The MAK Collection for Occupational Health and Safety

Azodicarbonamide

MAK Value Documentation – Translation of the German version from 2017

A. Hartwig[1,*], MAK Commission[2,*]

1 *Chair of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Institute of Applied Biosciences, Department of Food Chemistry and Toxicology, Karlsruhe Institute of Technology (KIT), Adenauerring 20a, Building 50.41, 76131 Karlsruhe, Germany*

2 *Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Kennedyallee 40, 53175 Bonn, Germany*

***** *email: A. Hartwig (andrea.hartwig@kit.edu), MAK Commission (arbeitsstoffkommission@dfg.de)*

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Azodicarbonamide / 1,2-diazenedicarboxamide

MAK Value Documentation

A. Hartwig[1,*], MAK Commission[2,*]

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Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has evaluated azodicarbonamide [123-77-3] to derive a maximum concentration at the workplace (MAK value), considering all toxicological endpoints. Available publications and unpublished study reports are described in detail. The critical effect is the occurrence of reactions in the airways in exposed workers. In the view of the Commission, the present inhalation studies in animals are not appropriate for the derivation of a MAK value as human airways are probably more sensitive than rodent airways. The workplace investigations show no clear correlation of exposure and frequency of respiratory symptoms. Nevertheless, from the NOAEC of 36 µg/m³ for lung function measurements in a small group of injection-molding workers in the plastics industry, a MAK value of 20 µg/m³ I is established. Due to the incomplete information on the relevant exposure scenario and the uncertainty regarding first occurrence of workplace related symptoms, this value should be considered as preliminary. As local effects are critical, azodicarbonamide is assigned to Peak Limitation Category I and the default excursion factor of 1 is designated. Since developmental toxicity studies are not available, Pregnancy Risk Group D is assigned. There are no carcinogenicity studies and azodicarbonamide is not regarded as germ cell mutagen. Skin contact is not expected to contribute significantly to systemic toxicity. The metabolism of azodicarbonamide and its reactivity do not point to stable protein binding in vivo and therefore, skin or respiratory sensitization is unlikely. Clear-cut clinical findings or positive results from animal studies on skin sensitization are not available, but the negative results in experimental animals are difficult to evaluate due to the low solubility of the compound. Although azodicarbonamide affects the respiratory tract in several case reports with positive bronchial provocation tests, sensitization by a known mechanism is not adequately verified. Additionally, it appears questionable whether a mono-causal relationship of respiratory symptoms and azodicarbonamide exposure can really be assumed. In conclusion, a sensitizing effect of azodicarbonamide is not sufficiently proven.
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Keywords

azodicarbonamide; 1,1′-azobiscarbamide; 1,1′-azodiformamide; C,C′-azodi(formamide); azodicarboxamide; 1,2-diazenedicarboxamide; diazene-1,2-dicarboxamide; diazenedicarboxamide; ADCA; 1,1′-azobis(formamide); mechanism of action; toxicokinetics; metabolism; (sub)acute toxicity; (sub)chronic toxicity; irritation; allergenic effects; reproductive toxicity; fertility; genotoxicity; carcinogenicity; peak limitation; prenatal toxicity; germ cell mutagenicity; absorption through the skin; sensitization; occupational exposure; maximum workplace concentration; MAK value; toxicity; hazardous substance

Author Information

[1] Chair of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Department of Food Chemistry and Toxicology, Institute of Applied Biosciences, Karlsruhe Institute of Technology (KIT), Adenauerring 20a, Building 50.41, 76131 Karlsruhe, Germany

[2] Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Kennedyallee 40, 53175 Bonn, Germany

* Email: A. Hartwig (andrea.hartwig@kit.edu), MAK Commission (arbeitsstoffkommission@dfg.de)

Azodicarbonamide

MAK value (2017)	**0.02 mg/m³ I (inhalable fraction)**
Peak limitation (2017)	**Category I, excursion factor 1**
Absorption through the skin	**–**
Sensitization	**–**
Carcinogenicity	**–**
Prenatal toxicity (2017)	**Pregnancy Risk Group D**
Germ cell mutagenicity	**–**
BAT value	**–**
Synonyms	1,1′-azobiscarbamide 1,1′-azodiformamide C,C′-azodi(formamide) azodicarboxamide 1,2-diazenedicarboxamide diazene-1,2-dicarboxamide diazenedicarboxamide ADCA
Chemical name	1,1′-azobis(formamide)
CAS number	123-77-3
Structural formula	$H_2N–CO–N=N–CO–NH_2$
Molecular formula	$C_2H_4N_4O_2$
Molar mass	116.1 g/mol
Melting point	decomposes at > 200 °C (ECHA 2016)
Vapour pressure at 20 °C	2.51×10^{-10} hPa (SRC 2014)
log K_{OW}[1)]	–1.7 (SRC 2014)
Solubility	35 mg/l water at 20 °C (SRC 2014) 33 mg/l water at 20 °C (ECHA 2016)

1) Octanol/water partition coefficient.

Azodicarbonamide is used as an auxiliary agent for the foaming of plastics and rubber products. During thermal decomposition, azodicarbonamide breaks down into gases (nitrogen, carbon monoxide, carbon dioxide and ammonia) as well as solids or sublimates such as biurea (1,2-hydrazine dicarboxamide), urea, urazole (1,2,4-triazolidine-3,5-dione), isocyanuric acid (1,3,5-triazine-2,4,6(1H,3H,5H)-trione), cyamelide (1,3,5-trioxane-2,4,6-triimine) and semicarbazide (hydrazine carboxamide) (Prakash et al. 1975; Stadler et al. 2004). As a result of different particle sizes or the addition of stabilizers or activators, the actual decomposition temperature of azodicarbonamide, of usually about 200 °C to 215 °C, is highly variable in the mixtures used for foaming, ranging from about 150 °C to 200 °C (Exelby et al. 1993; Heck and Peascoe 2011). Zinc salts, and in particular zinc oxide, are the primary activating additives, besides other metal and heavy metal salts, including chromium(III) salts and chromates (FBC Limited 1982; Toyo Rayon Company Limited 1972). During a chemical embossing process, foamable mixtures of PVC and azodicarbonamide were printed with dicarboxylic acid anhydrides before heating, inhibiting the thermal decomposition of azodicarbonamide in those areas (Hunter 1983). In addition, dicarboxylic acid anhydrides may have been used as stabilizers or to achieve particularly dry azodicarbonamide qualities (Otsuka Chemical Company Limited 2002; Otsuka Kagaku Yakuhin Kabushiki Kaisha 1981). In the plastics industry, azodicarbonamide is added to mixtures together with other blowing agents. For this reason it is particularly common in the plastics industry that, in addition to the exposure to a mixture of substances resulting from the different additives used in plastics raw materials, there is also potential exposure to the above-mentioned additives.

Azodicarbonamide is used, or rather has been used since 1962 in a concentration of up to 45 mg/kg to increase the gas binding capacity and pliability of dough by accelerating the (oxidative) maturing process of wheat flour (Joiner et al. 1963). It is no longer authorized for use as a food additive in the EU.

Toxic Effects and Mode of Action

The critical effect of azodicarbonamide is the triggering of airway reactions in exposed workers. Azodicarbonamide does not cause irritation of the rabbit skin, but induces reversible swelling and redness of the conjunctivae in the rabbit eye as a result of mechanical irritation.

The available data are not sufficient to demonstrate sensitizing effects of azodicarbonamide on the skin or the respiratory tract.

After inhalation exposure for 13 weeks, no effects were observed in male mice at 50 mg/m^3 or in male rats and female mice up to 100 mg/m^3. The terminal body weights were reduced in male mice at 100 mg/m^3 and above, and the serum levels of the thyroid hormones T_3 and T_4 were increased in male rats at 200 mg/m^3 and above. Effects on the respiratory tract of the animals were not found. After oral administration of high doses (1000 mg/kg body weight), kidney damage resembling pyelonephritis was observed in female rats.

In a 1-generation study in rats, oral treatment did not affect the fertility of the rats.

Azodicarbonamide induced mutagenicity in bacteria, but there were no mutagenic effects in mammalian cells. Indicator tests for clastogenicity yielded negative results in mammalian cells, but chromosomal aberrations and polyploidy were induced. There was no induction of clastogenic or polyploid effects in the bone marrow of mice in vivo. Azodicarbonamide did not induce mutations in Drosophila.

Studies of the developmental toxicity or carcinogenicity of azodicarbonamide are not available.

Mechanism of Action

Azodicarbonamide is a reactive substance; its reactivity is based on the electrophilic character of the disubstituted N=N double bond. The reactivity of azodicarbonamide, other azodicarboxamides and the even more reactive azodicarboxylic acid esters in chemical syntheses are not suitable for the estimation of the reactivity of azodicarbonamide under physiological conditions.

Several factors indicate that (under physiological conditions) azodicarbonamide does not form sufficiently stable conjugates with proteins Dicarbonic acid anhydrides are hard electrophiles and prefer to react with amino groups. By contrast, it is very probable that azodicarbonamide, as a soft electrophile, under physiological conditions will only or preferentially react with soft and highly polarizable nucleophiles, such as thiol residues, in a reaction analogous to the Michael addition of α,β-unsaturated carbonyls. This mechanism is similar to the reaction of some α,β-unsaturated carbonic acid amides (for example, the relatively thiol-specific N-ethyl maleic acid imide). Unlike these substances, the conjugates formed by azodicarbonamide are not sufficiently stable, but the reaction of the primary adduct to the N=N double bond with an additional thiol group ultimately leads to oxidation of the SH groups to form disulfides and to the reduction of azodicarbonamide to yield biurea. In biological systems, rapid oxidation of reduced glutathione (GSH) to oxidized glutathione (GSSG) takes place. The reaction is also the basis of the use of azodicarbonamide as a flour-maturing additive for the oxidation of the free sulfhydryl (SH) groups in gluten (Tsen 1963).

- This preferred reactivity is the basis for the use of diamide (N,N,N′,N′-tetramethyl derivative of azodicarbonamide) as a relatively selective thiol oxidant in cell systems and in vivo studies (Kosower et al. 1969; Kosower and Kosower 1995). Diamide appears to oxidize SH groups of peptides and proteins (far) more slowly and primarily with the formation of mixed peptide-glutathione disulfides. Diamide can also oxidize other oxidizable substances (for example NADH), but again far more slowly than the free SH group in GSH (Harris and Biaglow 1972; Kosower et al. 1972, 1977). None of the extensive literature that has been published on the substance since the beginning of its use in the 1970s includes information pertaining to conjugation with amino groups.
- Likewise, protein conjugation appears to have been neither observed nor detected in the toxicological studies carried out with azodicarbonamide in rats, mice or guinea pigs or in the in vivo studies on the antiviral effect of azodicarbonamide.

