Recommendation from the Scientific Committee on Occupational Exposure Limits for 4,4'-Diaminodiphenylmethane [MDA]

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Employment, Social Affairs and Inclusion

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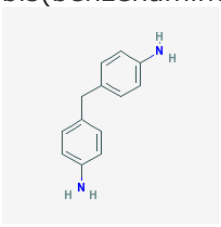
8-hour TWA	:	not feasible to derive a health-based limit (see Recommendation)
STEL (15 mins)	:	not feasible to derive a health-based limit (see Recommendation)
Notation	:	"Skin"
SCOEL carcinogen group	:	A (non-threshold genotoxic carcinogen)
Biological monitoring	:	Biological Guidance Value (BGV) = 1 µg/L urine (see Recommendation)

Substance identification:
4,4'-Diaminodiphenylmethane

Synonyms:
4,4'-Methylenedianiline (MDA), p,p'-methylenedianiline, 4,4'-methylene-bis(benzenamine).



Structural formula: $H_2N\text{-}C_6H_4\text{-}CH_2\text{-}C_6H_4\text{-}NH_2$
($C_{13}H_{14}N_2$)

EU-classification:

Carc. 1B	H350	May cause cancer.
Muta. 2	H341	Suspected of causing genetic defects.
STOT SE 1	H370**	Causes damage to organs.
STOT RE 2 *	H373**	May cause damage to organs through prolonged or repeated exposure.
Skin Sens. 1	H317	May cause an allergic skin reaction.
Aquatic Chronic 2	H411	Toxic to aquatic life with long lasting effects.

CAS No.:	101-77-9
Molecular weight:	198.26
Melting point:	92°C
Boiling point:	398°C
Conversion factor:	1 ppm = 8.22 mg/m^3; 1 mg/m^3 = 0.12 ppm

This summary document is based on documentations of IARC (1986), DFG (1996, 2007), NTP (2002) and supplemented by a recent literature search of SCOEL.

1 Occurrence, use and occupational exposure

4,4'-Diaminodiphenylmethane (also called (methylene dianiline, MDA) is a chemical intermediate in the closed-system production of 4,4'-diaminodiphenylmethane diisocyanate (MDI) and polyisocyanates. It is also used as a cross-linking agent for epoxy resins, in the determination of tungsten and sulphates, as an analytical agent, as a corrosion inhibitor, as an antioxidant and curative agent in rubber and to prepare azo dyes. Primary routes of occupational exposure are inhalation and skin contact.

Airborne exposure to MDA appears as aerosol. Potential exposure occurs during production, packaging and reprocessing of the chemical and during its use in epoxy resins (NTP 2002).

2 Health significance

4,4'-Diaminodiphenylmethane is hepatotoxic in man and animals. It also causes kidney damage with proteinuria and increased blood urea levels, hyperglycaemia and/or glycosuria and eye damage. In cats, the main symptoms of 4,4'-diaminodiphenylmethane intoxication are methaemoglobinaemia with Heinz body formation, reduced haemoglobin and erythrocyte levels and degeneration of the retina leading to blindness. There are also reports of ECG changes in man.

4,4'-Diaminodiphenylmethane is mutagenic in the Ames test after metabolic activation and is effective in a test for DNA repair in rat hepatocytes (UDS test). In vivo after intraperitoneal administration, it causes sister chromatid exchange in the hone marrow cells of the mouse and DNA strand breaks in the rat liver. It is carcinogenic in animal studies.

In man, 4,4'-diaminodiphenylmethane is a contact allergen, and there are indications that it can cause photosensitization (DFG 1996).

2.1 Toxicokinetics/biomonitoring

The available information shows that 4,4'-diaminodiphenylmethane is absorbed via the dermal, oral and inhalation routes. *N*-acetylation apparently represents the detoxification pathway, whereas *N*-hydroxylation, indicated from *in vitro* studies, can result in potentially toxic intermediates. 4,4'-diaminodiphenylmethane and its *N*-acetylated metabolites are mainly excreted in urine (UNEP 1999).

Kenyon *et al* (2004) have quantitatively assessed the permeability of 4,4'-diaminodiphenylmethane through human and rat skin. The compound was readily absorbed into and through the skin and was found to be bioavailable. After application of 0.1 mg 4,4'-diaminodiphenylmethane, 4% penetrated through latex and nitrile gloves, respectively.

Wellner *et al* (2008) conducted diffusion cell experiments for some aromatic amines, including 4,4'-diaminodiphenylmethane. Excised human skin was exposed to different amine concentrations in vehicles containing water and solvents. Recovery in the receptor fluid was about 15% over 24 h for 4,4'-diaminodiphenylmethane.

4,4'-Diaminodiphenylmethane is subject to metabolic *N*-oxidation. This pathway leads to formation of haemoglobin adducts. After hydrolysis of the sulfinic acid amides formed in the reaction between the arylnitroso compound and the cysteine in haemoglobin, both the diamine and the monoacetyl diamine can be detected (Bailey *et al* 1990; Farmer and Bailey 1989; Neumann *et al* 1993).

Studies of the drug metabolizing enzymes of rat liver demonstrated that 4,4'-diaminodiphenylmethane induces both epoxide hydrolase and ethoxyresorufin-O-deethylase (CYP1A1) activities and, at the same time, reduces the activity of aldrin epoxidase (Wu *et al* 1989).

2.1.1 Human data

Robert *et al* (1995) determined 4,4'-diaminodiphenylmethane, *N*-acetyl-4,4'-diaminodiphenylmethane and *N,N*'-diacetyl-4,4'-diaminodiphenylmethane. While mono-acetyl-4,4'-diaminodiphenylmethane represented more than 50% of total 4,4'-diaminodiphenylmethane compounds, 4,4'-diaminodiphenylmethane and diacetyl-4,4'-diaminodiphenylmethane were lower than 15% and 3% respectively. Urinary half times were 9-14 hours.

