure dorsally led to mild necrosis and very severe marginal oedema and produced mild reddening of the ear after 24 hours. After 8 days, auricular skin reactions were unchanged whereas the dorsal skin in some cases showed severe necrosis, sometimes with leather-like changes and marginal mild erythema. The substance was thus corrosive in this study (BASF, 1973 b).

An 80% aqueous formulation (w/w) of undiluted 2-butyne-1,4-diol was applied to the dorsal skin of rabbits (White Vienna; 2 males, one female) for 4 hours in accordance with OECD guideline No. 404. Reactions included redness and oedema at 24 hours, in some cases associated with haemorrhages and moderate to severe oedema extending beyond the area of exposure at 48 and 72 hours, and scaling of the skin after 8 days. Therefore, the 80% concentration of the substance had an irritant effect (BASF, 1986 a).

In a skin irritation study conducted in accordance with OECD guideline No. 404, 6 rabbits (small white Himalayan; 3 males, 3 females) had 0.5 g of > 99% pure solid 2-butyne-1,4-diol applied to the clipped dorsal skin as an undiluted powder under a patch. After a 4-hour exposure period, the dressing was removed and the residual chemical rinsed off and the skin reactions were evaluated at 1, 24, 48 and 72 hours and 6, 8, 10 and 14 days after exposure. After only one hour mild reddening and severe oedema extending beyond the area of exposure were observed in 5 out of 6 animals. At 24 hours, the reddening was markedly increased. At 48 hours, the application site was dark red in one rabbit and had a dark-red marbled appearance in 4 rabbits. At 72 hours, the area of exposure was severely necrotic and a dark red to black colour in 5 out of 6 animals and in some cases there was severe oedema extending beyond the area of exposure. At days 6 and 8, the animals additionally had black scabs and necrosis as well as eschar formation. At days 10 and 14, 2 out of 6 rabbits were without findings, whilst at both examinations 4 rabbits had black scabs and necrosis, which by day 14 had partly dropped off in 2 animals and partly transformed into scar tissue in 2 animals. The report further states that necrosis formation occurred only after an exposure period of 4 hours, but not after 3 minutes. The study director calculated a primary irritation index of 6.21 based on the readings obtained at 1 to 72 hours and evaluated 2-butyne-1,4-diol as severely irritating (Hüls, 1985 a). Pure solid 2-butyne-

1,4-diol was corrosive to the skin in this study because at 14 days only 2 rabbits were without skin reactions while 4 out of 6 rabbits still had black, partially sloughing scabs and necrosis which transformed into scarry tissue in 2 rabbits.

Repeated application of 2-butyne-1,4-diol (30% solution, 10-day exposure) to the skin of the rabbit caused severe reddening, hardening, scab formation and sloughing of the epidermis (no further details; Stasenkova and Kochetkova, 1965).

Due to the corrosive potential of 2-butyne-1,4-diol at higher concentrations, it is mandatory to label solutions with a 2-butyne-1,4-diol content in excess of 50% as corrosive and concentrations between 25 and 50% as irritant (EC, 2004).

Eye irritation studies

No irritation was observed 10 minutes or 1, 3 or 24 hours after instillation of one drop of a 30% aqueous solution of pure 2-butyne-1,4-diol into the conjunctival sac of the rabbit eye (number of exposed animals unspecified) in an eye irritation study. Administration of undiluted, approx. 30% 2-butyne-1,4-diol resulted in barely perceptible mild reddening, which in the study director's interpretation represented "practically no irritation" (no further details; BASF, 1959 a, 1987 b).

Lacrimation, pronounced hyperaemia of the sclera and narrowing of the palpebral fissure were observed following instillation of one drop of a 30% aqueous solution of 2-butyne-1,4-diol into the conjunctival sac of a rabbit. After the second instillation, oedema of the mucous membrane of the eye was observed (no data on the time of the second instillation) and on the following day, purulent conjunctivitis developed. The effects described were completely reversible after 6 to 8 days (Stasenkova and Kochetkova, 1965).

Instillation of a 0.1 ml volume of an aqueous solution of approx. 34% 2-butyne-1,4-diol and approx. 4% unchanged hexamethylenetetramine into the conjunctival sac of 5 male rabbits and one female rabbit (White Vienna) resulted in slight opacity of up to half of the entire cornea in 3 out of 6 rabbits together with increased reddening of the conjunctivae in 2 rabbits by the end of the 72-hour observation period. The study director calculated a

primary irritation index of 3 according to the 1973 Federal Register and evaluated the substance as nonirritating in this study. No healing of the corneal lesions was observed (BASF, 1981 a). Therefore, the 34% aqueous solution of 2-butyne-1,4-diol was irritating to corrosive in this study.

An inadequately documented study reported that 2-butyne-1,4-diol was not irritating to the rabbit eye at concentrations below 1 mg/l water. Higher concentration levels of 2-Butyne-1,4-diol could be irritating to the eye (no further details; Knyshova, 1968).

A single instillation into the conjunctival sac of the rabbit eye (number of exposed animals unspecified) of 50 mm^3 or 50 mg of 2-butyne-1,4-diol (no further details) or a product consisting of 94% 2-butyne-1,4-diol and 6% hexamethylenetetramine produced mild redness, severe oedema and a greasy film after 1 and 24 hours, with the latter product additionally causing slight opacity. The effects showed slight improvement at 24 hours and were completely reversible by 8 days, and therefore 2-butyne-1,4-diol was not irritating in either study (BASF, 1973 a, b).