Nevertheless, it was assumed that the antiviral effect of the electrophilic azodicarbonamide was caused by covalent binding to a cysteine SH group at the zinc bind-

ing site of the viral nucleocapsid p7 (NCp7) protein (Huang et al. 1998; Maynard et al. 1998). Nuclear magnetic resonance spectroscopy with equimolar concentrations showed that disulfide bridges are formed after zinc is released, inducing the unfolding of the molecule and ultimately the formation of oligomeric products. The authors stated that, following the reaction with azodicarbonamide, the NMR spectrum of the protein corresponded to the spectrum obtained after adding EDTA to the zinc-containing protein (Rice et al. 1997). Quantum chemistry calculations have shown that only protonated azodicarbonamide reacts with the zinc binding site of NCp7 (Topol et al. 2001).

In a different study, azodicarbonamide induced the displacement of the zinc cation and the formation of disulfide bonds at the zinc binding site of the Z protein of the lymphocytic choriomeningitis virus (Garcia et al. 2006).

Another publication explained the in vitro antiviral effect of a substituted azodicarboxamide (2-[[(2-phenylethyl)amino]carbonyl]diazene carboxylic acid, 2-acetyl-1-[3-(dimethylamino)-3-oxopropyl]hydrazide; 255873-65-5; molecular weight 376.41) against the human hepatitis A virus by the formation of covalent bonds with cysteine in the active centre of the viral C3 cysteine proteinase. In this publication, a respective adduct was detected by mass spectroscopy after the substituted azodicarboxamide reacted with the peptide in dimethyl sulfoxide. Apart from this possible adduct, no other reaction products were found in the mass spectrum. In aqueous systems, enzyme activity was inhibited by 50% after the addition of a 100-fold excess of the azodicarboxamide. The deactivation of the enzyme could not be reversed by subsequent addition of N-acetylcysteine. However, the deactivation did not occur if stoichiometric amounts of N-acetylcysteine had been added prior to the azodicarboxamide (Hill and Vederas 1999).

The investigations do not or do not clearly show whether azodicarbonamide or other substituted azodicarboxamides actually remain bound to the proteins investigated after an initial reaction with the cysteine residues of these proteins.

The form of azodicarbonamide most commonly used as a blowing agent has a particle diameter of less than 10 µm. After inhalation exposure to such a dust, the soluble fraction of azodicarbonamide that reaches the lower respiratory airways may lead to a decrease in the GSH concentration in the alveolar fluid or to a shift in the GSH redox balance by forming GSSG. Likewise, phagocytosis of the dust particles by the alveolar macrophages may induce a corresponding intracellular effect. Although GSH oxidation is in general reversible, in the case of inadequate compensation, it might cause oxidative stress or increase existing oxidative stress. In addition, a large number of regulatory processes and signalling pathways might be affected by a shift in the GSH redox balance or the associated rise of protein S-glutathionylation (Biswas and Rahman 2009; Go et al. 2015; Hoffman et al. 2015). For example, the glucose intolerance and the slight formation of methaemoglobin observed among HIV-positive patients treated with azodicarbonamide may also be the result of a chronic disturbance in the GSH redox balance.

In addition, direct effects on sensory receptor proteins may occur if these can be activated or deactivated by oxidative (or electrophilic) substances. For example, oxidative activation of the transient receptor protein A1 (TRPA1) of the pulmonary epithelium by hydrogen peroxide was investigated in mice and its activation by diamide was investigated in perfused guinea pig lung (Bannenberg et al. 1994; Bessac et al. 2008).

Toxicokinetics and Metabolism

Absorption, distribution and elimination

Male F344 rats were exposed once by inhalation to ^{14}C-azodicarbonamide concentrations of 1.5 or 150 mg/m^3 for 3 hours, 20 mg/m^3 for 5.5 hours or 25 mg/m^3 for 6 hours. The mass median aerodynamic diameter (MMAD) was in a range between 1.8 and 3.4 µm. At the end of exposure, the initial body burden was determined as the sum of the radioactivity equivalents in the body of the animal without the fur. About 30% of the inhaled material was deposited in the respiratory tract. After 72 hours, about 58% to 84% of the initial body burden had been removed from the lungs by mucociliary clearance and had reached the gastrointestinal tract, and 25% to 40% (on average 33%) was found in the urine and body at the three low concentrations. The authors themselves did not calculate the systemic absorption by inhalation. It is assumed that the initial body burden is equivalent to the amount deposited in the respiratory tract. Therefore, absorption by inhalation was about 33% of the deposited dose and thus 10% of the inhaled dose (Mewhinney et al. 1987).

After a single oral dose of 0.1 mg/rat, 33% of the administered dose was absorbed. Azodicarbonamide was eliminated with the urine, exhaled air (2%) and faeces (67%) within 72 hours. However, because of its low molecular size, azodicarbonamide was not excreted with the bile and was thus not absorbed. Therefore, in terms of the absorption of the deposited dose, inhalation exposure is quantitatively similar to oral exposure. After intratracheal instillation of 0.1 mg/animal, 88% was eliminated with the urine, 0.8% with the exhaled air and 9.8% with the faeces. Absorption was thus 90% (Mewhinney et al. 1987).

In the 13-week study described in Table 2, the levels of azodicarbonamide and its only metabolite biurea were investigated in the lungs and kidneys of the male F344 rats sacrificed after the last exposure (see Section "Inhalation"). The substance itself was not detected in either organ, whereas biurea was found in the lungs, but not in the kidneys. The amount of biurea in the lungs did not increase linearly with the increase in the exposure concentration. The authors assumed that about 10% of the inhaled azodicarbonamide entered the lungs and bronchi. At the high concentration of 200 mg/m^3, the amount of biurea in the lungs and bronchi was about 66% of the azodicarbonamide absorbed during the last day of exposure and thus about 6.6% of the administered amount. Therefore, azodicarbonamide was rapidly eliminated from the lungs at the concentrations tested in this study (Medinsky et al. 1990).

Experimental in vivo or in vitro data for the absorption of azodicarbonamide through the skin are currently not available.

Fluxes of 0.015, 0.0007 and 0.0023 µg/cm^2 and hour were calculated for a saturated aqueous solution using the models of Fiserova-Bergerova et al. (1990), Guy and Potts (1993) and Wilschut et al. (1995), respectively. Assuming the exposure of a 2000 cm^2 surface area of skin for 1 hour, this would correspond to absorbed amounts of 0.03, 0.0015 and 0.0046 mg, respectively.

Metabolism

Azodicarbonamide is effectively reduced to biurea by free thiol groups; these groups are oxidized to (mixed) disulfides (see Section "Mechanism of Action"). Biurea is thus the main metabolite and was detected also in kinetics studies in rats. Also in vitro, after the addition of ^{14}C-labelled azodicarbonamide to fresh blood from untreated rats, the total amount of radioactivity was detected in the HPLC peak of biurea in the first analysis after only 5 minutes. The reduction of azodicarbonamide is limited by the capacity of the thiol groups. Radioactivity in the HPLC peak of the parent substance was found only after pre-incubation of the blood with 5 mg unlabelled azodicarbonamide/ml (Mewhinney et al. 1987).

Effects in Humans

There are no data available for irritation of the skin or eyes, developmental toxicity, genotoxicity or carcinogenicity.

Single exposures

Dose-dependent methaemoglobin formation was observed in one volunteer after oral administration. The maximum was reached 1.5 hours after treatment, and the methaemoglobin level returned to the normal range 4 hours after ingestion of the substance (no other details; Goebel et al. 2001).

Repeated exposure

According to the authors, the maximum methaemoglobin level of 3.5%, which was determined in 2 HIV-positive patients after 3 daily doses of 3 g azodicarbonamide, is not clinically relevant (no other details; Goebel et al. 2001).

In a phase I/II study with 15 AIDS patients, 3 daily doses of 2 g azodicarbonamide was defined as the maximum tolerated oral dose. At higher doses (129 to 142 mg/kg and day), some patients developed nephrotoxicity (renal colic, nephrolithiasis with necrosis of the papillae, and increased creatinine) while in others, glucose intolerance or azodicarbonamide-related cytotoxic effects on CD4 cells were found (Goebel et al. 2001).

Case reports described very rare reactions on the skin and, after repeated exposure to azodicarbonamide by inhalation, mainly airway reactions that were probably not allergic in origin (see Section "Allergenic Effects").

There are four studies available (Ferris et al. 1977; NIOSH 1985 a, b; Slovak 1981; Whitehead et al. 1987) that linked symptoms among workers exposed to azodicarbonamide to person-related or workplace-related exposure. The studies included no or only very incomplete information about the concentration of possible decomposition products or other substances used at the plants.

In a facility manufacturing azodicarbonamide, the 151 persons who worked there in 1980 or had worked there at some time since the plant began operation in 1966

were divided by the author into 3 groups based on the results of a standardized questionnaire, the available occupational health documentation and examination of the clinical case history: a) workers with asthma assumed to have been caused by azodicarbonamide, b) asymptomatic workers who had worked in the facility for more than 1 year, and c) workers without exposure to azodicarbonamide or known respiratory allergens. All 28 workers of group a) did not have asthma or other respiratory diseases before they started work at the factory. Symptoms (shortness of breath, tightness of the chest, wheezing, coughing, rhinitis, and conjunctivitis) developed in more than half of the persons within the first 3 months and in a total of 21 of 28 workers within the first year of employment. Immediate reactions ordual reactions were reported by 6 workers in each case, and another 16 workers reported delayed reactions. The symptoms became worse after prolonged exposure to azodicarbonamide in 13 of the 28 workers. Within 3 months, more than half of the 13 workers who continued to be exposed to azodicarbonamide after the diagnosis developed bronchial hyperreactivity to irritants that had been tolerated before. This hyperreactivity persisted in 5 of 8 of these persons although they avoided being exposed to azodicarbonamide for more than 3 years. Details of the "historical" dust concentrations in the plant are not available; the author merely assumed that the concentration levels were similar to the current values. At the time of the investigation, the workers were exposed to 2 to 5 mg/m^3 "on average", but no respiratory symptoms were observed at the workplace. Likewise, workplace-related measurements of the forced expiratory volume in one second (FEV_1) and the forced vital capacity (FVC) before and after the shift did not reveal respiratory obstruction in any of the 3 groups. However, a healthy-worker effect is assumed because, according to the author, about half of the workers with symptoms were transferred to a different workplace by reason of their work-related respiratory disease and because the remaining workers had presumably applied their own "avoidance strategy". The negative results obtained from the measurements of the peak expiratory flow (PEF) in 11 workers with previous symptoms led the author to conclude that these workers had not been exposed at the time (as a result of protective measures that had been taken or after they had changed jobs within the plant); however, dust measurements were not carried out. Prick tests with 0.1%, 1% and 5% azodicarbonamide in dimethyl sulfoxide yielded exclusively negative results in groups a) and b) (Slovak 1981).