4,4'-Diaminodiphenylmethane and its monoacetyl and diacetyl derivatives were detected at the end of the work shift in the urine of exposed workers (Cocker *et al* 1986 a). The level of monoacetyl-4,4'-diaminodiphenylmethane was between 20% and 160 % higher than that of the unmetabolised substance (Cocker *et al* 1986b, 1988, UNEP 1999).

2.1.1 Animal data

After intraperitoneal injection into mice, 4,4'-diaminodiphenylmethane appeared in the blood where it reached its peak concentration after about 10 minutes. The elimination half-life was determined as 3.2 hours (Tortoreto *et al* 1983).

After oral administration to rats of a 4,4'-diaminodiphenylmethane dose of 50 mg/kg, the N-acetyl derivative and, to a lesser extent, the N,N'-diacetyl derivative were found in the urine. Elimination of the substance was complete within 72 hours (Tanaka *et al* 1985).

Chen *et al* (2008) conducted experiments to isolate, characterise, and quantify 4,4'-diaminodiphenylmethane metabolites excreted into bile in both male and female bile duct-cannulated Sprague Dawley rats. The rats were dosed by gavage with [^{14}C]-4,4'-diaminodiphenylmethane. HPLC analyses indicated numerous metabolites in both sexes, and male rats excreted greater amounts of glutathione and glucuronide conjugates than females. Electrospray-MS and NMR spectra of HPLC fractions revealed that the most prominent metabolite found in bile of both sexes was a glutathione conjugate of an imine metabolite of a 4'-nitroso-4,4'-diaminodiphenylmethane. Seven other metabolites were identified, including acetylated, cysteinyl-glycine, glutamyl-cysteine, glycine, and glucuronide conjugates.

According to animal experiments in rats, the polymorphic enzyme *N*-acetyltransferase 2 (NAT2) is involved in the acetylation of 4,4'-diaminodiphenylmethane, and rapid acetylators (F344 rats) responded to a moderately toxic dose of 4,4'-diaminodiphenylmethane with a more pronounced hepatotoxic effects than slow acetylators (WKY rats; Zhang *et al* 2006).

Kautiainen *et al* (1998) performed a study by treatment of mice with 4,4'-diaminodiphenylmethane and dosing tritiated or deuterated 4,4'-diaminodiphenylmethane, with identification of products of reaction with haemoglobin, after enzymatic hydrolysis of the globin and enrichment of the adducts. The main adduct, about 50% of the total amount of 4,4'-diaminodiphenylmethane associated with haemoglobin, was characterised by MS and was shown to be a reaction product of 4,4'-diaminodiphenylmethane and the amino group of *N*-terminal valine in Hb. It appeared likely that the quinonoid 4,4'-diaminodiphenylmethane -imine adduct to valine was formed by an attack of a metabolite formed through peroxidative oxidation

of 4,4'-diaminodiphenylmethane, in analogy with earlier observed oxidations of some other aromatic amines, e.g. benzidine. The formation of the adduct was confirmed by incubating 4,4'-diaminodiphenylmethane with valine methyl ester *in vitro* in the presence of H_2O_2 and lactoperoxidase.

2.1.2 Biological monitoring

For some time now, the internal exposure of workers to 4,4'-diaminodiphenylmethane has been measured via the excretion of free and conjugated 4,4'-diaminodiphenylmethane in urine. Despite extensive studies, exact correlations between inhaled 4,4'-diaminodiphenylmethane concentrations and the urinary excretion of 4,4'-diaminodiphenylmethane are not available. This is not last due to the predomonance of skin absorption, the percentage of which cannot be quantitatively determined in the total exposure to 4,4'-diaminodiphenylmethane.

In the industrial practice, the excretion of 4,4'-diaminodiphenylmethane after occupational contact has been often judged on the basis of so-called "empirical values", relying on industrial best-practice values at that time (Fairhurst 1993, DFG 1994). For instance, applying a "yardstick concept", biological limits of 88 µg 4,4'-diaminodiphenylmethane per L urine in the UK. (Cocker *et al* 1994), 72 µg/L by NIOSH (United States; NIOSH 2002) and 50 µg/L in France (Robert *et al* 1996) were recommended. Somewhat later in Germany, industrial biological monitoring data of a major manufacturer of 4,4'-diaminodiphenylmethane for the years 1998-2005 were compiled, and an empirical value, based on feasibility at that time, was derived of 10 µg/L 4,4'-diaminodiphenylmethane in urine (DFG 2007).

Based on an analytical detection limit of 0.5 µg/L of 4,4'-diaminodiphenylmethane in urine, it has been stated that persons that are not occupationally exposed, do not exceed this analytical detection limit. On this basis, a Biological Reference Value (BAR) is now being proposed in Germany of 0.5 µg/L 4,4'-diaminodiphenylmethane in urine (DFG 2011). This means that any analytically detectable quantity of 4,4'-diaminodiphenylmethane in urine is regarded indicative of an occupational exposure increment. The underlying analytical method used acid hydrolysis of the conjugates, extraction, derivatisation and GC/MS/NCI analysis. With HBFA derivatisation/capillary gas chromatography and ECD detection, a routine method has been described and evaluated in detail earlier, with a detection limit of 1 µg 4,4'-diaminodiphenylmethane per L urine (DFG 1994a).

Another method of biological monitoring is based on the metabolic pathway leading to formation of haemoglobin adducts (see 2.1). After hydrolysis of the sulphinic acid amides formed by the reaction between the arylnitroso metabolite and the cysteine in haemoglobin, the free diamine is detected. An analytical method has been evaluated by DFG (2000), based on GC/MS/NCI. However, the industrial experience with this methodology is still limited, as it is more sophisticated than the urinary analysis mentioned above.