In a further study, which was carried out in accordance with OECD guideline No. 405, the introduction of approx. 47 mg (0.1 ml) of the ground solid substance into the eyes of rabbits (White Vienna; 2 males, one female) caused only slight redness and swelling of the conjunctivae, effects which were completely reversible after 72 hours. Therefore 2-butyne-1,4-diol did not have an irritant effect (BASF, 1986 b).

In another study, 4 New Zealand rabbits had a single 100 mg dose of 2-butyne-1,4-diol (approx. 99% pure) administered into the conjunctival sac of one eye. Reactions were scored according to the Draize method at 1, 24, 48 and 72 hours as well as up to 7 days after treatment. 2-Butyne-1,4-diol caused lacrimation and slightly closed lids in all animals after one hour. There was minimal conjunctival erythema at 24 and 48 hours, but this was reversible in the further course of the study (Jedrychowski et al., 1992 a). 2-Butyne-1,4-diol was thus not irritating to the eye in this study.

In an eye irritation study conducted in accordance with OECD guideline No. 405, the introduction of 100 mg pure undiluted ground solid 2-butyne-1,4-diol (> 99%) into the conjunctival sac of 3 male and 3 female rabbits (small white Himalayan) was observed to produce a mild to moderate irritant effect. The respective grades of irritation for the cornea, iris and con-

junctivae were 1.61, 0.5 and 2.28, and there was grade 0.56 chemosis. One out of 6 animals developed corneal opacity of grade 2 on a 4-point scale, which was irreversible even after 21 days. Five out of 6 had no findings at 21 days (no further details; Hüls, 1985 b). In one rabbit, the undiluted pure substance was corrosive to the cornea in this eye study.

Due to the corrosive potential of 2-butyne-1,4-diol at higher concentrations, it is mandatory to label solutions with a 2-butyne-1,4-diol content in excess of 50% as corrosive and concentrations between 25 and 50% as irritant (EC, 2004).

7.4 Sensitisation

2-Butyne-1,4-diol (99.2% pure) was tested for skin sensitisation potential in female guinea pigs (281 to 379 g at the start of the study) as a 25% solution in physiological saline in a maximisation test in accordance with OECD guideline No. 406. Very slight erythema developed after 24 hours during the induction phase in both the controls (5/20) and the test animals (14/18; 2 animals died intercurrently). No reactions occurred in the controls after the challenge treatment, while in the treatment groups a slight erythematous reaction was observed in 1/18 guinea pigs after 24 hours, an effect which viewed as coincidental by the investigators. 2-Butyne-1,4-diol had no skin-sensitising properties in this study (RCC, 1990).

Another study also found 2-butyne-1,4-diol to be devoid of skin sensitising effects. The study was conducted as a Magnusson and Kligman maximisation test in male and female Hartley albino guinea pigs (403 ± 48 g; 22 test animals, 8 control animals). Based on preliminary studies, intradermal induction was carried out with 2% solutions, dermal induction with 20% solutions and challenge with 5% and 20% solutions. None of the test animals developed allergic contact dermatitis (no further details; Jedrychowski et al., 1992 a).

2-Butyne-1,4-diol (solid, > 99% pure) was investigated for its skin-sensitising properties in a Magnusson and Kligman study in accordance with OECD guideline No. 406 by treating 20 male albino guinea pigs in comparison with a control group of 10 animals. Intracutaneous induction was performed using 0.5% 2-butyne-1,4-diol (dissolved in paraffin oil), while epidermal challenge treatment was carried out with 25% 2-butyne-1,4-diol (dissolved in H_2O). A positive response was observed in 5 out of 20 animals at 24 and

48 hours, and hence the compound was ascribed a sensitising effect (Hüls, 1985 c). In accordance with the “EG-Kennzeichnungsleitfaden” (EC labelling directive) the substance was evaluated as not sensitising because a chemical is classified as sensitising only if 30% or more animals show positive responses (BASF, 1992 c). However, 5 out of 20 animals had clearly positive responses at both readings, with clear negative responses in the control group. Therefore the study indicates a weak sensitising potential for 2-butyne-1,4-diol.

Ten guinea pigs were intradermally injected 3 times with 0.1 ml of a 10% aqueous solution of 2-butyne-1,4-diol while an eleventh guinea pig was intradermally injected once with 0.1 ml of a 40% and twice with 0.1 ml of a 10% aqueous solution. A challenge test was performed 14 days after the last injection by applying the substance to the intact or abraded skin. Definite positive reactions of the intact skin were observed upon challenge in 5/11 animals (2/5 animals had strong erythema with oedema, 2/5 had moderate erythema and 1/5 had mild erythema at day 1 after the treatment; 5/5 animals had strong erythema with oedema at 2 days, 2 of which were with residual mild effect at 5 days and one had a necrotic spot up to day 8 after challenge). One other animal out of 11 had a questionably positive reaction. Upon challenge of the abraded skin, 2 of the animals with a positive reaction in the previous experiment showed sensitisation reactions while the other 3 had questionably positive reactions. When the intact or abraded skin was rechallenged 3 weeks after the first challenge, treatment of the intact skin produced positive reactions in 6/11 animals and questionably positive reactions in another 2 animals, while treatment of the abraded skin produced positive reactions in 5 of the 6 positive animals and questionably positive reactions in another 2, i.e. 6 to 8/11 animals showed sensitisation reactions. The same animals showed no positive reaction after a second challenge with 10% formaldehyde solution. In a preliminary study, 9/10 animals died on day 1 after intradermal injection of 0.1 ml of a 40% aqueous solution of 2-butyne-1,4-diol (equivalent to approx. 88 to 98 mg/kg body weight). The one surviving animal was slightly heavier and therefore had only received a dose of approx. 79 mg/kg body weight. This animal (which was the eleventh animal in the study described above) survived another two intradermal injections of 10% aqueous solution. Based on these results, intradermal injections in the main study were carried out with 10% solution, a treatment which was survived by all 10 animals. Ne-