In a plant manufacturing plastic floor coverings, some workers reported nose bleeds, irritation of the mucous membranes and skin reactions. Therefore, an initial group of 11 workers was questioned about their symptoms. Further investigations were carried out because of the high frequency of reported symptoms (eye and nose irritation, (nocturnal) coughing, shortness of breath, wheezing, and tightness of the chest). In addition, exposure levels were determined 7 times, with azodicarbonamide being detected only twice in concentrations of 2.1 and 3.1 mg/m^3. In the main study carried out 6 weeks later, direct exposure levels of 0.15 to 12 mg/m^3 (3 measurements; ϕ 3.8 mg/m^3) and of about 0.6 to 4.8 mg (5 measurements; ϕ 1.6 mg/m^3) were determined in two buildings. Indirect exposure levels (at a distance from the workplaces at which azodicarbonamide was handled) of a maximum of 0.03 mg/m^3 (11 measurements) were recorded in one building and of a maximum of 0.1 mg/m^3 (14 measurements) in the second building. The workers used dispos-

able breathing masks during direct exposure and in some cases also during indirect exposure. The findings in a group of 30 workers who had (previously) been exposed to azodicarbonamide, most of whom had symptoms, were compared with those in a group of 16 workers without previous exposure. The (average) results of the pre- and postshift lung function tests carried out in the workers exposed to azodicarbonamide did not differ significantly from those of the workers not exposed. There was a certain relationship between the results of the lung function test and the exposure, but individual measurements were not documented and the (mean) decrease of about 2% to 3% in the FEV_1 and FVC was very small. Likewise, the two groups did not differ in the frequency of the symptoms reported at the time (NIOSH 1985 b). It cannot be determined how many of the examined workers had previously reported respiratory symptoms.

Likewise, another study included case reports of workers with symptoms of the respiratory organs. In December 1983, 227 of a total of 242 workers of a plastics moulding plant underwent spirometric examination. The highest average FEV_1 and FVC values were determined in the 60 workers who earlier had been employed in injection moulding (4.16 litres and 3.37 litres), while the 110 workers currently employed (4.3 litres and 3.35 litres) and the 57 workers who were never employed there (4.08 litres and 3.25 litres) had somewhat lower values. The exposed workers reported a higher incidence of respiratory symptoms than the workers not exposed. Most striking was the "finding" that the "risk" for the combination of symptoms "wheezing and shortness of breath"—based on the frequency of symptoms reported—was about 6 to 8 times lower for persons who worked in the plant only before or after azodicarbonamide (n = 34) was used than for those who worked there while azodicarbonamide (n = 136) was being used. Static readings taken about 7 months earlier yielded airborne concentrations of up to 1800 µg/m³ and personal air samplers recorded exposures of up to 13.6 µg/m³ (presumably without the use of breathing masks) and of up to about 280 µg/m³ (presumably with the use of breathing masks) (Table 1). However, there are no data as to whether symptoms occurred at the workplace also after exposure to these concentrations.

In March 1984, personal air monitoring was carried out in 32 workers. The values for the exposed injection-moulding workers were between 1 and 368 µg/m³ (on average: 36.1 µg/m³); the second highest concentration was 57 µg/m³. The highest concentration of 752 µg/m³ was recorded for a worker who mixed azodicarbonamide with plastic resins and binders at his workplace while wearing a breathing mask. Average lung function values were obtained for 17 exposed injection-moulding operators before and after the shift (see below). Coughing was reported by 7 of the 17 workers, but it cannot be determined whether these data refer to the day of the lung function tests. One of the 7 workers reported that his symptoms were severer on Monday than on the preceding Sunday. Three workers reported workplace-related wheezing, which in 2 of the workers was severer than on the preceding Sunday. However, the work-related symptoms of 2 workers with tightness of the chest were not severer than on the preceding Sunday. The lung function tests revealed statistically significant, but only very small decreases in the average values for FEV_1 (–141, –35 and –30 ml) and FVC (–120, –8 and –50 ml) in the exposure ranges 0 to 20, 21 to 40 and > 40 µg/m³ (Table 1). Likewise, the unpublished individual measurements that were made available (Whitehead 2017) did not

demonstrate any consistent trend for a concentration–effect relationship. Spirometric examinations did not yield a decrease in the FEV_1 or FVC values of more than 2% for the 3 workers who subjectively reported severer symptoms at the workplace. The authors also suggested that there may be a healthy-worker effect, and the use of azodicarbonamide had been drastically reduced about 4 or 5 months before the workplace-related lung function tests (NIOSH 1985 a; Whitehead et al. 1987).

Shortly after a company started grinding azodicarbonamide, 10 of 11 workers reported workplace-related airway reactions (coughing, shortness of breath, and nocturnal coughing). Workplace-related investigations carried out in 3 workers on the Monday following a 4-day period away from work revealed an average decrease in FVC and FEV_1 levels over the shift of about 17% and 21%, respectively. On the following Friday and subsequent Monday, the (average) decreases in lung function parameters were at the most only about half as high in 4 workers in each case. A similar trend was observed also for the only worker who underwent lung function tests at all 3 times (no other details). The azodicarbonamide concentrations were between 0.7 and 2.1 mg/m^3 (4 personal air samples, see Table 1). However, there is no information about other irritants or potentially sensitizing substances that may have been present (Ferris et al. 1977).

These studies provide no clear relationship between respiratory symptoms and the airborne concentrations determined at the workplace (see also Table 1). The detailed measurements of the exposure to azodicarbonamide that were reported by NIOSH (1985 b) were derived from workers who used personal breathing masks and are therefore unsuitable for deriving a MAK value. Taking the objectified measurements of the lung function values into account,the average exposure of the three groups of 36 $\mu g/m^3$ (0.036 mg/m^3) reported by Whitehead et al. (1987) is considered to be the NOAEC. However, regarding the work-related symptoms, that were subjectively reported by the workers, a NOAEC cannot be derived .

Published data from registers

From 1989 to 1991, a total of 1528 cases of occupational asthma were reported in the United Kingdom under the SWORD project (Surveillance of Work-Related and Occupational Respiratory Disease Project; McDonald et al. 2000) including 17 cases in which azodicarbonamide was specified as the cause. In the subsequent periods from 1992 to 1994 and from 1995 to 1997, the number of reported cases decreased to 11 from a total of 2857 and to 2 from a total of 3002 cases, respectively (McDonald et al. 2000). According to a statistical survey, in the period from 1994 to 2000, the Department for Work and Pensions examined a total of 8 cases of occupational disability resulting from respiratory disease caused by azodicarbonamide; 4 and 3 cases were reported in 1994 and 1995, respectively, and another case was recorded in 1998 (no other details; HSC 2001). In the period from 2003 to 2014, none of the 1670 recorded cases were caused by azodicarbonamide (HSE 2015).

A review of a total of 2602 cases of occupational asthma recorded in the Finnish Registry of Occupational Diseases (FROD) of the Finnish Institute of Occupational Health (FIOH) from 1989 to 1995 did not mention azodicarbonamide as a causative agent (Karjalainen et al. 2000). A later publication reported 9 cases caused by azodicarbonamide among the 5591 cases registered between 1986 and 2002. The

9 cases were recorded in 1991, 1993, 1994, 1997 (2 cases), 1998, 1999 (2 cases), and 2002 (Piipari and Keskinen 2005). Between 2000 and 2012, the Dutch National Centre for Occupational Diseases recorded one case of occupational asthma in an analyst of a pharmaceutical company (no other details; ECHA 2012). The Korea Work-Related Asthma Surveillance programme reported 2 cases of occupational asthma caused by azodicarbonamide, without giving further details, among a total of 236 work-related asthma cases recorded from 2004 to 2009. Among the 236 cases, 13 were classified as irritant-induced and 9 were classified as asthma exacerbating under workplace conditions. This was the first time that azodicarbonamide has been linked to a case of asthma in Korea (Kwon et al. 2015). It is not possible to establish a correlation between the cases included in these registries and the relevant exposure, or to carry out a plausibility check.

No cases have been reported in Germany (see, for example, Latza and Baur (2005)), although exposures at the same level seem to have occurred. However, exposure data have been available only since the late 1980s.

From 1996 to 2011, occupational physicians did not report any cases of occupational asthma induced by azodicarbonamide to the British OPRA (Occupational Physicians Reporting Activity) register (ECHA 2012). The THOR (The Health and Occupation Research Network) and THOR-GP (The Health and Occupation Reporting network in General Practice) programmes, which maintain a register of cases reported by pulmonary specialists or general practitioners, did not record any case caused by azodicarbonamide between 2000 and 2014 and between 2000 and 2011, respectively (ECHA 2012; THOR 2015). A total of 948 cases were recorded between 1990 and 1997 by the Surveillance Scheme of Occupational Asthma (SHIELD) established by the Midland Thoracic Society in the West Midlands, but again there was no mention of occupational asthma induced by azodicarbonamide (Di Stefano et al. 2004).

Likewise, azodicarbonamide was not explicitly mentioned as the cause of asthma in the French surveillance programme ONAP (Observatoire National des Asthmes Professionnels) among 2178 cases recorded between 1996 and 1999 (Ameille et al. 2003).

Data for respiratory diseases induced by azodicarbonamide cannot be found in further publications referring to a smaller number of cases that were recorded in the respective registers. However, these registers too do not provide full information about specific causes: Australia (SABRE, Surveillance of Australian workplace Based Respiratory Events); from November 1997 to April 2001, a total of 170 cases of occupational asthma (Elder et al. 2004) and New South Wales; from June 2001 to December 2008, a total of 89 cases (Hannaford-Turner et al. 2010),

South Africa (SORDSA, Surveillance of Work-related and Occupational Respiratory Diseases); October 1996 to December 1999, a total of 324 cases (Esterhuizen et al. 2001 a, b),

Michigan and New Jersey (SENSOR, Sentinel Event Notification System for Occupational Risks); 535 cases from 1988 to 1992 and 768 cases from 1993 to 1995 (Jajosky et al. 1999; Reilly et al. 1994),

California; 444 cases between 1993 and 1996 (Reinisch et al. 2001),

Quebec; 214 cases between 1983 and 1988 (Lagier et al. 1990), and

Singapore; 90 cases between 1983 and 1999 (Kor et al. 2001).