2.2 Acute toxicity

2.2.1 Human data

Hepatotoxicity with jaundice in man was first associated with 4,4'-diaminodiphenylmethane exposure in 1965 when it became clear that the 84 patients with hepatitis in a local epidemic had all eaten bread which had been baked with flour accidentally contaminated with 4,4'-diaminodiphenylmethane (the "Epping jaundice incident").

The first symptoms were severe colicky pains in the upper abdominal region, followed after 1 to 2 days by nausea, shivering, rigor, raised temperature and finally progressive jaundice. The obstructive icterus was accompanied by pale stools, dark urine and intense itching. At this time the liver was soft and enlarged. Some patients developed a transient erythematous rash during the attacks of shivering, one patient a purpuric rash which persisted for several days. Clinical examination revealed increased serum alkaline phosphatase activity, increased serum transaminase values in all patients and increased bilirubin in some. Liver biopsies from seven of the patients revealed cell infiltration in the portal zones, which were variously expanded and inflamed, and in some cases a markedly increased incidence of liver cell mitosis, cholangitis, bile duct congestion and liver cell damage. Haematuria was seen in one patient.

Within a few weeks, 82 of the persons were free of symptoms but in 2 patients the disorder persisted for up to 3 months. Analysis of the residual bread and flour provided only a vague indication of the levels of 4,4'-diaminodiphenylmethane contamination because they were variable; analysis of one bread sample revealed an amine level of about 0.26% (Kopelman 1981, Kopelman *et al* 1966a, 1966 b).

Two years after the accident, liver function tests and questionnaires answered by 43 of the patients did not reveal progressive liver disease; however, food intolerance was reported by 18% of these persons (Kopelman 1981).

Roy *et al* (1985) described the case of a 28-year old man who accidentally ingested several mouthfuls of a liquid containing 4,4'-diaminodiphenylmethane (dissolved with potassium carbonate and gamma-butyrolactone in unspecified proportions). In addition to the symptoms described above such as icterus and ECG changes, he also suffered from a visual disorder. Symptoms developed 2 days after his admission to hospital with increased transaminase and bilirubin levels followed on the third day by marked icterus and erythema multiforme which regressed within 2 days.

2.2.2 Animal data

4,4'-Diaminodiphenylmethane is acutely toxic for rats, rabbits, cats and dogs: single doses administered to rats to determine the LD_{50} caused marked liver and kidney damage with massive proteinuria and spieen enlargement; intraperitoneal doses of 400 mg/kg or more caused clouding of the cornea. In rabbits, single oral 4,4'-diaminodiphenylmethane doses of 500 mg/kg body weight caused an increase in blood sugar and blood urea, as well as progressive proteinuria; in dogs, doses of 100 mg/kg led to vomiting, jaundice, severe functional disorders of the liver, glucosuria (without increased blood sugar) and proteinuria. Cats were particularly sensitive to the effects of 4,4'-diaminodiphenylmethane: doses of 100 mg/kg, which were often lethal, resulted not only in jaundice and bilirubinaemia but also in liver damage, anaemia, methaemoglobinaemia with Heinz-body formation, hyperglycaemia (but without glucosuria) and irreversible blindness. Kidney damage led to increased blood urea and proteinuria. Visual disorders sometimes developed after single oral doses of as little as 25 mg/kg and liver and kidney damage after only 10 mg/kg (Hofmann *et al* 1966a, 1966b, Schilling von Canstadt *et a1* 1966, Schmidt *et al* 1974). After gastric intubation of male albino rats with single 4,4'-diaminodiphenylmethane doses of 600, 250, 200, 50 or 20 mg/kg body weight or eight doses of 50, 20 or 8 mg/kg within 10 days, necrosis was found in the periportal area and the interlobular bile ducts in the livers of the high dose group animals (600-200 mg/kg), increased incidence of mitosis, Kupffer cell hyperplasia, marked reduction in glycogen levels and even total absence of glycogen and slight peripheral fatty degeneration. In the kidneys, vacuolation, cystic degeneration and cell necrosis were found in the tubule epithelia in the renal cortex, perivascular oedema and highly dilated tubules in the medulla.

After the medium doses (50-20 mg/kg), mitotic activity and incorporation of radioactive thymidine were markedly increased in the hepatocytes and the bile duct epithelia, and various enzyme activities (SDH, $NADH_2$-reductase, LDH, acid phosphatase) were decreased in the liver; the activities of glucose-6-phosphate dehydrogenase and alkaline phosphatase were increased. No changes were seen in the animals given 8 mg/kg. In addition, the oral 4,4'-diaminodiphenylmethane doses of 50 to 600 mg/kg caused oedema and parenchymal degeneration in the heart muscle, brain and testes in some animals. Additional heat stress (warmth and humidity) did not change the effects of 4,4'-diaminodiphenylmethane significantly (Gohlke and Schmidt 1974, Schmidt *et al* 1981).

Daily oral 4,4'-diaminodiphenylmethane doses of 5 to 20 mg/kg, administered to dogs for one to five days, led to jaundice and increased serum alkaline phosphatase activity. Dogs given smaller daily doses of 4,4'-diaminodiphenylmethane for 7 weeks were unaffected and without increased serum parameters. Histological examination of the livers, however, revealed inflammatory changes (Rowe 1974).

Single 4,4'-diaminodiphenylmethane doses of 300 mg/kg administered to dogs in gelatine capsules caused vomiting and diarrhoea. The animals died within 3 to 4 days (Dion 1978).