cropsies of the 9 fatalities revealed no significant systemic change. The investigators evaluated 2-butyne-1,4-diol as definitely sensitising to the skin, a conclusion which was verified by a second challenge test 3 weeks after the first one (no further details; Haskell Laboratory, 1966). The study was not conducted in accordance with current guidelines, but it is nonetheless also indicative of the chemical's sensitising effect in animals.

In a study of the sensitising properties of 2-butyne-1,4-diol, which was not carried out in accordance with current guidelines, 7 white rabbits (2 of which were controls) were treated with undiluted 2-butyne-1,4-diol, of which 0.5 g was applied to a 4 cm x 4 cm area of clipped skin every day for 10 days, with the untreated skin of the contralateral side of the body serving as a control. The 10 applications were repeated 3 times at intervals of 15 to 20 days, with a new skin section on the same side of the body being treated each time. In the first series of treatments, the skin irritation became more intense after 5 to 7 applications, as evidenced by an increase in hyperaemia and haemorrhages as well as tears in the skin. Marked xeroderma, skin thickening and profuse scaling were observed after 8 to 10 applications, with the skin appearing normal again after 15 to 20 days. In the second series of treatments, the skin effects occurred on day 2 to day 3 and were more marked. The skin reaction in the third series of treatments was somewhat weaker again. Histopathological examination of the treated rabbit skin after the first series of 10 applications showed congestion of the skin with haemorrhages and round-cell infiltration around vessels and hair follicles. On examination after the end of the second and third series of treatments, focal thinning of the epidermis was observed with partial shedding of the skin and diffuse round-cell infiltration. The investigators concluded from the results that 2-butyne-1,4-diol had both a direct skin irritant effect and a skin sensitising effect (Stasenkova and Kochetkova, 1965). The study was not carried out in accordance with a validated protocol. The somewhat more pronounced skin reaction seen in the second series of treatments could possibly also be explained by varying sensitivity of the different areas of skin treated and is not an unequivocal indication that the substance has sensitising potential.

7.5 Subchronic and chronic toxicity

In a 6-month oral study, the administration of 2-butyne-1,4-diol to male rats (6 per group) at dose levels of 0 (controls), 0.04, 0.2 or 2 mg/kg body

weight did not result in any effects on weight, behaviour, general condition or blood parameters (haemoglobin, numbers of erythrocytes, leucocytes and thrombocytes and prothrombin time). At 2 mg/kg body weight, delayed development of conditioned reflexes occurred with a 40% increase in the latent period. In addition, reduced cholinesterase and SH enzyme activities and increased transferase (transaminase) activity were seen, together with an altered serum protein profile. In the brain, the high dose led to a decrease in the number of Nissl bodies and an increase in neuroglia as well as reduced SH enzyme activity. The liver showed fatty dystrophy, areas of sclerotic growth and reduced glycogen levels, while in other organs, patchy hyperaemia occurred. The 0.2 and 0.04 mg/kg body weight dose levels were without effect (no further details; Knyshova, 1968). The study was not conducted in accordance with current guidelines and, due to inadequate reporting, is suitable for evaluation purposes only to a limited extent.

In a 6-month inhalation study, 20 rats (180 to 200 g) inhaled the aerosol of a 5% aqueous solution of 2-butyne-1,4-diol for 4 hours daily, 6 times a week. The concentration was 0.008 to 0.01 mg/l air (equivalent to 8 to 10 mg/m³; droplet size ≤ 2 µm). Twenty animals served as controls. General condition and body weight gain were unaffected. After 4 months, the neuromuscular stimulation threshold had fallen to 6.0 ± 0.2 mA compared with 8.9 ± 0.1 mA in the controls. The difference was also detectable in months 5 and 6 of the study ($p < 0.02$). Towards the end of month 6, the haemoglobin content was slightly reduced at 78.3 ± 1.2% compared with 85.2 ± 1.3% in the controls, while the numbers of erythrocytes and leucocytes were within the normal physiological range. At the end of the sixth month of exposure, the arterial blood pressure was on average 70.0 ± 3.5 mmHg compared with 98.2 ± 1.5 mmHg in the controls ($p < 0.01$). The amount of urine produced daily was almost the same in the treated and control rats at 6 ± 1.2 ml, as was the protein content of the urine at 57.4 ± 4.4 mg on average. In contrast, the daily excretion of hippuric acid was increased towards the end of month 6 from 128 ± 3.8 mg in the controls to 158 ± 7.2 mg ($p < 0.05$). At the end of the study, half of the animals were killed. Gross pathology revealed no remarkable findings. Microscopic examination revealed minimal catarrhal bronchitis and mild hypertrophy of the musculature of the bronchial walls and their arteries. Hyperplasia of the lymphatic tissue was present around the large bronchi, and the alveolar walls were slightly thickened due to proliferation of lymphocytic and histio-