Table 1 Exposure data for azodicarbonamide (ADCA)

Year / activity	Exposure: static [mg/m³]	Exposure: personal air sampling or breathing zone [mg/m³]	Comments	Symptoms	References
1971 / grinding of ADCA and other raw materials	–	personal air sampling: shift levels: **0.7**, **2.0** and **2.1** (1st Monday) and **1.9** (Friday)	dust particle diameter: 3.9 µm (before grinding) and 2.2 µm (after grinding): in addition, Al_2O_3, SiO_2, sulfanilamide, processed	"acute symptoms" ("cold", productive cough, mainly at night); determinations in 3 workers before and after the shift on Monday and 4 workers on Friday and on the following Monday: FVC ø: –16.6%, –5.0% and –5.7% FEV_1 ø: –20.8%, –8.3% and –10.4%	Ferris et al. 1977
1980 / manufacture of ADCA (no other details)	–	personal air sampling: **2–5** (no other details, values determined only gravimetrically)	a summary of all cases that occurred between 1966 and 1980 after "presumably the same level of exposure"	28 of 151 workers: shortness of breath, tightness of the chest, wheezing, coughing (11×), rhinitis (8×), and conjunctivitis (7×); currently no symptoms among non-symptomatic workers; determination of FEV_1 and FVC before and after the shift without effects; workers previously with symptoms who may have no longer been exposed at the time of the investigation	Slovak 1981
1983 / manufacture of PVC floors	4× negative, 1× in traces and 1× **2.1** and **3.1** (12–53 minutes)	–	probably investigated with a product that was not used in normal production	in 11 workers interviewed: frequent (no other details) reports of eye and nose irritation, (nocturnal) coughing, shortness of breath, wheezing or tightness of the chest	NIOSH 1985 b

Table 1 (continued)

Year / activity	Exposure: static [mg/m³]	Exposure: personal air sampling or breathing zone [mg/m³]	Comments	Symptoms	References
1983 / manufacture of PVC floors	indirect exposure: not detectable up to **0.1** (25 air samples)	direct exposure (short-term: 7–56 minutes) in 2 buildings: **0.15–12** (median: 3.8; n = 3 with breathing masks) and **0.59–4.8** (median: 1.6; n = 5 with breathing masks)	combined direct (with breathing masks) and indirect exposure (without breathing masks): **0.04–1.7 mg/m³** (median: 0.36; n = 6); 8 hours TWA: **0–0.91 mg/m³** (median: 0.08; n = 8 with breathing masks during direct exposure)	**case history**: 18 of 30 exposed persons reported respiratory symptoms (eye/nose irritation, (nocturnal) coughing, wheezing, shortness of breath, tightness of the chest); the symptoms were considered to be non-occupational in 3 persons, occupational, but not related to ADCA in 7 persons, and related to ADCA in 8 persons; 10 of the 18 had a prior history of allergies (no other details); 26 of 30 exposed persons reported symptoms of the eyes or upper respiratory tract (considered to be related to ADCA in 18); no data about the decisive exposure; **current** exposures: no differences between workers with symptoms (s) and workers not exposed (n) with regard to symptoms before and after the shift; lung function tests before and after the shift: FEV_1: on average: –0.087 l (s) compared with –0.048 l (n) FVC: on average: –0.116 l (s) compared with –0.043 l (n)	NIOSH 1985 b

Table 1 (continued)

Year / activity	Exposure: static [mg/m³]	Exposure: personal air sampling or breathing zone [mg/m³]	Comments	Symptoms	References
1983 / plastic moulding (various areas)	not detectable up to **0.01** (33 air samples); **0.11**, **0.12**, **0.13**, **0.22**, **1.43** and **1.80** (6 air samples taken at various mixing and loading workplaces)	breathing zone (injection-moulding machine): not detectable up to **0.014** (26 air samples); breathing zone (mixing of the raw materials probably with breathing masks): **0.13**, **0.19** and **0.28** (one air sample in each case)	total dust: 0.03–3.0 mg/m³ (median: 0.81); frequently also high total dust concentration in spite of low exposure to ADCA, in particular at the injection-moulding machines	case reports: respiratory symptoms (irritation, coughing, wheezing, shortness of breath, headaches), no allocation of symptoms to the relevant exposure; relative risk for combination of wheezing and shortness of breath about 6–8 times higher among 136 workers who may have potentially been exposed than among 34 workers not exposed	NIOSH 1985 a; Whitehead et al. 1987
1984 / plastic injection moulding	–	personal air sampling (1× mixing workplace with breathing masks): **0.75**; (2× mixing workplace without breathing masks): 0.012 and 0.057; personal air sampling (injection-moulding machines without breathing masks): **0.001–0.014** (12 air samples); **0.022–0.036** (10 air samples); **0.043–0.057** (6 air samples) and **0.37** (1 air sample) (total median: 0.025; median for injection-moulding machines: 0.036)	investigation after transition from manual handling to an enclosed pneumatic system; no data for the current total dust concentrations	for ADCA concentration ranges (breathing zone, injection-moulding machines: 0–20, 20–40 and > 40 µg/m³) only very low decreases in the average values (7, 6 and 4 measurements) before and after the shift for FEV_1 (–141, –35 and –30 ml) and FVC (–120, –8 and –50 ml)	NIOSH 1985 a; Whitehead et al. 1987

Table 1 (continued)

Year / activity	Exposure: static [mg/m³]	Exposure: personal air sampling or breathing zone [mg/m³]	Comments	Symptoms	References
1993 / toll manufacturer / milling	day shift: ø **1.21**; night shift: ø **0.27**	day shift: ø **10.8**; night shift: ø **2.55**		workers of the night shift complained about respiratory symptoms (no other details)	ECHA 2012
1995 / toll manufacturer / milling	traces up to **1.16** (TWA)	traces up to **4.14** (TWA); **18.9** and **20.1** (short-term values during cleaning)	follow-up of the above investigation after the introduction of personal protective equipment	no data	ECHA 2012
1994–2000 / manufacture, filling	15 full shift values (7× static sampling, 8× personal air sampling); 90% percentile: **2.7**; 50% percentile: **0.32**; 5 air samples < **0.04**		since 1995 use of personal protective equipment (P1 or P2 masks)	no data	OECD 2007
1997 / manufacture, milling	traces up to **0.33** (TWA)			no data	ECHA 2012
2003 / manufacture of PVC floors	0–**0.23** (short-term)	**1.76** (short-term, 12 minutes, 1 air sample)		no data	ECHA 2012
2009 / manufacture of ADCA dispersions	**0.64–3.0** (TWA)	**15.1** (short-term, 45 minutes, 1 air sample)		no data	ECHA 2012

FEV_1: forced expiratory volume in one second
FVC: forced vital capacity
TWA: time weighted average

In 1996, the United Kingdom established a maximum exposure limit (MEL) of 1 mg/m^3 and a short-term exposure limit (STEL) of 3 mg/m^3 (HSE 1996). At about the same time, azodicarbonamide ceased to be manufactured in the United Kingdom; since then the substance has been imported. The number of reported cases of respiratory diseases caused by exposure to azodicarbonamide decreased as described. However, there are no data about the actual exposure concentrations or whether the concentrations decreased further after the introduction of protective measures or changes in the conditions of use.

Allergenic effects

Sensitizing effects on the skin

Following accidental exposure to azodicarbonamide (no other details), a worker who had suffered from occasional eczema of the forearms and legs during the previous 10 years developed eczema over various parts of the body, but not on the face or hands. A 2+ reaction to 1% azodicarbonamide in petrolatum, but not to a 0.1% formulation, was observed in a patch test after 48 hours and 72 hours. Patch tests were also carried out in 5 of 10 other workers who had complained about skin problems while handling azodicarbonamide; the results were all negative (Bonsall 1984).

A 58-year-old textile worker who used foam earplugs during his work developed recurrent inflammatory reactions in the external auditory canals. After 48 hours and 96 hours, the patch test provoked 2+ reactions to the foam and 1% and 5% azodicarbonamide in petrolatum, but not to a 0.1% formulation. A group of 10 control persons did not respond to patch tests with 1% and 5% formulations. According to the authors, this was only the second notification the manufacturer had received after selling "millions" of these earplugs for over a decade (Yates and Dixon 1988). There are no data available as to whether undecomposed azodicarbonamide could still be found in the foam.

Another positive patch test result with azodicarbonamide was reported for a 59-year-old baker, suffering from bakers' allergy, who had worked for 36 years and had had eczema on his arms and face for at least 20 years. Information was not provided about the test concentration or the reaction outcome or about whether the tested product might have been a mixture of azodicarbonamide and wheat starch (Nava et al. 1983).

Sensitizing effects on the airways

A 56-year-old worker of a plant manufacturing azodicarbonamide developed a cough, shortness of breath and wheezing 7 years after starting his job (monitoring the quality of finely ground azodicarbonamide powder). Although he was given medication, the symptoms progressively worsened during the following 3 years, especially in the evening. The patient was admitted to hospital with a cough, dyspnoea, and wheezing; the FEV_1 was 1.78 l (67.7% of the normal value) and moderate non-specific bronchial hyperreactivity was observed ($PC_{20(methacholine)}$: 0.85 mg/ml). Immediately after local treatment with budesonide (400 µg twice daily) and salbu-

tamol (as required) for 4 weeks, oral doses of theophylline (200 mg twice daily) and bambuterol (10 mg once daily) and prednisolone treatment for 2 weeks, the FEV_1 (2.58 l) had returned almost to the normal level and the non-specific bronchial hyperreactivity was markedly reduced ($PC_{20(methacholine)}$: 4.69 mg/ml). Although the report did not include all the data, the provocation test that was carried out after 1 week without treatment caused a 22% decrease in the FEV_1 5 hours after exposure for 10 minutes to a 1:1 mixture of azodicarbonamide and lactose. The patient complained of coughing and tightness of the chest. The non-specific bronchial hyperreactivity increased again 2 days after the provocation test ($PC_{20(methacholine)}$: 0.47 mg/ml). Prick tests with 0.1%, 1% and 5% azodicarbonamide in dimethyl sulfoxide yielded negative results, whereas a patch test with 1% and 5% azodicarbonamide in petrolatum led to a positive result (2+ reaction after 48 hours and 96 hours). The provocation test carried out in two asthma patients without exposure to azodicarbonamide, but with similar non-specific bronchial hyperreactivity ($PC_{20(methacholine)}$: 3.91 and 5.13 mg/ml), did not reveal substantial changes in the FEV_1 (Kim et al. 2004).

One worker with atopic diathesis and one non-atopic worker had been employed in a plastics plant for about 4 years. During this time, they were exposed to azodicarbonamide for about 1 or 2 weeks 3 or 4 times a year. After only a few months, both developed upper airway symptoms (irritation of the eyes and nose) during work, followed by (nocturnal) asthma attacks. After a period of 1 month without exposure, a bronchial provocation test was carried out in both workers with a 1:1 mixture of azodicarbonamide and lactose for 15 seconds. The worker with atopic diathesis developed a delayed asthmatic response beginning after 3 hours with a maximum decrease in the FEV_1 of 24% after 6 hours. In addition, non-specific bronchial hyperreactivity was increased in the worker after provocation ($PC_{20(histamine)}$: 2 mg/ml → 0.28 mg/ml (after 5 days) and 0.84 mg/ml (after 19 days)), which returned to normal only after 6 weeks. The provocation test produced a dual reaction in the second worker with a decrease in the FEV_1 of 26% after 30 minutes and 23% after 5 to 6 hours. In this patient, non-specific bronchial hyperreactivity ($PC_{20(histamine)}$: 0.5 mg/ml → 1.2 mg/ml) was not increased after the test. A control person with atopic diathesis and non-occupational asthma did not produce an asthmatic response to 15-minute provocation. A prick test was not carried out because of the low solubility of azodicarbonamide (Malo et al. 1985; Pineau et al. 1985).