Kanz *et al* (1992) positioned bile cannulas in Sprague-Dawley male rats under pentobarbital anesthesia. After 1 h of control bile collection, each rat was given 250 mg 4,4'-diaminodiphenylmethane/kg (50 mg/ml) po in 35% ethanol or 35% ethanol only; bile was collected for a further 4 hr. Groups of rats were also examined for liver injury and biliary function at 8 and 24 hr after 4,4'-diaminodiphenylmethane dosing. Four hours after 4,4'-diaminodiphenylmethane administration, main bile duct cells were severely damaged with minimal damage to peripheral bile ductular cells. Focal periportal hepatocellular necrosis and extensive cytolysis of cortical thymocytes occurred by 24 hr. Serum indicators of liver injury were elevated by 4 hr and continued to rise through 24 hr. By 4 hr, biliary protein concentration was increased 4-fold while concentrations of biliary bile salt, bilirubin, and glutathione were decreased by approximately 80, 50, and 200%, respectively.

4,4'-Diaminodiphenylmethane also induced a striking effect on biliary glucose with an approximately 20-fold increase. Bile flow was diminished by 40% at 4 hr; three of five rats had no bile flow by 8 hr and none had any bile flow by 24 hr. These results were interpreted to indicate that 4,4'-diaminodiphenylmethane rapidly diminishes bile flow and alters the secretion of biliary constituents and is highly injurious to biliary epithelial cells.

Experiments with inducers and inhibitors of oxidative CYP-mediated metabolism suggested that the acute hepatotoxicity of 4,4'-diaminodiphenylmethane required oxidative bioactivation. The damage was both dose- and time-dependent (Bailie *et al* 1993).

Kwon *et al* (2008) performed a toxicogenomics study in the mouse liver after treatment with 4,4'-diaminodiphenylmethane with a low (10 mg/kg bw) or high (100 mg/kg bw) dose. The treatment increased liver-toxicity-related enzymes in blood and induced bile-duct cell injury, followed by regeneration. Many genes associated with liver toxicity and diseases belonged to one of these categories. The chemokine-mediated Th1 pathway was implicated in the inflammatory process. The genes associated with oxidative stress, apoptosis, and cell-cycle regulation were dynamically responsive to 4,4'-diaminodiphenylmethane treatment. The Wnt/beta-catenin signaling pathway was considered to be responsible for the reconstitution process of the 4,4'-diaminodiphenylmethane-injured liver.

2.3 Irritation and corrosivity

Application of one drop of 4,4'-diaminodiphenylmethane into the rabbit eye led to lacrimation, conjunctival oedema and blepharospasm. Application of more dilute solutions of the substance produced the same symptoms in a weaker form (Schmidt *et al* 1974).

2.4 Sensitisation

4,4'-Diaminodiphenylmethane is described in several publications as a potent contact and occupational allergen, for example in the production of polyurethane, rubber, epoxy resins and many other products (Agrup and Fregert 1969, Breit 1969, Emmet 1976, Goldmann 1963, Jolanki *et al* 1987, Malten 1972, Melli *et al* 1983, van Joost *et al* 1987, Wallenstein *et al* 1977). Cross-reactions with other para-amino compounds, for example, p-phenylenediamine, or with azo dyes are increasingly observed (Gailhofer and Ludvan 1987, 1989, Massone *et al* 1991, Van Joost *et al* 1987). Likewise, contact dermatitis which is not of occupational origin, caused by exposure to polyurethane or epoxy resin adhesives or after wearing elasticised underwear, is increasingly attributed to 4,4'-diaminodiphenylmethane (Alomar 1986, Nigro *et al* 1988).

4,4'-Diaminodiphenylmethane has also been suggested as the cause of acute photosensitivity in a telephone service installer (LeVine 1983).

More recently, Liipo and Lammintausta (2008) carried out patch testing with 4,4'-diaminodiphenylmethane in 1595 patients. 4,4'-Diaminodiphenylmethane reactions were seen in 17 (1.1%) patients. Six of these 4,4'-diaminodiphenylmethane-positive patients reacted also to p-phenylenediamine and two to epoxy chemicals.

Three cases of allergic contact dermatitis to 4,4'-diaminodiphenylmethane were recently reported by Grimalt *et al* (2009).

2.5 Repeated dose toxicity

2.5.1 Human data

In the years 1972 and 1973, hepatitis developed in 6 of about 300 workers who coated the walls of an atomic power station with epoxy resin. They all became ill within 2 days to 2 weeks of beginning the work. Clinically, the symptoms were like those of viral hepatitis. Serum transaminase activities were increased and in some cases the bilirubin values as well. At this workplace, liquid epoxy resin was mixed with a dry powder which contained 4,4'-diaminodiphenylmethane, and the mixture was then applied to the walls either with a bricklayer's trowel or a spray gun. The authors were of the opinion that in spite of observance of standard protective measures (not described), it was possible for the workers to inhale the substance in the closed rooms, to swallow it while eating or smoking and to absorb it through the skin. Workplace analyses were, however, not carried out (Williams *et al* 1974).

Acute hepatitis developed in 4 of 6 workers after they had laid an epoxy resin floor-covering containing 4,4'-diaminodiphenylmethane as the hardener. Their symptoms were like those described above; after they had recovered and returned to work, 2 of the workers became ill a second time and their convalescence period was then prolonged (Bastian 1984).

2.5.2 Animal data

Nose-only inhalation of a 4,4'-diaminodiphenylmethane aerosol (0.44 ± 0.09 mg/L), 4 hours daily on 5 days per week for 2 weeks produced no visible symptoms in guinea pigs (albino and pigmented), not even after challenge by dermal application of a solution of 4,4'-diaminodiphenylmethane in polyethylene glycol (20 or 200 mg/mL). Neither skin irritation nor allergic reactions were seen. The most prominent histopathological findings were degeneration of the inner and outer segments of the photoreceptor cells and the pigmented epithelial cell layer of the retina in both kinds of guinea pig. Apart from small pulmonary granulomas and slight granulomatous pneumonitis, no tissue lesions were seen in the lungs, liver or kidneys of most of the exposed animals (Leong *et al* 1987).