cytic cells and polynuclear lymphocytes. There were no particular histologically abnormal findings in the other organs examined. The remaining rats were sacrificed one month after the end of the study. On necropsy and histopathological examination, treatment-related findings were no longer evident, and the changes observed immediately after the end of the study thus proved to be reversible. The concentration of 0.008 to 0.01 mg/l air (equivalent to 8 to 10 mg/m³) was therefore described as the “threshold concentration” by the investigators. Based on these studies, they recommended a provisional workplace limit value of 0.001 mg/l, equivalent to 1 mg/m³ air (no further details; Stasenkova and Kochetkova, 1965). The study was not conducted in accordance with current guidelines and, due to inadequate reporting, is suitable for evaluation purposes only to a limited extent.

7.6 Genotoxicity

7.6.1 In vitro

2-Butyne-1,4-diol (> 99% pure; dissolved in DMSO) was tested for mutagenic activity in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 at concentrations ranging from 20 to 5000 µg/plate in the absence and presence of metabolic activation (S9 mix from Aroclor 1254-induced rat liver). 2-Butyne-1,4-diol showed neither cytotoxic nor mutagenic effects in this study (BASF, 1981 b).

In a further Salmonella/microsome assay, 2-butyne-1,4-diol (purity unspecified) was investigated in *Salmonella typhimurium* strains TA 97, TA 98, TA 100 and TA 1535 in the concentration range from 100 to 10000 µg/plate in the absence and presence of metabolic activation (each assay was performed with 10% and 30% S9 mix from both induced rat liver and induced hamster liver) in comparison with a vehicle control (water) and a positive control in the preincubation test. 2-Butyne-1,4-diol was clearly not mutagenic (no further details; NTP, 1998).

A further in-vitro assay for structural chromosome aberrations was carried out with 2-butyne-1,4-diol (99.5% pure) in Chinese hamster V79 cells. The concentrations tested were 860, 300 and 50 µg/ml in two independent experiments without metabolic activation and 300, 100 and 10 µg/ml in three

independent experiments with metabolic activation (S9 mix from Aroclor 1254-induced rat liver). Chromosomes were prepared after 7 (high concentration), 18 (all three concentrations) and 28 hours (high concentration), and 100 to 200 metaphases were scored per culture. Cell growth was inhibited by 860 µg/ml (without metabolic activation) and 300 µg/ml (with metabolic activation). In one of the three independent experiments with metabolic activation, and restricted to the 18-hour preparations for the intermediate and high concentrations, aberration rates were increased up to 2-fold, a finding which could not be explained and was not seen when an additional 200 cells were scored. Furthermore, the effect was not reproduced in the other two independent experiments. None of the other test groups had significant increases in chromosome aberrations. 2-Butyne-1,4-diol thus possessed no genotoxic activity in this study (CCR, 1989, 1991).

7.6.2 In vivo

A micronucleus assay was conducted in groups of 5 male and 5 female mice given 2-butyne-1,4-diol in a single intraperitoneal dose of 17.5, 35 or 70 mg/kg body weight. Preliminary studies had shown the high dose to be close to the maximum tolerated dose. The test substance (99.5% pure) was dissolved in deionised water, which also served as the vehicle control. At 24 or 48 hours after dosing, femoral bone marrow preparation was carried out and 2000 polychromatic erythrocytes/animal were examined for micronuclei. Twenty-four-hour preparations were performed for all three dose levels, while 48-hour preparations were carried out only for the top dose. The number of normochromatic erythrocytes was not increased relative to the number of polychromatic erythrocytes, indicating that 2-butyne-1,4-diol had no cytotoxic effect on bone marrow cells. Whilst the positive control substance cyclophosphamide produced clear positive results following intraperitoneal administration of 30 mg/kg body weight, 2-butyne-1,4-diol caused no enhancement in the frequency of micronuclei at any dose level or preparation interval and hence was not clastogenic (RCC, 1998).

7.7 Carcinogenicity

Groups of 10 albino mice (S strain, 7 to 9 weeks old) had a total dose of 4 or 40 mg 2-butyne-1,4-diol (dissolved in acetone, application volume 0.3

ml) applied to the clipped dorsal skin once weekly for 10 weeks. In addition, the mice had croton oil (0.5% in acetone, application volume 0.3 ml) applied to the skin once a week for 18 weeks, beginning on the third day after the first dose of 2-butyne-1,4-diol. Three control groups, each consisting of 20 animals, were treated with croton oil alone for 18 weeks. The treated area of skin was inspected weekly for tumour growth, with tumours of 1 mm and above included. One week after the end of treatment (week 19), control group survival was 57/60. In 3/57 animals, a total of 7 tumours had occurred, which were shown to be benign papillomas on histopathological examination. With additional administration of 2-butyne-1,4-diol, survival was 19/20 (no details of the cause of death of one animal that died in the low dose group), and one animal in the low dose group developed a benign papilloma. One of the 3 control groups was maintained for lifespan observation on completion of the 18-week croton oil treatment, but no further tumours developed and no malignant changes to the papillomas occurred during the observation period. Moreover, no increase in the incidence of tumours was found in the lung, which was examined histopathologically in the treated groups. Thus 2-butyne-1,4-diol had no tumour-initiating effect in this initiation/promotion study with croton oil (no further details; Roe, 1957; Pereira, 1982). It should be noted that a study duration of 19 weeks is too short to permit a definite conclusion regarding the tumour-initiating effect of 2-butyne-1,4-diol.

