4,4’-Methylene bis (2-chloroaniline)

Health-based recommendation on occupational exposure limits

To: the State Secretary of Social Affairs and Employment
No. 2018/25, The Hague, December 7, 2018

Health Council of the Netherlands

contents

samenvatting

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid (SZW) heeft de Gezondheidsraad gezondheidskundige advieswaarden afgeleid voor de beroepsmatige blootstelling aan de kankerverwekkende stof 4,4’-methyleenbis(2-chlooraniline). Dit advies is tot stand gekomen in de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS). Op www.gezondheidsraad.nl staat meer informatie over de taken van deze vaste commissie van de Gezondheidsraad.
De samenstelling van de commissie is te vinden achterin dit advies.

Gebruik van 4,4’-methyleenbis(2-chlooraniline)

4,4’-Methyleenbis(2-chlooraniline) wordt hoofdzakelijk gebruikt als verhardingsmiddel voor polyurethaan prepolymeren bij het vervaardigen van producten van gietbare urethaanrubber. Vooral mensen die werkzaam zijn in de kunststof- en rubberindustrie kunnen ermee in aanraking komen.

Gezondheidskundige advieswaarden op basis van extra risico op kanker

Voor kankerverwekkende stoffen die geclassificeerd zijn in categorie 1A of 1B en directe schade aan het DNA veroorzaken (stochastisch genotoxisch werkingsmechanisme) kan geen blootstellingsniveau worden afgeleid waar onder geen kankerverwekkende effecten kunnen optreden. Om voor deze stoffen toch een grenswaarde te kunnen bepalen, heeft de minister van SZW risiconiveaus vastgelegd. Deze risiconiveaus betreffen het extra risico op kanker door beroepsmatige blootstelling gedurende het arbeidzame leven. Als advieswaarden schat de commissie de concentraties van een stof in de lucht die overeenkomen met die risiconiveaus, uitgaande van 40 jaar beroepsmatige blootstelling.

Streefrisiconiveau en verbodsrisiconiveau

Het streefrisiconiveau is 4 op 100.000.
Dat betekent dat tot en met 4 extra gevallen op 100.000 beroepsmatig blootgestelde mensen geen extra beschermende maatregelen genomen hoeven te worden. Het verbodsrisiconiveau is 4 op 1.000. Dat betekent dat het niveau van 4 extra gevallen op 1.000 beroepsmatig blootgestelde mensen niet overschreden mag worden.

Geraadpleegde onderzoeken

Er zijn geen onderzoeken beschikbaar met blootstelling aan 4,4’-methyleenbis(2-chlooraniline) en het optreden van kanker bij de mens die geschikt zijn voor het afleiden van gezondheidskundige advieswaarden. Er zijn verschillende dieronderzoeken gedaan naar het optreden van kanker door blootstelling aan 4,4’-methyleenbis(2-chlooraniline).
De commissie heeft deze onderzoeken

beoordeeld en de meest geschikte geselecteerd. In dat onderzoek werden ratten levenslang blootgesteld aan 4,4’-methyleenbis (2-chlooraniline) via het voer en kregen ze verschillende soorten tumoren. Het aantal longtumoren is door de commissie gebruikt voor het afleiden van de gezondheidskundige advieswaarden.

Advies aan de staatssecretaris

De commissie schat de concentratie van 4,4’-methyleenbis(2-chlooraniline) in de lucht die samenhangt met een extra kans op kanker van 4 per 100.000 (het streefrisiconiveau) gelijk aan 0,026 milligram (mg)/per kubieke meter lucht (m^3). Een extra risico op kanker van 4 per 1.000 (het verbodsrisiconiveau) komt overeen met een concentratie van 2,6 mg/m^3. Beide schattingen gaan uit van een 40 jaar beroepsmatige blootstelling.

Verder adviseert de commissie om een huidnotatie (H-aanduiding) toe te passen voor 4,4’-methyleenbis(2-chlooraniline) omdat huidopname van deze stof substantieel kan bijdragen aan het risico op kanker.

executive summary

At the request of the Ministry of Social Affairs and Employment, the Health Council of the Netherlands has derived health-based advisory values for 4,4’-methylene bis (2-chloroaniline). This advisory report has been composed by the Dutch Expert Committee on Occupational Safety (DECOS). More information on the tasks of this permanent committee of the Health Council of the Netherlands can be found at www.gezondheidsraad.nl. The members of the Committee are listed on the last page of this report.

Use of 4,4’-methylene bis (2-chloroaniline)

4,4’-Methylene bis (2-chloroaniline) is primarily used as a curing agent for polyurethane pre-polymers in the manufacture of castable urethane rubber products. Particularly workers in the plastic and rubber industry can be exposed to this substance.

Advisory values based on extra risk of cancer

For carcinogenic substances that have been classified in category 1A or 1B and directly interact with DNA (stochastic genotoxic mechanism), no exposure level can be derived below which no carcinogenic effects can occur. To be able to set occupational exposure limits for these substances, the Minister of Social Affairs and Employment has determined risk levels. These risk levels relate to the extra risk of cancer due to lifetime occupational exposure. As advisory values, the Committee estimates the concentrations in the air that correspond to these risk levels, taking into account 40 years of occupational exposure.

Target risk level and prohibitive risk level

The target risk level is 4 per 100,000. This means that for concentrations leading up to 4 extra cancer cases per 100,000 occupationally exposed people, no additional protective measures need to be taken. The prohibitive risk level is 4 per 1,000. This means that the concentration leading to 4 extra cancer cases per 1,000 occupationally exposed people, must not be exceeded.