Long-term oral administration (185 doses in 37 weeks) of 4,4'-diaminodiphenylmethane caused liver damage with the first signs of cirrhosis and blood damage in cats given as little as 3 mg/kg per dose. Kidney damage was not seen in this dose range. Cats died after 61 or 81 4,4'-diaminodiphenylmethane doses of 10 mg/kg body weight. In rats given 70 oral doses of 50 mg/kg (3 times weekly for about 6 months), the main effect was liver damage with transiently increased sulphobromophthalein retention and liver cirrhosis in most animals (Hofmann *et al* 1966 a).

In contrast, administration of an oral 4,4'-diaminodiphenylmethane dose of 8 mg/kg body weight (which had no effect as a single dose) on each of 5 days weekly for 6 weeks caused only transient liver changes in male albino rats. After 10 days the incidence of hepatocyte mitosis was increased, after 6 weeks there were large hepatocytes with giant nuclei and hyperchromatism of the nuclear membrane and cells in. the bile duct epithelia with large, vesicle-like nuclei. Two weeks after the end of treatment, differences between treated, and control animals were no longer detectable (Gohlke 1978).

When 4,4'-diaminodiphenylmethane was administered to male albino rats on 5 days per week for 16 weeks, daily doses of 3.2 mg/kg had no effect, 8 mg/kg had slight effects an the liver, producing swelling of the hepatocytes with nuclear enlargement and increased incidence of mitosis, and doses of 20 mg/kg were clearly hepatotoxic, producing cirrhotic changes, adenoma-like bile duct hyperplasia and hyperplastic nodules; 2 animals of 120 developed haemangiomas. The average age of the exposed animals (11.3 months) and the controls (12.5 months) was conspicuously low in these studies (Gohlke 1978). Atrophy of the liver parenchyma and increased spleen weights with hyperplasia of the lymphatic System were reported in Wistar rats given oral doses of 83 mg/kg daily for 12 weeks (Pludro *et al* 1969).

In castrated female Sprague-Dawley rats given 4,4'-diaminodipheny1methane doses of 150 mg/kg by gavage, daily for 5 to 14 days, hypertrophy was detected in the adrenals, uterus and thyroid. The thyroid weights practically doubled during the period of treatment while the body weights of the animals were reduced by about 17 % relative to the control values. The thyroid follicles in the treated animals contained little or no colloid. There was extensive lipid accumulation in the adrenal cortex (Tullner 1960).

Rats given 4,4'-diaminodiphenylmethane in the diet at a concentration of 1000 mg/kg for 12 weeks developed bile duct proliferation with concurrent oval cell and inflammatory cell infiltration, fibrosis and dilation of the smooth endoplasmic reticulum in the liver (Miyamoto *et al* 1977).

Likewise, in rats given 1 000 mg 4,4'-diaminodiphenylmethane (purity >98 %) per kg diet for 8, 16, 24, 32 or 40 weeks, changes were not seen in any organ apart from

the liver where bile duct proliferation, periportal inflammatory oval cell infiltration and fibrosis developed. The bile duct proliferation began in week 8 and increased in severity until week 24, whereas cirrhosis-like inflammatory oval cell infiltration was most prominent in week 40. Neither hyperplastic nodules nor tumours were found. Simultaneous with the bile duct proliferation, the levels of serum gamma-glutamyl transpeptidase and alkaline phosphatase were increased. After the end of treatment, the changes regressed at a rate dependent on the duration of administration of the substance. In the group treated for 8 weeks, the liver findings were normal again after 40 weeks, whereas after the 16-week and 24-week treatments slight changes were still detectable at this time. The serum transaminase values, which had been increased during exposure, returned to normal during the recovery period (Fukushima *et al* 1979).

Three female dogs were given 5 weekly 4,4'-diaminodiphenylmethane doses of 4, 6 or 8 mg/kg; the animal given the highest dose died after 93 doses. Autopsy revealed oedematous nephritis and liver cirrhosis. The medium dose animal was killed 3 weeks after the 76th dose; histological examination revealed oedematous nephritis and fatty degeneration in the liver. After 382 doses of 4 mg/kg, histological examination demonstrated the beginnings of liver cirrhosis but no nephritis (Dion 1978).

In dogs given gelatine capsules containing 70 mg technical grade or pure 4,4'-diaminodiphenylmethane, 3 times weekly (with occasional pauses) for about 4 to 7 years (approximate total dose per dog 40 to 67 g, about 4.0 to 6.26 g/kg body weight), the serum alkaline phosphatase values were increased; the occasional weight losses were made up rapidly after treatment was discontinued. Histological examination revealed mainly slight to severe toxic effects on the livers which were yellow to pale brown with rough and granular surfaces. The lesions ranged from enlarged liver cells and slight structural changes to degeneration, portal fibrosis, liver cell necrosis, haemosiderosis, cell infiltration of the portal areas, dilated bile ducts and thickened bile. Some animals also had slight changes in the kidneys, bladder, spleen and lungs. However, tumours were not seen (Deichmann *et al* 1978).

Dugas *et al* (2004) treated male and female rats with 25 mg 4,4'-diaminodiphenylmethane/kg or vehicle once per week for 17-22 wk. Though concentric fibrosis around bile ducts of the liver was noted, vascular medial hyperplasia was also prominent. Morphometric analysis of histologic sections revealed that in male rats, vessel wall area increased relative to lumen area in hepatic arteries by 22 wk. However, in female rats, wall areas of both hepatic and pulmonary arteries increased relative to lumen area by 17 wk. In both male and female rats, increased wall thickness was localized to the medial layer; no intimal changes were noted. *In vitro* treatment of vascular smooth muscle cells (VSMC) with 25-100 µM 4,4'-diaminodiphenylmethane resulted in increased DNA synthesis and VSMC proliferation. To test whether the observed alterations in cell cycle control involved VSMC-mediated metabolism of 4,4'-diaminodiphenylmethane to electrophilic intermediates, cells were treated with 4,4'-diaminodiphenylmethane or 4,4'-diaminodiphenylmethane plus 50 µM *N*-acetylcysteine (NAC). Co-incubation with NAC afforded dramatic protection against 4,4'-diaminodiphenylmethane-induced VSMC proliferation. Though 4,4'-diaminodiphenylmethane had no appreciable effect on levels of reduced glutathione, oxidised glutathione, or oxidant production, 4,4'-diaminodiphenylmethane increased glutathione-*S*-transferase activity in VSMC. These data were taken to indicate that 4,4'-diaminodiphenylmethane can initiate VSMC proliferation, possibly via VSMC-mediated metabolism of 4,4'-diaminodiphenylmethane to reactive intermediates.