The United States National Toxicology Program (NTP) is planning to conduct a carcinogenicity study of 2-butyne-1,4-diol (NTP, 2005).

7.8 Reproductive toxicity

In a preliminary study to an embryotoxicity/teratogenicity study based on OECD guideline No. 414, which was conducted to identify the maternally toxic dose, groups of 5 pregnant Wistar rats were administered 2-butyne-1,4-diol (98.9% pure, dissolved in double-distilled water) at dose levels of 20, 40 or 60 mg/kg body weight by oral gavage on days 6 to 15 post coitum. The high dose group showed the following statistically significant signs of maternal toxicity up to day 16 p.c.: decreases in food consumption and body weight from days 6 to 8 p.c., increases in total protein and albumin, decreases in thromboplastin time, serum cholinesterase activity and triglycerides, and increases in absolute and relative kidney and liver

weights. The intermediate dose group also showed a significant increase in serum albumin, significant decreases in thromboplastin time and serum cholinesterase activity and significant increases in absolute and relative liver weights. In the low dose group, there was a statistically significant increase in albumin, and thus dose-dependent maternal toxicity was observed. There were no differences in the reproductive parameters studied between the treated and control groups. Based on these results, dose levels of 10, 40 and 80 mg 2-butyne-1,4-diol/kg body weight/day were chosen for the subsequent embryotoxicity/teratogenicity study (BASF, 1992 b).

Groups of 18 to 22 pregnant Wistar rats were given aqueous solutions of 2-butyne-1,4-diol (98.9%, volume 10 ml/kg body weight) at dose levels of 10, 40 or 80 mg/kg body weight by gavage from day 6 to 15 p.c., while the control group (22 pregnant females) received the vehicle only. The study was carried out in accordance with OECD guideline No. 414. On day 20 p.c., the dams were assessed by gross pathology and the foetuses were removed, weighed and sexed and further examined for organ and skeletal abnormalities. Signs of maternal toxicity caused by 2-butyne-1,4-diol in the high dose group included a statistically significant reduction in food intake during the first half of the study period from day 6 to 8 p.c. (by approx. 21% compared with the controls), statistically significant body weight loss between days 6 and 8 p.c. and one intercurrent death on day 8 p.c. with preterminal vaginal haemorrhages, apathy, poor general state and piloerection. At gross pathology, the animal that died had a mottled liver and marginal emphysema of the lungs. Another animal showed piloerection on days 8 and 9 p.c. There was an increase in the number of foetuses per litter with accessory 14th ribs among the high-dose foetuses. This was evaluated by the investigators as a marginal sign of developmental toxicity in the embryos, representing a manifestation of nonspecific stress on the dams, not a teratogenic effect. The intermediate and low dose groups showed no signs of either maternal toxicity or embryotoxicity/foetotoxicity, and thus the NOAEL was 40 mg/kg body weight/day for the dams and foetuses (BASF, 1995; Hellwig et al., 1997).

2-Butyne-1,4-diol was administered to groups of 25 male and 25 female Wistar rats (F_0 parental generation) in their drinking water at concentrations of 0 (controls), 10, 80 or 500 ppm (equivalent to 0 (controls), approx. 1, 7.6 or 40 mg/kg body weight/day) in an extended one-generation reproductive toxicity study in accordance with OECD guideline No. 415. The scope of

the study was expanded to include the following parameters as required by OECD guideline No. 416 and US EPA guideline OPPTS 870.3800: oestrus cycle, sperm parameters, determination of organ weights in selected offspring parental animals, extensive histology and signs of sexual maturation. The F_0 generation animals were allowed to mate no earlier than 76 days after the beginning of treatment. The resulting offspring (F_1 generation) were reared until day 21 after parturition. The parental F_0 animals and their offspring were then killed, with the exception of one male and one female pup from each litter of the F_1 generation. The latter were raised to sexual maturity and then sacrificed. Animals received drinking water containing 2-butyne-1,4-diol throughout the entire study. Both the state of health of the parents and their offspring, and mating behaviour were monitored on a daily basis. Water and food consumption and parental (F_0) body weights were recorded at regular intervals during premating phases, gestation and lactation, and the same data were also collected for the selected F_1 offspring. The results are shown in Table 5.