A 50-year-old worker reported having experienced episodes of dyspnoea and dermatitis of the face during the 10 years he had worked in a plastics processing plant. About 9 months after he began to grind and mix blowing agents in addition to pigments he developed asthmatic symptoms. Dyspnoea occurred about 5 or 6 hours after starting work, and occasionally only later that night. In addition, he was exposed to more than 30 substances including pre-polymerized polyurethanes, formaldehyde and melamine formaldehyde resins; however, the provocation test results were all negative (no other details). In a provocation test with azodicarbonamide (no other details), no clear decrease in the FEV_1 occurred up to about 4 hours after provocation, but the patient reported asthmatic symptoms later that night (about 14 hours after provocation). The worker was replaced by a worker who had previously worked as a baker. After about 1 month, the second worker developed asthmatic symptoms also at the end of the shift or a few hours later. A provocation test with azodicarbonamide powder (no other details) produced a continuous decrease

in the FEV_1, which reached a maximum of 22% 3 hours and 40 minutes after the 40-minute provocation. No more respiratory symptoms occurred in the following year under improved working conditions. However, accidental re-exposure to azodicarbonamide induced another asthmatic reaction. A third worker at a different plant developed asthmatic symptoms after he started to work in a process in which azodicarbonamide was used for about 2 weeks a year; the symptoms started as soon as exposure to azodicarbonamide began. A fourth, 54-year-old worker at the same plant, who had had to discontinue an apprenticeship in a bakery because he had chronic eczema and asthma attacks, developed an asthma attack immediately after his first contact with azodicarbonamide in the plastics plant (no other details) (Normand et al. 1989).

A worker who operated a moulding press for acrylonitrile-butadiene-styrene/polyurethane plastics developed work-related rhinitis about one year after azodicarbonamide had been used in the plant. This first occurred in the afternoons of work days; later, the worker complained of a dry cough and dyspnoea in the evenings and during the night. A 1-hour provocation test with 100 g azodicarbonamide heated to 37 °C on a moving glass plate did not lead to changes in the FVC or FEV_1 during the subsequent 24 hours. However, 3 exposures carried out on non-consecutive days under workplace conditions resulted in a continuous decrease in the FEV_1, which 4 to 6 hours after the end of shift was only 50% of the initial value before the shift. On the following morning, the FEV_1 had almost returned to the initial value determined the day before. If the worker was exposed, again 3 times, after previous administration of disodium cromoglycate, there was no marked decrease in the FEV_1 on any of these days and there was an average reduction of about 10% (Valentino and Comai 1985).

A worker in the azodicarbonamide production was accidentally exposed to an azodicarbonamide concentration that was higher than that usually encountered at the workplace. He developed rhinitis with gradually increasing nocturnal attacks of coughing about 3 weeks later. A provocation test or other allergological investigations were not carried out. No other workplace-related respiratory symptoms occurred when exposure to azodicarbonamide was avoided as far as possible (Alt and Diller 1988).

Studies of exposure to azodicarbonamide at the workplace and the prevalence

In a total of 4 studies of workers from plants producing or processing azodicarbonamide who reported respiratory symptoms (see Section "Repeated exposure"), workplace-related lung function tests, but no further pulmological-allergological investigations were carried out. In one of the studies, prick tests with non-conjugated azodicarbonamide yielded negative results in workers with symptoms and workers without symptoms (Slovak 1981). In a second study, it was not possible to detect specific IgE against azodicarbonamide human serum albumin conjugate. Specific IgG was found only at a dilution of 1:10, but not at the usual dilution of 1:50. Furthermore, the differences between exposed workers and those not exposed were only slight. Likewise, when compared with the laboratory controls, the specific IgG was not markedly increased in any group (NIOSH 1985 a). However, the method

was not described in detail, and there are no data regarding the preparation of the azodicarbonamide human serum albumin conjugates or as to whether conjugates were actually formed.

Summary

There are only very few data available for the sensitizing effects of azodicarbonamide on the skin. As the origin and purity of the substance investigated in the patch tests were not reported, there is insufficient documentation to show whether these few cases were caused by azodicarbonamide.

Likewise, there are only few studies available for the sensitizing effects of azodicarbonamide on the airways that include well-documented clinical findings with details of bronchial provocation tests. With one exception, these were all carried out before 2000. The duration of provocation varied widely between 15 seconds (Malo et al. 1985) and up to 40 minutes (Normand et al. 1989). In one case (Valentino and Comai 1985), workplace-related provocation tests were carried out 3 times, but exposure to a mixture of substances cannot be ruled out. With the exception of the study of Malo et al. (1985), the reactions are not late reactions that occured only a few hours after provocation, but reactions with a "progressive" course that seem to begin immediately after the provocation and reach their maximum only after a few hours. Reactions which follow such a course have been observed, for example after provocation with di-isocyanates, but a similar form has also been described after provocation with some irritants (for example cleaning agents containing chlorine/hypochlorite solutions) (see, for example, Burge et al. (2012)). Only 3 cases, in which the symptoms and the findings in the bronchial provocation test were sufficiently documented, may provide evidence of an immunological genesis. The origin and purity of the substance investigated in the provocation test were not reported in either these cases or in the other cases described. Therefore, and keeping the mentioned possible co-exposure in mind, the case reports do not provide sufficient evidence of an immunological effect of azodicarbonamide on the airways. Furthermore, azodicarbonamide is a fine dust with a particle size range of about 1 to 30 μm, which in the case of chronic inflammatory bronchitis might induce non-specific provocation reactions at sufficiently high concentrations.

The 4 available studies from 4 plants that reported workplace-related symptoms in exposed workers (see Section "Repeated exposure") and the data from registers are not suitable for the evaluation of the genesis of the airway reactions to azodicarbonamide because no data or no detailed data for positive immunological findings are available. The listed registers include also many "cases" caused by other substances that have no or questionable sensitizing effects on the airways, such as formaldehyde, "paints", "metal-working fluids", "cleaning agents", chlorine, ammonia and sulfur dioxide.

Animal Experiments and in vitro Studies

Acute toxicity

Inhalation

The 4-hour inhalation of azodicarbonamide aerosol (MMAD: 5.8 µm) yielded an LC_{50} above 6100 mg/m³ in male and female rats (5 animals per group). At the end of exposure, 8 of 10 animals had dyspnoea, but no mortality was observed. The microscopic examination did not reveal any unusual findings 14 days after the end of exposure (Mewhinney et al. 1987).

Guinea pigs were exposed to azodicarbonamide concentrations of 19, 58 or 97 mg/m³ in a plethysmograph for 1 hour. Some lung function parameters were determined before, during and up to 24 hours after the head-only exposure, and histological examination of various tissues was carried out at the end of exposure. A slight, concentration-related decrease in the respiratory minute volume (by about 12%, 15% (read from a graph) and 24% compared with that in control animals after exposure to 19, 58 or 97 mg/m³, respectively) and in the high exposure group decreases in the tidal volume and respiratory frequency (a measure of sensory irritation in the RD_{50} test with mice) were found; however, there were no unusual histopathological findings in the respiratory tract. The authors stated that the respiratory pattern did not change in the RD_{50} test, as it is the case for typical irritants of the upper respiratory tract. According to the authors, the results suggest that azodicarbonamide has a very low irritation potential (Shopp et al. 1987). Therefore, this study is regarded only as providing evidence that azodicarbonamide causes irritation.

Oral administration

In 2 studies with 10 and 15 male Wistar rats, no mortality occurred after treatment with doses of 2500 mg/kg body weight. The clinical symptoms observed in the animals were indicative of a poor general state of health (OECD 2007). Other studies yielded LD_{50} values of above 4000 mg/kg body weight in male and female Alderley Park rats and of above 5000 mg/kg body weight in male Wistar rats (OECD 2007).

Dermal application

The dermal dose of 500 mg/kg body weight was tolerated by 5 male Wistar rats without any signs of toxicity or mortality (OECD 2007). In a screening study, systemic toxicity was not observed in 1 female rabbit at a dose of 2000 mg/kg body weight (OECD 2007).

Subacute, subchronic and chronic toxicity

Inhalation

Details of all the studies described below can be found in Table 2.

Table 2 Effects of azodicarbonamide after repeated inhalation

Species, strain, number per group	Exposure	Findings	References
rat, F344, 5 ♂, 5 ♀ additionally 5 ♂, 5 ♀ methaemoglobin, acetylcholinesterase in blood	**12 exposures**, 6 hours/day, 5 days/week, 0, 2, 10, 50, 100, 200 mg/m³ (dust aerosol, particle size: 1.89–2.45 µm, MMAD: 2.13 µm)	**100 mg/m³**: ♂: **NOAEC**; **200 mg/m³**: ♂: liver weights ↓, terminal body weights ↓; no histopathological findings; ♀: NOAEC; no effect on methaemoglobin formation or acetylcholinesterase levels in blood	Medinsky et al. 1990
rat, F344, 10 ♂, 10 ♀	**13 weeks**, 6 hours/day, 5 days/week, 0, 50, 100, 200 mg/m³ (dust aerosol, particle size: 1.33–2.43 µm, MMAD: 2.38 µm)	**50 mg/m³**: ♂, ♀: effects on the lungs (lung weights ↑, bronchial and mediastinal lymph nodes enlarged, perivascular accumulation of lymphocytes, hyperplasia of the alveolar epithelium, non-suppurative inflammation of the alveoli) possibly resulting from a virus infection; **100 mg/m³**: ♂: **NOAEC**; **200 mg/m³**: ♂: T_3 and T_4 ↑ (50% and 40% ↑ compared with the levels in controls); ♀: **NOAEC**; histopathology: control and high dose groups, exception: lungs, all groups here	Medinsky et al. 1990
mouse, B6C3F1, 5 ♂, 5 ♀ additionally 5 ♂, 5 ♀ methaemoglobin, acetylcholinesterase in blood	**12 exposures**, 6 hours/day, 5 days/week, 0, 2, 10, 50, 100, 200 mg/m³ (aerosol, particle size: 1.89–2.45 µm, MMAD: 2.13 µm)	**100 mg/m³**: ♂, ♀: **NOAEC**; **200 mg/m³**: ♂, ♀: liver weights ↓, terminal body weights ↓; no histopathological findings; no effect on methaemoglobin formation or acetylcholinesterase levels in blood	Medinsky et al. 1990

Table 2 (continued)

Species, strain, number per group	Exposure	Findings	References
mouse, B6C3F1, 10 ♂, 10 ♀	**13 weeks**, 6 hours/day, 5 days/week, 0, 50, 100, 200 mg/m^3 (aerosol, particle size: 2.33–2.45 µm, MMAD: 2.38 µm)	**50 mg/m^3**: ♂: **NOAEC**; **100 mg/m^3**: ♂: terminal body weights (93% of the control values) ↓; ♀: **NOAEC**; **200 mg/m^3**: ♂, ♀: liver weights ↓, terminal body weights ↓ (♀: 94%, ♂: 91% of the controls); histopathology: control and high dose groups	Medinsky et al. 1990
guinea pigs, Hartley, 10, no other details	**4 weeks**, 6 hours/day, 5 days/week, 0, 51, 200 mg/m^3 sensitizing effects on the airways	**up to 200 mg/m^3**: no histopathological changes in the nasal cavity, larynx, trachea, lungs, tracheobronchial or popliteal lymph nodes, skin; body weights unchanged: no airway hyperreactivity	Gerlach et al. 1989

MMAD: mass median aerodynamic diameter; T_3: triiodothyronine; T_4: thyroxine

In 13-week inhalation studies, the NOAEC of azodicarbonamide was 50 mg/m³ for male mice, 100 mg/m³ for male rats and female mice and 200 mg/m³ for female rats. The terminal body weights of mice were reduced, and the thyroid hormones T_3 and T_4 in the serum of male rats of the group exposed to 200 mg/m³ were increased. Higher concentrations were not tested. The authors concluded that it was more likely that an unknown virus or viral antigen was the cause of the effects observed in the lungs of rats exposed to 50 mg/m³ (increased lung weights, enlarged bronchial and mediastinal lymph nodes, perivascular accumulation of lymphocytes and the occurrence of inflammatory cells) than an antigen effect caused by azodicarbonamide because these effects were not observed in the two higher exposure groups (Medinsky et al. 1990).