2.6 Genotoxicity

The classical genotoxicity studies with 4,4'-diaminodiphenylmethane have been reviewed by McQueen and Williams (1990).

After metabolic activation, 4,4'-diaminodiphenylmethane is mutagenic in the Ames test in *Salmonella typhimurium* TA100 (Andersen *et al* 1980, Cocker *et al* 1986b, Darby *et al* 1978, Klopman *et al* 1985, Lavoie *et al* 1979, McCarthy *et al* 1982, Messerly *et al* 1987, Parodi *et al* 1981, Rao *et al* 1982, Shimizu *et al* 1982, Takemura and Shimizu 1978, Tanaka *et al* 1985). In strains TA98 and TA1538, 4,4'-diaminodiphenylmethane is not or only weakly mutagenic (Darby *et al* 1978, Klopman *et al* 1985, Lavoie *et al* 1979, Messerly *et al* 1987, Parodi *et al* 1981, Rannug *et al* 1984, Rao *et al* 1982, Takemura and Shimizu 1978). 4,4'-Diaminodiphenylmethane was activated more effectively by rat liver microsomes induced with phenobarbital than by those induced with Aroclor (Rao *et al* 1982). After activation with PCB-induced rat liver microsomes, 4,4'-diaminodiphenylmethane was mutagenic in *S. typhimurium* TA100 at concentrations of 10-1 000 µg/plate; in TA98 the substance was less mutagenic (Rao *et al* 1982).

The metabolites, *N*-acetyl-4,4'-diaminodiphenylmethane and *N,N'*-diacetyl-4,4'-diaminodiphenylmethane, were not mutagenic in this test system (Cocker *et al* 1986b, Tanaka *et al* 1985).

With the alkaline elution method it was demonstrated that 4,4'-diaminodiphenylmethane at concentrations of 1 to 3 mM caused DNA strand breaks in Chinese hamster V79 cells (Swenberg 1981). Clearly positive results were obtained with 4,4'diaminodiphenylmethane in one DNA repair test with rat hepatocytes (UDS test) (Mori *et al* 1988), negative results in another (Mirsalis *et al* 1989). Pretreatment with inducers of hepatic monooxygenases increases the sensitivity of the DNA repair test in rat hepatocytes and produces clearly positive results with 4,4'-diaminodiphenylmethane (Shaddock *et al* 1989).

Intraperitoneal injection of 4,4'-diaminodiphenylmethane doses of 9 or 18 mg/kg body weight into male Swiss mice caused a dose-dependent increase in sister chromatid exchange (Parodi *et al* 1983). Likewise, in the bone marrow cells of BALB/c mice, a significant increase in sister chromatid exchange was seen after the highest 4,4'-diaminodiphenylmethane dose of 35 mg/kg (the dose range tested was 1-35 mg/kg) (Gorecka-Turska *et al* 1983).

An increase in the level of DNA strand breaks in the liver was found after intraperitoneal injection of a 4,4'-diaminodiphenylmethane dose of 0.37 mmol/kg (74 mg/kg) into male rats (Parodi *et al* 1981).

Schütze *et al* (1996) studied DNA adducts after application of radiolabelled 4,4'-diaminodiphenylmethane to rats. The DNA-binding potency appeared in the range of weakly genotoxic compounds. The major adducts found in the liver did not correspond to previously synthesised standards. However, it was possible to release 4,4'-diaminodiphenylmethane and 4,4'-diaminodiphenylmethane-d4 from DNA of rats dosed with 4,4'-diaminodiphenylmethane and/or 4,4'-diaminodiphenylmethane-d4 using strong base hydrolysis.

Martelli *et al* (2002) reported on experiments in primary cultures of hepatocytes and thyreocytes from rats and humans. After exposure for 4 and 20 h to 4,4'-diaminodiphenylmethane concentrations ranging from 10 to 180 µM, a statistically significant increase in the frequency of DNA lesions was revealed by the Comet assay in primary hepatocytes and thyreocytes from donors of both species, the response being dose dependent up to 56-100 µM 4,4'-diaminodiphenylmethane. DNA

fragmentation was more marked after 4 than after 20 h exposure in all four cell types. DNA was damaged to a lesser extent in human hepatocytes and thyreocytes than in corresponding rat cells and in both species in hepatocytes than in thyreocytes. In both rat and human hepatocytes a 20-h exposure to the same 4,4'-diaminodiphenylmethane concentrations elicited a modest amount of DNA repair synthesis, as evaluated by autoradiography. Evidence of a partial reduction of DNA damage, and therefore of only partial DNA repair, was observed in rat hepatocytes and in rat and human thyreocytes incubated for 16 h in 4,4'-diaminodiphenylmethane-free medium after a 4 h 4,4'-diaminodiphenylmethane treatment. A 4-h exposure to 56, 100, and 180 µM 4,4'-diaminodiphenylmethane did not induce DNA lesions in primary cultures of cells from three rat organs, kidney, urinary bladder mucosa, and brain, which are resistant to 4,4'-diaminodiphenylmethane carcinogenic activity. Under the same experimental conditions evidence of DNA damage was absent in primary kidney and urinary bladder cells from human donors. The authors interpreted their results to indicate that 4,4'-diaminodiphenylmethane is activated to DNA-damaging reactive species by hepatocytes and thyreocytes in both rats and humans.