Table 5. Substance-related effects of 2-butyne-1,4-diol in an extended one-generation reproductive toxicity study in Wistar rats after administration in drinking water

Dose groups and parameters studied	F_0 parental animals	F_1 offspring raised until day 21 after parturition	F_1 offspring raised to sexual maturity
10 ppm			
Clinical observations including reproductive performance and sexual maturity	no substance-related adverse effects	no substance-related adverse effects	no substance-related adverse effects
Organ weights	no substance-related adverse effects	no substance-related adverse effects	not studied
Gross lesions and histopathological findings	no substance-related adverse effects	not studied	not studied
80 ppm			
Clinical observations including reproductive performance and sexual maturity	statistically significant decrease in water consumption during premating (males ca. 5%, females ca. 9%) and gestation (up to ca. 18%); no substance-related adverse effects on reproductive performance	no substance-related adverse effects	no substance-related adverse effects
Organ weights	statistically significant increases in absolute (males) and relative (both sexes) kidney weights and absolute and relative liver weights (females)	no substance-related adverse effects	not studied

Table 5. Substance-related effects of 2-butyne-1,4-diol in an extended one-generation reproductive toxicity study in Wistar rats after administration in drinking water			
Dose groups and parameters studied	F_0 parental animals	F_1 offspring raised until day 21 after parturition	F_1 offspring raised to sexual maturity
Gross lesions and histopathological findings	no substance-related adverse effects	not studied	not studied
500 ppm			
Clinical observations including reproductive performance and sexual maturity	statistically significant decrease in water consumption during premating (males ca. 17%, females ca. 29%), gestation (up to ca. 37%) and lactation (up to ca. 24%); statistically significant decrease in food consumption in females during premating (up to 9%), gestation (up to 6%) and lactation (up to 10%); statistically significant decreases in body weights/body weight gains in females towards the end of premating (ca. 10%, calculation based on entire premating period) and during gestation (not statistically significant) and lactation (ca. 64%); no substance-related adverse effects on reproductive performance	statistically significant decrease in mean body weight from day 14 after parturition until weaning (ca. 14% on postpartum day 21, both sexes combined); statistically significant decrease in body weight gains from postpartum day 7 until weaning (ca. 18%, calculated from postpartum day 4 to 21, both sexes combined)	statistically significant decrease in water consumption during the treatment period (weeks 0 to 3 in females, weeks 0 to 4 in males; ca. 22%, both sexes combined)
Organ weights	statistically significant increases in absolute and relative (both sexes) kidney weights and absolute and relative liver weights (females); statistically significant decreases in absolute and relative adrenal and thymus weights (females)	statistically significant decreases in absolute organ weights for brain (ca. 3%), thymus (ca. 23%) and spleen (ca. 22%); statistically significant increase in relative brain weight (ca. 15%) and decrease in relative thymus weight (ca. 9%)	statistically significant decrease in water consumption during the treatment period (weeks 0 to 3 in females, weeks 0 to 4 in males; ca. 22%, both sexes combined); statistically significant decrease in food consumption in males (ca. 10%); statistically significant decrease in body weight gains in males (ca. 9%); statistically significant decrease in body weights (up to 11%) in females during weeks 0 and 1 (nonsignificant during the remaining time)
Gross lesions and histopathological findings	no substance-related adverse effects	not studied	not studied

Based on the results of the clinical observations and the gross and histopathological examinations, 2-butyne-1,4-diol was devoid of adverse effects on the reproductive performance and fertility of the parental F_0 animals at all three dose levels. Substance-related adverse effects were observed neither with regard to oestrus cycle data, mating behaviour, conception, gestation, delivery, lactation or weaning nor with regard to sperm parameters, reproductive organ weights or gross pathological or micropathological lesions of the reproductive organs. Most parental F_0 animals were fertile. The scattered occurrence of individual infertile rats across the different dose groups did not suggest any relation to treatment. Signs of general, systemic toxicity in the parental F_0 animals were confined to the 80 and 500 ppm groups. In the 500 ppm group, reduced water consumption was noted for the parental F_0 animals during the premating phase and for the F_0 females during gestation and lactation. Impairment of body weight/body weight gain with concurrent lowered food consumption was observed in F_0 females during premating, gestation and lactation. In addition, the 500 ppm animals were found to have substance-related, statistically significant increases in absolute and relative kidney (males and females) and liver weights (females) and statistically significant decreases in absolute and relative adrenal and thymus weights (females). In addition, the 80 ppm group exhibited impaired water consumption during premating (males and females) and gestation (females) and statistically significant increases in absolute and relative kidney (males and females) and liver weights (females). Signs of systemic toxicity observed in the 500 ppm group of F_1 offspring reared to sexual maturation included reduced water consumption (males and females), impaired body weight/body weight gain with concurrent reductions in food consumption (males) as well as reduced body weights (females). Substance-related signs of developmental toxicity were observed in the progeny of the parental F_0 males and females only after administration of 500 ppm; this resulted in impairments of pup body weight data and causally related decreases in organ weights. In addition, a sign of general delay in physical development noted in the reared F_1 males and females was a delay in preputial separation and vaginal opening, respectively. The 10 and 80 ppm levels did not cause developmental toxicity in the progeny. The investigators evaluated the highest test concentration of 500 ppm (approx. 40 mg/kg body weight/day) as the NOAEL for reproductive performance and fertility of the parental F_0 animals, whilst the NOAEL for systemic toxicity was 10 ppm (approx. 1 mg/kg body weight/day). The

NOAEL for developmental toxicity (growth and physical development of the offspring) was given as 80 ppm (approx. 7.6 mg/kg body weight/day) for the F_1 progeny. Signs of developmental toxicity thus occurred only at a dose level which was also systemically toxic to the parental males and females. No impairment of reproduction (fertility) was observed (BASF, 1999).

7.9 Effects on the immune system

No information available.