Oral administration

Details of all the studies described below can be found in Table 3.

In a 90-day study, no effects were observed in male rats at 500 mg/kg body weight and day; in a 1-generation study, the NOAEL was 300 mg/kg body weight and day in pregnant female rats. The kidney was the target organ at high oral doses (BG Chemie 1993; Hatano Research Institute 2000).

Dermal application

There are no data available for dermal application.

Intraperitoneal injection

In Sprague Dawley rats given intraperitoneal injections of up to 200 mg/kg body weight and day, no effects on thyroid function were observed (Gafford et al. 1971). Details of this study can be seen in Table 4.

Table 3 Effects of azodicarbonamide after repeated oral administration

Species, strain, number per group	Exposure	Findings	References
rat, F344, 5 ♂, 5 ♀	**14 days**, range finding 5 days/week, 0, 625, 1250, 2500, 5000, 10 000 mg/kg body weight and day	**625 mg/kg body weight**: ♂, ♀: **NOAEL**; ≥ **1250 mg/kg body weight**: ♂, ♀: mortality ↑; ♂: nephritis, nephrosis; ≥ **2500 mg/kg body weight**: ♀: nephritis, nephrosis	BG Chemie 1993
rat, Sprague Dawley, 7–12 ♂	**1 week** (1%, 10%), **10 days** (5%), **4 weeks** (10%), 0%, 1%, 5%, 10% in the diet (about 0, 750, 3750, 7000 mg/kg body weight and day) only thyroid function control group with low iodine level in the diet	≥ **3750 mg/kg body weight** (10 days or 4 weeks): uptake of iodine (24 hours) slightly increased, iodine bound to serum protein slightly increased (questionable effect because of great variability in various control groups); no thyroid enlargement (exception: 7000 mg/kg body weight after 1 week; not reproducible in a second 1-week study, not even after treatment for 4 weeks)	Gafford et al. 1971
rat, F344, 10 ♂, 10 ♀	**90 days**, daily ♂: 0, 100, 500, 2500 mg/kg body weight and day ♀: 0, 200, 1000, 5000 mg/kg body weight and day	**500 mg/kg body weight**: ♂: **NOAEL**; **1000 mg/kg body weight**: ♀: **NOAEL**; **2500 mg/kg body weight**: ♂: mortality (10/10), animals that died with kidney damage (pyelonephritis, "concrements", deposited crystals in renal tubules); **5000 mg/kg body weight**: ♀: mortality (2/10)	BG Chemie 1993
rat, Sprague Dawley, no other details	**1-generation study** OECD 415 ♂: 98 days, ♀: 2 weeks before mating, during mating and gestation up to day 20 of lactation, 0, 100, 300, 1000 mg/kg body weight and day	**300 mg/kg body weight**: ♀: **NOAEL**; **1000 mg/kg body weight**: ♂: **NOAEL**; ♀: effects on the kidneys (kidney damage resembling pyelonephritis)	Hatano Research Institute 2000

Table 3 (continued)

Species, strain, number per group	Exposure	Findings	References
mouse, B6C3F1, 5 ♂, 5 ♀	**14 days**, range finding 5 days/week, 0, 625, 1250, 2500, 5000, 10 000 mg/kg body weight and day	**≥ 625 mg/kg body weight**: ♂: kidney damage (crystals in renal tubules and bladder, tubular nephrosis, changes of the renal papilla and renal pelvis); **≥ 1250 mg/kg body weight**: ♂, ♀: mortality ↑; ♀: kidney damage (see above)	BG Chemie 1993
mouse, B6C3F1, 10 ♂, 10 ♀	**13 weeks**, 5 days/week ♂: 0, 78, 156, 312, 625, 1250 mg/kg body weight and day ♀: 0, 156, 312, 625, 1250, 2500 mg/kg body weight and day	**1250 mg/kg body weight**: ♂: **NOAEL**; **2500 mg/kg body weight**: ♀: **NOAEL**	BG Chemie 1993

Table 4 Effects of azodicarbonamide after repeated intraperitoneal injection

Species, strain, number per group	Exposure	Findings	References
rat, Sprague Dawley, 8 ♂	**1 week**, 0, 2, 20, 200 mg/kg body weight and day, only thyroid function feed with low iodine level	**200 mg/kg body weight**: mortality (5/8), anorexia, loss of weight, haematuria; thyroid: no changes in weights, uptake of iodine, and iodine bound to plasma protein, TSH levels unchanged in the plasma	Gafford et al. 1971

Local effects on skin and mucous membranes

Skin

Azodicarbonamide did not cause irritation of the rabbit skin (BG Chemie 1993).

After 24-hour semi-occlusive application of 50 or 500 mg azodicarbonamide to the inside of the ears of 2 New Zealand White rabbits, no effects on the skin were recorded during the 7-day observation period (OECD 2007).

Likewise, no irritation of the rabbit ear was observed after exposure to 50 mg of a mixture of 90% azodicarbonamide and 10% zinc salt of benzene sulfinic acid after semi-occlusive application for 24 hours (BG Chemie 1993; OECD 2007).

Azodicarbonamide was applied 6 times occlusively to the shaved dorsal skin of 3 rabbits per group as a 50% aqueous preparation or 50% suspension in acetone. Mild erythema and scaling were observed after the second treatment, but these symptoms were no longer detected after the fourth application (BG Chemie 1993).

Eyes

Azodicarbonamide induced reversible swelling and redness of the conjunctivae in the rabbit eye as a result of mechanical irritation (BG Chemie 1993).

The instillation of 50 mg of a mixture of 90% azodicarbonamide and 10% zinc salt of benzene sulfinic acid into the conjunctival sac of the eyes of 2 New Zealand White rabbits caused redness and swelling of the conjunctivae, which persisted for 3 days and were reversible within the 7-day observation period. In the cornea there were no reactions (BG Chemie 1993; OECD 2007).

Amounts of 50 µl of a 25% aqueous azodicarbonamide suspension were instilled into 1 eye of each of 3 rabbits. After 1 hour, 2 animals had mild irritation of the conjunctivae, which was reversible after 24 hours. The fluorescein test yielded negative results in all animals (BG Chemie 1993).

Airways

In the head-only inhalation study previously described in Section "Inhalation", guinea pigs were exposed to azodicarbonamide concentrations of up to 97 mg/m^3 in a plethysmograph for 1 hour. Slight changes in lung function parameters were found

but there were no unusual histopathological findings in the respiratory tract. According to the authors, this indicates that azodicarbonamide causes only very slight irritation (Shopp et al. 1987).

Allergenic effects

Sensitizing effects on the skin

Negative results were obtained with azodicarbonamide in a local lymph node assay (LLNA) carried out with groups of 5 female CBA/J mice according to OECD Test Guideline 429. The substance was used in 10%, 25% and 50% formulations in propylene glycol. Stimulation indices of 1.2, 1.2 and 1.1 showed that it was not possible to achieve a 3-fold increase in the stimulation index compared with the value for the controls in any of the cases (WIL Research Europe 2014).

A maximization test carried out in 10 female Dunkin Hartley guinea pigs according to OECD Test Guideline 406 yielded negative results.In this test, intradermal induction was carried out with 10% azodicarbonamide in an aqueous formulation containing 1% carboxymethyl cellulose. Topical induction (after 24-hour open application of 10% sodium dodecyl sulfate in petrolatum) and challenge treatment were carried out with 50% azodicarbonamide formulations in the same vehicle. There were no erythematous or oedematous effects in any animal after either topical induction or challenge treatment. The sensitivity of the strain had been verified with hexyl cinnamaldehyde about 3 months before (WIL Research Europe 2014).

Likewise, a Bühler test in only 10 guinea pigs with a 50% aqueous suspension of azodicarbonamide did not lead to positive results (ECHA 2016).

In an earlier study of skin sensitizing effects that was not carried out according to guidelines, 0.1 ml 1% azodicarbonamide in dimethyl formamide was applied daily to the outer surface of the ears of only 4 Alderley Park guinea pigs for 3 days. On day 7 of the test, various concentrations of 0.2 ml formulations of azodicarbonamide (no other details) were applied to the shaved flanks of the animals, but no skin reactions were observed 24 hours later (Stevens 1967).

Sensitizing effects on the airways

Groups of male Hartley guinea pigs inhaled an azodicarbonamide aerosol for 4 weeks (0, 51 or 200 mg/m^3; 6 hours a day; 5 days a week). Provocation treatment was carried out in groups of 10 animals 1 week before and 2 days after the 4-week induction treatment using an aerosol with the same azodicarbonamide concentrations, and the specific airway conductance was determined. Further groups of 10 animals were tested with a histamine aerosol for non-specific bronchial hyperreactivity before and after the 4-week exposure to azodicarbonamide. Intradermal skin tests with 0.05%, 0.1%, 0.5% and 1% azodicarbonamide (in physiological saline) were carried out in 5 animals of every group 1 week later. The injection sites were examined after 15 minutes and after 6, 24, 48 and 72 hours. The 4-week exposure did not result in either sensitizing effects on the airways or non-specific bronchial hyperreactivity. In both dose groups, 6 of 10 animals, and in the control group, 5 of 10 animals reacted with a more pronounced change in the specific airway conduc-

tance than before induction treatment (no other details). The skin test yielded no immediate reactions, but erythematous reactions were observed in all groups. In the groups of animals that had been provoked with azodicarbonamide, there were no significant differences in the severity of these reactions to the 1% azodicarbonamide formulation. The diameter of the reactions in the animals provoked with histamine was the smallest in the control animals and the largest in the animals pre-treated with an azodicarbonamide concentration of 200 mg/m^3 (average of 4.2 mm compared with 2.4 mm in the control group and 3.6 mm in the low dose group). According to the authors, the observed erythematous reactions resulted from a foreign body reaction caused by azodicarbonamide. The exposure to azodicarbonamide by inhalation did not influence body weights or cause histopathological changes (Gerlach et al. 1989).