2.7 Carcinogenicity

2.7.1 Human data

In a cohort of 595 power generator workers potentially exposed to 4,4'-diaminodiphenylmethane as a curing agent of an epoxy system, the overall standardised cancer incidence ratio (SIR) among males (n = 550), however, was only 0.52 [95% confidence interval (CI) 0.16-1.21] based on five observed cases. One male urinary bladder cancer case was found in comparison to 0.6 expected (SIR 1.67; 95% CI 0.04-9.31). This case was identified in an unexposed subcohort. High levels of 4,4'-diaminodiphenylmethane metabolites were ascertained in the urine of currently exposed workers, probably following percutaneous absorption. It was noted that limitations of the study in regard to the size of the cohort, age and cancer latency precluded a definite risk assessment (Seldén *et al* 1992).

Between 1967 and 1976, 10 workers at a plant in Ontario that used 4,4'-diaminodiphenylmethane as an epoxy hardener developed acute jaundice. This group was followed from the date of intoxication through to the end of 1991 for cancer incidence by matching with the Ontario Cancer Registry. At the time of publication (1994), one pathologically confirmed bladder cancer has developed [expected number based on provincial incidence rates: 0.64 for all cancers, 0.05 for bladder cancer] (Liss and Guirguis 1994).

2.7.2 Animal data (evaluation of IARC 1986)

Groups of 50 male and 50 female B6C3F1 mice, 12 weeks of age, were given 0.015% (150 mg/kg) or 0.03% (300 mg/kg) 4,4'-diaminodiphenylmethane dihydrochloride (98.6% pure) in the drinking-water for 103 weeks, followed by one week without treatment prior to terminal sacrifice. Groups of 50 male and 50 female mice receiving drinking-water adjusted with 0.1 N HCl to pH 3.7 (equivalent to the pH of the 0.03% 4,4'-diaminodiphenylmethane dihydrochloride solution) served as controls. Survival at termination of the study was 40/50 (80%) control, 39/50 (78%) low-dose and 32/50 (64%) high-dose males and 40/50 (80%) control, 38/50 (76%) low-dose and 37/50 (74%) high-dose females. An increased incidence of follicular-cell adenomas of the thyroid was observed in high-dose animals: 0/47 control, 3/49 (6%) low-dose and 16/49 (33%) high-dose males ($p < 0.001$) and 0/50 control, 1/47 (2%) low-dose and 13/50 (26%) high-dose females ($p < 0.001$). In addition, a dose-related incidence of thyroid-gland follicular-cell hyperplasia was observed in both males and females, and

2/50 high-dose females developed thyroid follicular-cell carcinomas. An increased incidence of hepatocellular adenomas occurred in females: 3/50 (6%) controls, 9/50 (18%) low-dose and 12/50 (24%) high-dose animals ($p = 0.01$, Fisher exact and Cochran-Armitage trend tests), but not in males. Increased incidences of hepatocellular carcinomas were observed in treated males [10/49 (20%) controls, 33/50 (66%;;$p < 0.001$) low-dose and 29/50 (58%; $p < 0.001$) high-dose animals] and in treated females [1/50 (2%) controls, 6/50 (12%) low-dose and 11/50 (22%; $p = 0.002$, Fisher exact and Cochran-Armitage trend tests) high-dose animals] (Weisburger *et al* 1984).

A group of 20 female Sprague-Dawley rats, 40 days old, received 30 mg (maximum tolerated dose) 4,4'-diaminodiphenylmethane dihydrochloride [purity unspecified] in 1 mL sesame oil by gastric intubation every three days for 30 days (total dose, 300 mg/rat) and were observed for a further nine months. A group of 140 female rats receiving sesame oil alone served as negative controls and a group of 40 females receiving single doses of 18 mg 7,12-dimethylbenz[a]anthracene (DMBA) served as positive controls. Survival after nine months was 14/20 in the 4,4'-diaminodiphenylmethane dihydrochloride-treated group, 127/ 140 in the negative-control group and 19/40 in the DMBA-treated group. Mammary lesions were found in 5/132 negative controls (three carcinomas, one fibroadenoma, five hyperplasias), 29/29 DMBA-treated (75 carcinomas, ten fibroadenomas, 47 hyperplasias) and 1/14 4,4'-diaminodiphenylmethane dihydrochloride-treated (one hyperplasia) animals (Griswold *et al* 1968).

Groups of eight male and eight female rats [strain and age unspecified] received four or five doses of 20 mg/rat 4,4'-diaminodiphenylmethane [purity not stated] by gastric intubation over a period of less than eight months and were observed until death. One hepatoma and a haemangioma-like tumour of the kidney were found in a male rat after 18 months. An adenocarcinoma of the uterus was found in one female after 24 months. Most animals had varying degrees of liver fibrosis and inflammation (Schoental 1968).

Groups of 50 male and 50 female Fischer 344/N rats, six weeks old, were given 0.015% (150 mg/kg) or 0.03% (300 mg/kg) 4,4'-diaminodiphenylmethane dihydrochloride (98.6% pure) in the drinking-water for 103 weeks, followed by one week without treatment, after which time the animals were sacrificed. Groups of 50 males and 50 females receiving drinking-water adjusted with 0.1 N HC1 to the pH 3.7 (equivalent to the pH of the 0.03% 4,4'-diaminodiphenylmethane dihydrochloride solution) served as controls. There was no significant effect on survival in males or females. The incidences of thyroid follicular-cell carcinomas in high-dose animals were significantly increased over those in controls: 0/49 control, 0/47 low-dose and 7/48 high-dose males ($p < 0.012$, life-table test) and 0/47 control, 2/47 low-dose and 17/48 high-dose females ($p < 0.001$). A significant increase in the incidence of liver neoplastic nodules was also observed in male rats: 1/50 controls, 12/50 low-dose ($p = 0.002$) and 25/50 high-dose ($p < 0.001$) animals, and a statistically non-significant increase in these lesions was seen in treated females: 4/50 controls, 8/50 low-dose and 8/50 high-dose (Weisburger 1984, Lamb *et al* 1986).