7.10 Neurotoxicity

In a 5-day study, groups of 5 male and 5 female Wistar rats received 2-butyne-1,4-diol at 5, 10 or 20 mg/kg body weight/day by oral gavage (for a detailed account of the study, see Section 7.2). All animals were tested for neurological function before the first dose, 24 hours after the first dose and 24 hours after the fifth dose. In addition to extensive observations, the following motor and sensory tests were performed on the animals: response to touch, coordination of movements (righting response), lid reflex, pupillary reflex, vision (visual placing response), hearing (startle response), olfaction, grip strength of forelimbs and hindlimbs, toe pinch, tail pinch and pain perception (hot plate test). No neurotoxicological changes were noted. Apart from a dose-dependent increase in male cholesterol levels, which attained statistical significance in the high dose group only, there were no other treatment-related changes. The *no adverse effect level* (NOAEL) in this 5-day study was thus 10 mg/kg body weight (see also Section 7.10; BASF, 1992 a).

A subsequent subacute inhalation neurotoxicity study was conducted in accordance with OECD guidelines for testing Nos. 412 and 413 and Directive 92/69/EEC and US EPA Health Effects Testing Guidelines 40 CFR §§ 798.6059, 798.6200 and 798.6400. In this study, groups of 16 male and 16 female Wistar rats underwent head-nose exposure to liquid aerosols of aqueous solutions of 2-butyne-1,4-diol (99.5% pure) at concentration levels of 0 (controls), 0.5, 5 or 25 mg/m³ for 6 hours per day. In order to investigate the concentration-time-response relationship, half of the animals served as a concurrent satellite group for 15 study days (10 exposures), while the other half of the animals were maintained for 30 study days (20

exposures). A detailed account of the study procedures and results is given in Section 7.2. Detailed neurotoxicological studies (functional observational battery) and motor activity measurements were performed in accordance with the above-mentioned guidelines in 5 animals/sex and group before the start of exposure, after 8 exposures (main and satellite groups) and after 18 exposures (main groups). Three animals per sex and group underwent perfusion fixation and subsequent neuropathological examination. In summary, none of the concentrations tested produced systemic toxicity. Neither the functional observational battery assessments and motor activity measurements nor the neurohistopathological examinations identified any statistically or biologically relevant neurotoxicological changes. However, the high and intermediate concentrations of 2-butyne-1,4-diol, 25 and 5 mg/m³, produced local irritant effects in the upper respiratory tract both after 10 and 20 exposures (larynx, trachea). When the results from the 5-day range-finding study and the satellite groups given 10 exposures were taken into account, there was no indication of cumulative systemic toxicity when the duration of exposure was extended to 20 exposures at concentration levels of up to 25 mg/m³. It was considered that the effects noted in the larynx and trachea should be interpreted as unspecific responses to local irritation due to the deposition of 2-butyne-1,4-diol aerosol in the aerodynamic traps presented by the larynx and tracheal bifurcation. The NOAEC for systemic toxicity, including neurotoxicity, was 25 mg/m³ while that for local toxicity to the upper respiratory tract was 0.5 mg/m³ (see also Section 7.2; BASF, 1998).

7.11 Other effects

2-Butyne-1,4-diol produced marked hypothermia in rats. Intraperitoneal injection of 2-butyne-1,4-diol at 0.408 mmol/kg body weight (equivalent to 35 mg/kg body weight) caused a fall in body temperature to approx. 35 °C 2.5 hours after administration, while intraperitoneal injection of 1.634 mmol/kg body weight (equivalent to 141 mg/kg body weight) caused body temperature to drop to 30.1 °C. The body temperature of the animals that received the low dose returned to normal within 12 hours. Animals given the high dose died within 2.5 to 3 hours after administration (Taberner and Pearce, 1974).

In order to study the possible tumour-inhibiting properties of 2-butyne-1,4-diol, 5 white mice were treated intraperitoneally with 2-butyne-1,4-diol at 5

mg/kg body weight/day for 7 days, starting 24 hours after subcutaneous transplantation of a Crocker sarcoma 180 into the axillary region (the previously determined intraperitoneal LD_{50} was 15 mg/kg body weight). Administration of 2-butyne-1,4-diol caused no inhibition of tumour growth compared with the control group (Carlson and Morgan, 1954).

The sleep- or anaesthesia-inducing effect of 2-butyne-1,4-diol was studied in 10 male albino rats (Sprague-Dawley or Holtzmann, weighing 250 to 350 g) and compared to that of its saturated congener 1,4-butanediol. The animals were administered an intraperitoneal dose of 5.5×10^{-3} mol/kg body weight in aqueous solution. Neuropharmacological effects were studied by observing gross behavioural changes as evaluated by the Irwin screen, and by recording other clinical effects. Signs of toxicity reported included marked depression of motor activity, marked vasodilation of the extremities, profuse diarrhoea and death after 2 hours (no further details; Sprince et al., 1966). The dose administered was equivalent to 473.5 mg/kg body weight and was thus almost 10-fold higher than the intraperitoneal LD_{50} in rats.