Structure–activity relationship

As azodicarbonamide is a reactive substance, it seems plausible that it would cause sensitizing effects. Several reviews of structure–activity relationships (SARs) for low-molecular respiratory allergens list azodicarbonamide as a potential respiratory allergen. The authors of one of the publications considered the substance not to be (sufficiently) electrophilic according to the original SAR rules. Therefore, they discussed whether azodicarbonamide (as well as ethanolamine, dimethylethanolamine, etc.) was "classified incorrectly" and considered sensitizing effects on the airways to be plausible based on a detailed review of the possible reactions of azodicarbonamide. They suggested that the mechanism responsible for protein reactivity was a Michael addition to the N=N double bond activated by the carbonyl groups (Enoch et al. 2010, 2011, 2012). The above-mentioned earlier study on the reaction of azodicarbonamide with SH groups in proteins of dough (Tsen 1963; see Section "Mechanism of Action") and a study on the antiviral effect of substituted azodicarbonamides (Hill and Vederas 1999; see Section "Mechanism of Action") are referred by the authors as evidence for the postulated protein binding.

Azodicarbonamide was included in other investigations on the structure–activity relationship of asthmagenic substances (Graham et al. 1997; Jarvis et al. 2005), but again together with substances which do not cause airway sensitization, such as substituted ethanolamines and dioctyl phthalate.

Summary

The maximization test, Bühler test and LLNA all led to clearly negative results; however, azodicarbonamide was only very slightly soluble in the vehicles that were used, therefore, a 10% aqueous suspension was used for intradermal induction in the maximization test. In experimental studies of (skin) sensitizing effects, low-molecular respiratory allergens yielded positive results in the LLNA insofar as findings are available (Kimber et al. 2007). In this respect, azodicarbonamide would be an exception if it were a respiratory allergen. However, because of the low solubility of the substance, a sensitizing potential cannot definitely be ruled out on the basis of the findings available for azodicarbonamide. The experimental studies of the sensitizing effects of azodicarbonamide on the airways in guinea pigs cannot be included in the evaluation because the study design was unsuitable.

The considerations on the structure–activity relationship do not provide clear evidence for the prediction of protein binding and a sensitizing potential of azodicarbonamide.

Reproductive and developmental toxicity

Fertility

In a 1-generation study, Sprague Dawley rats were treated with azodicarbonamide doses of 0, 100, 300 or 1000 mg/kg body weight and day. The animals were given gavage doses, corn oil was used as a vehicle, and the groups consisted of 25 animals per sex. The males were treated for 98 days, and the females were treated for 2 weeks before mating, during mating and gestation, and up to day 20 of lactation. Up to the high dose, there were no effects on reproductive performance or the development or viability of the offspring. Effects on the kidneys were recorded in the female parental animals of the high dose group. Cellular infiltrations of lymphocytes were observed in the interstitium of 4 of the surviving animals. Therefore, the systemic NOAEL was 1000 mg/kg body weight and day for male parental animals and 300 mg/kg body weight and day for female parental animals; the highest dose tested of 1000 mg/kg body weight and day was the NOAEL for fertility in both sexes (see Section "Oral administration"; Hatano Research Institute 2000; OECD 2007).

In a 3-generation study with rats which were given bread made of flour that had been treated with 100 mg azodicarbonamide per kg, effects on fertility, reproductive ability or lactation were not observed in any generation (Oser et al. 1965). As azodicarbonamide is completely converted to biurea during the baking process, this study is not suitable for the evaluation of the toxic effects of azodicarbonamide on reproduction.

The 90-day inhalation studies with F344 rats and B6C3F1 mice already described in Section "Inhalation" did not reveal any effects on the reproductive organs or sperm morphology up to the high concentration of 200 mg/m^3. The oestrus cycle of the female mice was unchanged; in 2 of 10 rats of the 200 mg/m^3 group, the cycle length was more than 7 days or could not be determined with certainty (Medinsky et al. 1990).

Developmental toxicity

There are no data available for developmental toxicity.

Genotoxicity

In vitro

Positive results were obtained in the Salmonella typhimurium strains TA100 and TA1535 in the presence and absence of a metabolic activation system. Concentrations of a maximum of 10 000 µg/plate were tested (no data for cytotoxicity). However, mutations were not induced in the strains TA97, TA98, TA102, TA1537 and TA1538 or in Escherichia coli WP2/pKM101 (BG Chemie 1993; Hachiya 1987; HSE 1996; OECD 2007).

A UDS test with primary rat hepatocytes yielded negative results even at cytotoxic concentrations. Concentrations between 0.2 and 6000 µg/well were tested (no other data). Cytotoxicity occurred at concentrations above 200 µg/well. The positive and negative controls yielded the expected results (HSE 1996; OECD 2007).

There was no induction of SCE in CHO cells . The concentrations used were 0, 20, 60, 200, 600 or 900 µg/ml without a metabolic activation system and 0, 60, 200, 600, 900 or 1200 µg/mln with a metabolic activation system. DMSO was used as the vehicle. Cytotoxicity was observed at the low concentration of 200 µg/ml and above; there were evidently delays in the cell cycle at this low concentration because the cells were incubated for 34 hours instead of 25 hours and the two higher concentrations were not evaluated. The concentrations of 600 and 1200 µg/ml were not evaluated with metabolic activation (NTP 1984 a).

According to the authors, a chromosomal aberration test with CHO cells yielded positive results. The cells were incubated for 21 hours and 45 hours without the addition of a metabolic activation system, and the tested concentrations were 0, 30, 150 and 300 µg/ml (21 hours) and 0, 26, 130 and 260 µg/ml (45 hours). In the presence of a metabolic activation system, the incubation period was 4 hours with concentrations of 3, 200 or 300 µg/ml (21 hours) and 3.2, 16 or 24 µg/ml (45 hours), with 17-hour and 41-hour recovery periods. DMSO was the vehicle, and mitomycin C, cyclophosphamide and carbendazim (evidence of polyploidy) were used as positive controls. The mitotic index was determined in a pretest. The solubility limit was reported to be 300 µg/ml. The positive controls yielded the expected increases in incidences. Without an activation system, 21-hour and 45-hour incubation led to a statistically significant increase in cells with aberrations at the highest concentrations tested of 300 µg/ml (without gaps) and 260 µg/ml (with gaps). After 45-hour incubation with 300 µg/ml, the mitotic index was 37.5% compared with that for the controls; therefore, it cannot be ruled out that the concentration of 260 µg/ml was cytotoxic. After 21 hours, the number of polyploid cells was not increased, whereas there was a statistically significant increase after 45 hours. According to the authors, after 45-hour incubation, this increase was within the range of the historical controls of the testing laboratory (no other details); therefore, the results were regarded as negative. In the presence of a metabolic activation system, there was an increase in cells with chromosomal aberrations after 21-hour incubation only at the low concentration (3 µg/ml). The cytotoxicity observed in several experiments of this test run was unusual: marked cytotoxicity was observed in the low concentration groups, whereas the cytotoxic effects were weaker at higher concentrations (no other details). The increase in clastogenic effects was also greatest at the low concentrations. There was no increase in polyploid cells. In the presence of a metabolic activation system, positive results were obtained also after 45-hour incubation. An increase in cells with chromosomal aberrations (without gaps) and the induction of polyploid cells were observed at the middle concentration of 16 µg/ml and above. Only the high concentration was clastogenic if the gaps are taken into account. Overall, azodicarbonamide was regarded as clastogenic and as a substance that induces polyploidy (ECHA 2016; Otsuka Chemical Company Limited 1989). It is assumed that the positive effects were caused by cytotoxic effects. However, this cannot be clarified conclusively because of the inadequate representation and description of the results, especially as the mitotic index was determined in pretests

rather than parallel to the main test. Furthermore, there was no concentration–effect relationship and some of the concentrations were close to the solubility limit. Therefore, this test is regarded as questionably valid.

Another test for the induction of chromosomal aberrations with CHO cells, which was carried out on behalf of the NTP, likewise led to positive results in the presence of a metabolic activation system. The test was carried out twice. Without the addition of a metabolic activation system, the first test with concentrations of 0, 402, 498, 600 and 720 µg/ml yielded negative results after an incubation period of 26.5 hours, and the second test yielded questionable results with concentrations of 0, 402, 498, 702 and 798 µg/ml and an incubation period of 26.8 hours. After treatment with 498 µg/ml, there was a 3.5-fold increase in the percentage of cells with aberrations. At the next higher concentration, the number was again in the range of that in the concurrent controls, and the high concentration was not evaluated. This was possibly due to cytotoxicity. With the addition of a metabolic activation system, concentrations of 0, 900, 1020, 1104 and 1290 µg/ml were tested in the first experiment with an incubation period of 18.7 hours (the high concentration was not evaluated). After treatment of the cells with 900 µg/ml, the percentage of cells with aberrations was increased 3.25-fold compared with that in the controls. Increases were observed also at the next two higher concentrations, but to a lesser extent than at the low concentration. In the second experiment with 0, 900, 1002 and 1104 µg/ml, there was a 12-fold increase in the percentage of cells with aberrations at the high concentration. The results of this test were assessed as weakly positive (NTP 1984 b). Although the same DMSO vehicle was used, the concentrations in this test were far above the solubility limit of 300 µg/ml described in the first test (Otsuka Chemical Company Limited 1989).

Mutation tests in mammalian cells ($TK^{+/-}$ test in mouse lymphoma cells and HPRT test in CHO cells) yielded negative results. The azodicarbonamide concentrations tested in the $TK^{+/-}$ test with mouse lymphoma cells were 73 to 150 µg/ml without and 106 to 261 µg/ml with the addition of a metabolic activation system. In the HPRT test, CHO cells were treated with concentrations between 5 and 500 µg/ml in the presence and absence of a metabolic activation system. In this test, the proportion of surviving cells was less than 10% after treatment with the high concentration. The concurrent positive and negative controls confirmed the validity of the test (BG Chemie 1993; HSE 1996; OECD 2007).

In vivo

The SLRL test with adult male Drosophila melanogaster led to negative results after the injection of 8000 or 10 000 ppm (HSE 1996; OECD 2007; Yoon et al. 1985).

A micronucleus assay in the bone marrow of male and female CD-1 mice, , yielded negative results after the administration of an oral azodicarbonamide dose of 5000 mg/kg body weight . In a pretest with 2 animals per group and sex, bent posture and ruffled fur were observed in the animals at 1250 mg/kg body weight and above up to 4 hours after administration. The vehicle used for the suspension was 1% methyl cellulose, the test group consisted of 19 males and 17 females, the control group included 15 males and 15 females and the positive control group treated with mitomycin C consisted of 5 animals per sex. The animals were sacrificed 24, 48 or

72 hours after treatment, the positive control animals after 24 hours. Clinical symptoms were not reported in the main test. The PCE/NCE ratio was unchanged. The tests with the positive controls led to the expected results (Otsuka Chemical Company Limited 1988).