A group of five female pure-bred beagle dogs, five to six months of age, received oral administrations of 70 mg 4,4'-diaminodiphenylmethane ('highly purified', dissolved in corn oil and placed in gelatinous capsules) thrice weekly. A further four female beagles received capsules containing 'crude' 4,4'-diaminodiphenylmethane (50% 4,4'-diamino-diphenylmethane; 50% higher molecular weight analogues). Total doses were 5.0-6.26 g/kg bw 'pure' 4,4'-diaminodiphenylmethane over periods of four-and-a-half to seven years, at which time there was one survivor, and 4.0-6.25 g/ kg bw 'crude' 4,4'-diaminodiphenylmethane over periods of four to seven years, at which time there

were two survivors. No tumour of the urinary bladder or liver was found (Deichmann 1978).

Groups of 25 male and 25 female Wistar rats [age unspecified] received subcutaneous injections of 30-50 mg/ kg bw 4,4'-diaminodiphenylmethane in physiological saline at one- to three-week intervals over a period of 705 days (total dose, 1.4 g/ kg bw). Mean survival times were 970 days for treated males and 1060 days for treated females, compared to 1007 days in controls. A total of 29 benign tumours [types unspecified] and 33 malignant tumours [types unspecified] were found in treated rats compared with 15 benign and 16 malignant tumours in controls. Four hepatomas were reported (Steinhoff and Grundmann 1970).

2.8 Reproductive toxicity

There is only one study in which pregnant animals were treated with 4,4'-diaminodiphenylmethane: a study of proliferative liver and gall bladder changes in new-born animals, in which five Wistar rats were given 4,4'-diaminodiphenylmethane hydrochloride in doses of 300 mg/kg per day by gavage from day 7 to day 20 of gestation and ten others 50 mg/kg from day 14 to day 20 of gestation. On day 21 of gestation the dams and foetuses were killed and the livers examined. In the first series, one dam contained six abiotrophic and abnormal pups; maternal toxicity was either not seen or not described. In the dams of the second series, liver discoloration was evident and the histological examination revealed proliferation in the bile ducts and in the periportal region, in the latter with initial signs of fibrosis. In the foetal livers, the whole liver parenchyma was so altered by fatty Infiltration that it was difficult to distinguish the bile ducts and portal region (Bourdelat *et al* 1983).

These findings provided no evidence for a teratogenic potential of 4,4'-diaminodiphenylmethane. Teratogenicity studies, which meet present-day requirements, are not available.

3. Recommendation

4,4'-Diaminodiphenylmethane is hepatotoxic and nephrotoxic, owing to its metabolism to biologically reactive intermediates. Its methaemoglobinaemia-forming potency is species-dependent. In man, 4,4'-diaminodiphenylmethane is a potent contact allergen, and there are indications that it can cause photosensitisation (DFG 1996).

4,4'-Diaminodiphenylmethane is mutagenic in the Ames test after metabolic activation and produces DNA damage. It is carcinogenic in animal studies, as tested by oral administration in rats, mice and dogs. Treatment-related increases in the incidence of thyroid follicular-cell adenomas and hepatocellular neoplasms were observed in both male and female mice. In rats, treatment-related increases in the incidences of thyroid follicular-cell carcinomas and hepatic nodules were observed in males, and thyroid follicular-cell adenomas occurred in females (IARC 1986). Experimentally, the minimal carcinogenic daily dose of 4,4'-diaminodiphenylmethane was 18 mg/kg (Weisburger *et al* 1984, Lamb *et al* 1986). There are no adequate epidemiological studies to support a carcinogenicity in humans, although the compound is being regarded as a possible human bladder carcinogen, based on animal experiments and by analogy to other aromatic amines.

Because of the experimentally proven carcinogenicity and genotoxicity 4,4'-diaminodiphenylmethane is grouped into the *SCOEL carcinogen group A* as a non-

threshold genotoxic carcinogen. Accordingly, the derivation of a health-based OEL is not possible.

For practical purposes, risk evaluations have been published using a pragmatic margin-of-exposure approach, to which reference can be made (Lewandowski *et al* 2005, as well as Bos *et al* 1998 and Brouwer *et al* 1998 for a dermal exposure model). Reference may also be made to a recent risk assessment of BAuA (2010).

Based on the proven skin permeability for 4,4'-diaminodiphenylmethane, a *skin notation* is required. The ease of skin penetration under practical workplace conditions argues in favour of application of biological monitoring methods.

Methods of biological monitoring analyse the urinary excretion of 4,4'-diaminodiphenylmethane (after acid hydrolysis of the conjugates), or the detection of specific adducts to haemoglobin (see 2.1.1). Although the latter method has the potential advantage to indicate specific exposures over a much longer period of time, published industrial experience with this method is very limited. For the urinalysis of (conjugated) 4,4'-diaminodiphenylmethane, reliable analytical methods have been evaluated for years, and tolerable biological exposure values based on technical feasibility have been recommended or issued in different EU countries (see 2.1.1). In general, these values show a decreasing trend with the date of issue; these may serve as a practical guidance.

Using HFBA derivatisation, gas chromatography and ECD detection an analytical detection limit of 1 µg/L urine has been evaluated (DFG 1994a). Any excretion of 4,4'-diaminodiphenylmethane above the detection limit is indicative of an external exposure, as the compound does not occur naturally or as an environmental pollutant (see 2.1.1). *On this basis, a Biological Guidance Value (BGV) can be recommended that is equivalent to the analytical detection limit of 1 µg/L urine.*

4. References

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