8 Experience in humans

A 20-year-old vehicle body builder, whose occupation involved the nickel-plating of electronic parts in galvanic baths, initially noticed acute, relatively clearly localised livid redness and swelling of his left lower arm. The eczema spread to the upper arms, shoulders and palms of the hands and later also to the temples and eyes in the further course. The axillary lymph nodes were swollen and painful on palpation. Symptoms included severe itching and general malaise. Treatment with antibiotics and anti-inflammatory drugs led to a complete recovery to the original condition. Skin lesions did not recur after the patient was transferred to a different workplace within the company. There was no indication of skin disease or allergy in the patient's medical history. Patch testing, which was performed according to the recommendations of the DKG (German Contact Dermatitis Research Group) or the DDG (German Society of Dermatology) using a standard test series supplemented with various substances for external use, the rubber series, substances used in metalworking and the patient's own gloves (worn and new), produced a questionable reaction to thiomersal in addition to a clear positive crescendo reaction to the worn rubber gloves, but not to a brand-new pair of the same gloves. The contamination

of the worn pair of gloves was due, *inter alia*, to a degreasing agent (Balcid BO). Patch-testing with 2-butyne-1,4-diol, a constituent, elicited a marked crescendo reaction, starting at a concentration of 1% in water. No positive test reactions were obtained when the chemical was tested in control patients with eczema and occupations in similar industries. In the reported case, the diagnosis was allergic contact dermatitis to 2-butyne-1,4-diol. The chemical is used as a gloss improver in nickel-plating baths but also as a starting material in the synthesis of plastics or as an intermediate in the manufacture of insecticides (Reinecke et al., 1999; Blaschke et al., 2001).

Investigations of the health of workers with direct contact with 2-butyne-1,4-diol diagnosed dermatitis, the intensity of which was dependent on the duration of contact and individual susceptibility (no further details; Stasenkova and Kochetkova, 1965).

A 54-year-old male worker, who had been employed for years in the storeroom of a galvanising department and who was involved in weighing out, packing and mixing various substances (such as solvents, paints, synthetic resins, hardeners, epoxy resins, isocyanates, acrylic resins, diaminodiphenylsulphone as well as 2-butyne-1,4-diol as an additive for nickel baths) developed an itchy dermatitis of the hands and lower arms although he wore rubber gloves. The patient was patch-tested with a standard patch test tray, the clinic's own patch test series and an additional test series comprising nonirritant concentrations of 47 substances to which the patient had occupational exposure. Only a 2% 2-butyne-1,4-diol solution caused a clear positive reaction after 48 hours, which increased in severity after 72 hours. The patient, whose task it was to prepare 10% solutions of 2-butyne-1,4-diol in water, reported exacerbation of his complaints merely on opening the 2-butyne-1,4-diol containers. Of 50 workers who had had contact with increasing amounts of 2-butyne-1,4-diol over 5 years, this was the only patient who developed contact allergy to 2-butyne-1,4-diol (Malten, 1980).

A 41-year-old woman developed itchy eczema of the face, hands and distal part of the arms on contact with 2-butyne-1,4-diol as a component of a cleaning agent. The symptoms appeared about 12 hours after contact. Standard patch tests were negative. In contrast, the test with a 10% dilution of the cleaning agent was positive. Tests performed with the various components of the product were positive only for 2-butyne-1,4-diol (0.01% in water); the purity of the tested 2-butyne-1,4-diol was > 99.9%. Patch tests

in 55 control subjects, in whom a 10-fold higher concentration of 2-butyne-1,4-diol was tested, did not produce any positive reactions. The investigators concluded that the female patient described above had a contact-allergic reaction to 2-butyne-1,4-diol, which was present in the cleaning agent at a concentration of 0.7% (Baadsgaard and Jørgensen, 1985).

Without giving further details, one source mentioned 4 cases of 2-butyne-1,4-diol-induced allergic contact dermatitis among 902 chemical industry employees referred to in 950 reports submitted to the BG Chemie during the period from 1 May 1955 to 31 December 1962 for the purpose collecting statistical data on skin diseases. However, allergic reactions were not confirmed by patch-testing (Goldmann, 1963 a, b).

Six out of 10 workers with accidental dermal exposure to 2-butyne-1,4-diol in the period from 1989 to 2000 and clinically suspected exposure-related allergic contact dermatitis, agreed to be patch-tested at the company's occupational medicine and healthcare protection department. Per patch test, pure 2-butyne-1,4-diol was tested together with a 50% technical-grade 2-butyne-1,4-diol solution as 0.5 and 1% aqueous solutions, respectively. Tests were also performed with formaldehyde, which is used as an intermediate in the synthesis of the chemical and is contained in the technical-grade product as a contaminant. Whereas formaldehyde was negative, sensitivity to 2-butyne-1,4-diol was clearly confirmed in all 6 patients tested (BASF, 2001 b).

The human olfactory threshold for 2-butyne-1,4-diol has been reported as 200 mg/l water (no further details; Knyshova, 1968).

9 Classifications and threshold limit values

In accordance with Annex I to Directive 67/548/EEC, the European Commission has classified 2-butyne-1,4-diol as sensitising by skin contact (R 43; EC, 2004).

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area ("MAK-Kommission") of the Deutsche Forschungsgemeinschaft has designated 2-butyne-1,4-diol with "Sh" for skin-sensitising substances and listed it in the "Yellow Pages" ("Substances being Examined for the Establishment of MAK Values and BAT Values") of the List of MAK and BAT Values 2004 on the suggestion of BG Chemie in order that a MAK value be established for the chemical (DFG, 2004).

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