Groups of 5 male and 5 female CD-1 mice were given intraperitoneal injections with azodicarbonamide doses of 0 or 150 mg/kg body weight. Corn oil was used as a vehicle, and the animals were sacrificed 30, 48 or 72 hours after treatment. Negative and positive controls were included. The dose given was the maximum tolerated dose because clinical signs of toxicity such as reduced activity were observed in the animals and there was an increase in mortality in the group that was supposed to be sacrificed 72 hours after treatment. In this group, 1 male and 3 females died. Further animals (number not specified) were treated so that this group could still be evaluated; of these animals, 1 male and 2 females died. A decrease in the PCE/NCE ratio observed in the animals that were examined 30 hours and 48 hours after treatment was regarded as a sign of cytotoxicity. There was no increase in the number of micronucleated polychromatic erythrocytes in the bone marrow (ECHA 2016; OECD 2007). Because of insufficient data, the study is included in the evaluation of genotoxic effects only with reservations. Another micronucleus test was carried out in 6 mice per group (sex not specified) with 0, 20, 40 or 80 mg/kg body weight. Treatment was carried out intraperitoneally, and olive oil was used as a vehicle. The animals were sacrificed 24 hours after the injection. A statistically significant increase in micronucleated erythrocytes was observed only in the middle dose group. Thus, there is no dose–response relationship, and the test result was regarded as negative. The number of polychromatic erythrocytes remained unchanged. Positive and negative controls yielded the expected results. An increase in mortality was reported at doses above 80 mg/kg body weight, but without giving any other information. This observation is consistent with the findings obtained in the above study with 150 mg/kg body weight (Hachiya 1987; HSE 1996; OECD 2007).

Summary

Azodicarbonamide induced base-pair mutations in bacteria, but was not mutagenic in mammalian cells. Indicator tests for clastogenicity yielded negative results in mammalian cells, but chromosomal aberrations and polyploidy were induced; one of the tests was regarded as questionably valid. Micronuclei were not observed in vivo after oral administration or intraperitoneal injection. Therefore, clastogenic or aneugenic effects on the bone marrow of mice are not assumed. Azodicarbonamide did not induce mutations in Drosophila.

Carcinogenicity

Carcinogenicity studies that were carried out according to accepted guidelines are not available.

Rats and dogs were given bread made of flour that had been treated with azodicarbonamide. The animals had no treatment-related clinical symptoms or organ changes. The tumour incidences were unchanged (Oser et al. 1965). This study is not suitable for the evaluation of the carcinogenic effects of azodicarbonamide be-

cause the doses given were low and azodicarbonamide is completely converted to biurea during the baking process (WHO 1999).

Other effects

Azodicarbonamide concentrations of up to 200 µmol/l had no cytotoxic effects in vitro on CD4-positive T-lymphocytes from human peripheral blood. The dose-dependent inhibition of cell proliferation and of the lymphocyte secretion of the cytokines interleukin-2 and interleukin-5 as well as of γ-interferon stimulated by dendritic cells or monoclonal antibodies against CD3 and CD28 was observed in vitro after azodicarbonamide doses of 50 to 200 µmol/l (Tassignon et al. 1999). In addition, azodicarbonamide doses in the same dose range inhibited the calcium mobilization and blastogenesis of the T lymphocytes stimulated by the monoclonal antibodies. It was possible to inhibit this effect by the addition of a calcium ionophore. Intraperitoneal injection of BALB/c mice with single azodicarbonamide doses of 100 mg/kg body weight led also in vivo to a decrease in interleukin-2 and interleukin-4 secretion induced 1 day later by intraperitoneal inoculation with 25 µg monoclonal CD3 antibodies of the hamster. In C57BL/6 mice, daily intraperitoneal injections of an azodicarbonamide dose of 50 mg/kg body weight resulted in the delayed rejection of skin allografts (MHC class II-incompatible or MHC class I-incompatible preparations of C57BL/6.CH-2^{bm12} mice or C57BL/6.CH-2^{bm1} mice) (Tassignon et al. 1999, 2001).

Exposure of SUP-T1 lymphoblasts to an azodicarbonamide concentration of 100 µmol/l for 2 hours induced a reduction in deoxyribonucleotide triphosphate of around 50%, possibly by inhibiting ribonucleotide reductase. There was hardly any effect on the proliferation of the cells after 24 hours. In addition, azodicarbonamide concentrations in the range from 50 to 200 µmol/l induced the inhibition of thymidine phosphorylation (IC_{50} = 70.5 µmol/l) (Fagny et al. 2002).

Manifesto (MAK value/classification)

The critical effect of azodicarbonamide is the induction of respiratory diseases in occupationally exposed workers.

MAK value. Results from animal studies are not suitable for the establishment of a MAK value as the respiratory tract of humans reacts more sensitively to the substance than that of rodents in the available inhalation studies.

After inhalation exposure for 13 weeks, no effects were found in mice up to 50 mg/m³; at 100 mg/m³ and above, the terminal body weight was reduced. Effects on the respiratory tract were not observed up to the highest concentration of 200 mg/m³ in either rats or mice. On the basis of the NOAEC of 50 mg/m³ a MAK value of 5 mg/m³ would be obtained (taking into consideration a possible increase in the effects with a prolonged exposure duration 1:2, increased respiratory volume 1:2, extrapolation of the data from animals to humans 1:2, using the preferred value approach).

However, effects on the respiratory tract occur in humans at lower concentrations and therefore the MAK value must be based on data in humans. However, the available results, especially those gained from the plastics processing industry, only allow the establishment of a provisional MAK value:

No clear correlation between the frequency of respiratory symptoms and the airborne concentrations measured at the workplace could be found in any of the investigations performed in factories (see Section "Effects in Humans"), although frequent exposure-induced symptoms have been reported. In regard to the potential exposure to a mixture of substances it is also unclear whether there was really (only) a monocausal relationship between the respiratory symptoms and exposure to azodicarbonamide. The detailed measurements of the exposure to azodicarbonamide in the publication of NIOSH (1985 b) are from employees who used personal breathing masks and are therefore unsuitable for deriving a MAK value. Exposure data from employees who did not use breathing masks can be found only in the investigations of NIOSH (1985 a) and Whitehead et al. (1987). In 17 injection-moulding workers investigated, lung function values for FEV_1 and FVC were determined before and after the shift parallel to the exposure measurements, and the results were assigned to 3 exposure groups (0–20, 21–40 and > 40 µg/m³). In these 3 groups, lung function was not or only slightly impaired, and there was no concentration–effect relationship for the average determined lung function values. Likewise, the unpublished individual measurements that were made available (Whitehead 2017) did not reveal a positive concentration–effect relationship. Taking these objectified measurements of the lung function values into account, the average exposure of the three groups of 36 µg/m³ (0.036 mg/m³) is therefore considered to be the NOAEC. However, as a NOAEC cannot be derived for the work-related symptoms which were subjectively reported by the workers, a MAK value of 0.02 mg/m³ I (20 µg/m³ I; inhalable fraction) has been established. As a result of the uncertainties regarding the exposure scenario and the concentration at which respiratory symptoms first occurred, and in view of the small cohort, this value should be regarded as provisional.

Peak limitation. As the airway reactions are evidently local effects, azodicarbonamide is classified in Peak Limitation Category I. The excursion factor is 1 because there is no information about the concentration at which these effects are first observed.

Prenatal toxicity. There are no developmental toxicity studies available. In a 1-generation study in rats, no foetotoxic effects were observed up to 1000 mg azodicarbonamide/kg body weight and day. Azodicarbonamide is therefore classified in Pregnancy Risk Group D.

Carcinogenicity. There are no data available that would justify classification of the substance in one of the categories for carcinogenic substances.

Germ cell mutagenicity. There are no data available for germ cell mutagenicity. Azodicarbonamide induces base-pair mutations in bacteria, but there are no mutagenic effects in mammalian cells. Indicator tests for clastogenicity yielded negative results in mammalian cells, but the induction of chromosomal aberrations and polyploidy were observed. One study was regarded as questionably valid. There was no induction of micronuclei in vivo after oral or intraperitoneal administration;

thus, clastogenic or aneugenic effects on the bone marrow of mice cannot be assumed. Azodicarbonamide did not induce mutations in Drosophila. There are no data available which would justify classification in a category for germ cell mutagens.

Absorption through the skin. There are no experimental studies available concerning the absorption of azodicarbonamide through the skin. In one animal study no signs of systemic toxicity were found after a single dermal application of 2000 mg/kg body weight. Model calculations for absorption through the skin under standard conditions yielded a maximum dermal absorption of 0.03 mg. In animal studies conducted with mice, a threshold value of at least 5 mg/m^3 may be derived for systemic effects; the systemic uptake (10% absorption by inhalation, 10 m^3) was approximately 5 mg. Because of the very large difference between the dermal absorption expected from model calculations even in the worst case and the maximal tolerable dose for prevention of systemic effects, a relevant contribution of percutaneous absorption to the systemic toxicity of the substance is not to be expected. Therefore, azodicarbonamide is not designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. There is only one plausibly documented clinical finding available which could indicate a possible skin sensitization potential for azodicarbonamide. However, information about the identity and the purity of the tested product are not given. Likewise, this information is also missing in the few other reports. Negative results were obtained in a local lymph node assay with mice and in a maximization test and a Bühler test with guinea pigs. These negative results should be considered within the context of the low solubility of azodicarbonamide in the solvents used. Positive results from animal studies are not available. Several case studies are available concerning the effects of azodicarbonamide on the airways. However, most of the studies had methodological shortcomings and the dust concentrations obtained in the bronchial provocation tests were not documented. An immunological origin and thus a potential for sensitization in the respiratory tract could be deduced from the findings in bronchial provocation tests in only three cases at most, but without proof of sensitization according to known mechanisms. Results of prick tests with (unconjugated) azodicarbonamide were negative in all cases and no specific IgE to azodicarbonamide conjugated to human serum albumin was detected. Studies at the workplace did not include substance-specific pulmological-allergological investigations, and also in the reported cases, which were recorded mainly in British registers of reportable diseases, information on diagnosis and exposure was lacking; it is therefore not possible to carry out a plausibility check. Although azodicarbonamide has been used as a blowing agent also in Germany for a long time and with similar exposures, no cases of sensitization have been observed. Azodicarbonamide is not listed as a cause of asthma , or only in individual cases, in several reporting systems from other regions. The decisive factor for the assessment of a possible sensitizing potential is, however, that none of the studies reported the origin, identity and purity of the substance that was causative at the workplace or used for diagnostics. In view of the numerous potential additives present during the production or use of azodicarbonamide, the substance is not clearly identifiable as the cause of the effects. Available results on metabolism and considerations on the

reactivity and mode of action of the compound do not clearly point to protein binding in vivo which would be a prerequisite for a sensitizing potential on the skin or airways. Altogether, the available data are not sufficient to demonstrate sensitizing effects for azodicarbonamide and the compound is neither designated with "Sh" nor with "Sa" (for substances which cause sensitization of the skin or airways).

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