Consulted research

There are no studies in humans available on exposure to 4,4’-methylene bis (2-chloroaniline) and the occurrence of cancer that are suitable for deriving health-based advisory values. There are different animal carcinogenicity studies available. The Committee has evaluated these studies and selected the most suitable one. In this study, rats exposed to 4,4’-methylene bis (2-chloroaniline) via feed during lifetime developed different types of tumours. The number of lung tumours has been used by the Committee to derive the health-based advisory values.

Recommendation to the State Secretary

The Committee estimates the concentration of 4,4’-methylene bis (2-chloroaniline) in the air that corresponds to an extra cancer risk of 4 per 100,000 (the target risk level) equal to 0.026 milligram (mg)/per cubic metre air (m^3). An extra risk of cancer of 4 per 1,000 (the prohibitive risk level) corresponds to a concentration of 2.6 mg/m^3. Both estimates are based on 40 years of occupational exposure.

In addition, the Committee recommends to apply a skin notation for 4,4’-methylene bis (2-chloroaniline) because skin absorption can contribute substantially to the risk of cancer.

01 scope

1.1 Background

At the request of the minister of Social Affairs and Employment, the Dutch expert Committee on Occupational Safety (DECOS), a committee of the Health Council of the Netherlands, performs scientific evaluations on the toxicity of substances that are used in the workplace. The purpose of these evaluations is to recommend health-based occupational exposure limits, which specify levels of exposure to airborne substances, at or below which it may be reasonably expected that there is no risk of adverse health effects.

For carcinogenic substances that directly interact with DNA (stochastic genotoxic mechanism), no exposure level can be derived below which no carcinogenic effects can occur. To be able to set a limit value for these substances, the Minister of Social Affairs and Employment has determined risk levels. These risk levels relate to the extra risk of cancer due to life time occupational exposure. The target risk level is 4 per 100,000. This means that for concentrations leading up to 4 extra cancer cases per 100,000 occupationally exposed people, no additional measures need to be taken. The prohibitive risk level is 4 per 1,000. This means that the concentration leading to 4 extra cancer cases per 1,000 occupationally exposed people, must not be exceeded.

DECOS estimates the concentrations in the air that correspond to these risk levels, taking into a 40-year occupational exposure. The Committee calculates risk-based advisory values for compounds which are classified by the European Commission or by the Committee as carcinogens in category 1A or 1B.

For the establishment of the risk-based advisory values, the Committee generally uses a linear extrapolation method, as described in the Committee’s reports *Calculating cancer risk* and *Guideline for the calculation of occupational cancer risk values*.[1,2] The linear model to calculate occupational cancer risk is used as a default method, unless scientific data would indicate that using this model is not appropriate.

In the next phase of the three-step procedure, the Social and Economic Council advises the Minister of Social Affairs and Employment on the feasibility of implementing a statutory occupational exposure limit at the target level. In the final step of the procedure, the Minister sets the statutory occupational exposure limit.

1.2 Committee and procedure

The present document contains the evaluation of the DECOS, hereafter called the Committee. The members of the Committee are listed at the end of this report.

In 2018, the president of the Health Council released a draft of the report for public review. The Committee has taken the comments received into account in deciding on the final version of the report. These comments, and the reply by the Committee, can be found on the website of the Health Council.

1.3 Data

The Committee’s recommendation has been based on scientific data which are publicly available. Data were obtained from the online databases Toxline and Medline, using carcino*, cancer, neoplastic, 4,4’-methylene bis (2-chloroaniline) and CAS registry number as key words.

In addition, reviews from the following organisations were consulted:

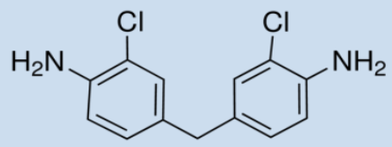
- Health Council of The Netherlands[3]
- European Scientific Committee on Occupational Exposure Limits (SCOEL)[4]
- International Agency for Research on Cancer (IARC)[5-7]
- Agency for Toxic Substances and Disease Registry (ATSDR)[8]
- National Toxicology Program (NTP)[9].

The last literature search was performed in April 2018.

02
identity, toxicity profile and classification

2.1 Uses, identity and physical and chemical properties

4,4'-Methylene bis (2-chloroaniline) is used for the production of polyurethane pre-polymers in the manufacture of castable rubber products. More information on the industrial uses, process and product categories, sectors of end use and environmental release categories of 4,4'-methylene bis (2-chloroaniline) is available in the disseminated registration dossier of this substance on the ECHA website.[10] Physico-chemical data shown below are derived from the Hazardous Substances Data Bank (HSDB) and IARC.[5,6,11]

Chemical name	:	4'4-Methylene bis (2-chloroaniline)
CAS number	:	101-14-4
EC number	:	202-918-9
IUPAC name	:	4-[(4-Amino-3-chlorophenyl)methyl]-2-chloroaniline
Synonyms	:	MOCA, MBOCA, 4,4'-Methylenebis(o-chloroaniline; 2,2'-dichloro-4,4'-methylenedianiline, 3,3'-dichloro-4,4'-diamino-diphenylmethane; methylene-bis-ortho-chloroaniline
Physical description and colour	:	solid, colourless crystals
Molecular formula	:	$C_{13}H_{12}Cl_2N_2$
Structure	:	
Molecular weight	:	267.15
Melting point	:	110 °C
Boiling point (101.3 kPa)	:	378.9 °C
Density	:	1,440 kg/m^3
Solubility	:	Slightly soluble in water (13.9 mg/l); very soluble in benzene, diethyl ether and ethanol; soluble in (g/100 ml): trichloroethylene (4.2), toluene (7.5), ethoxyethyl acetate (34.4), methyl ethyl ketone (43.0), tetrahydrofuran (55.5), dimethylformamide (61.7), and dimethyl sulfoxide (75.0)
Octanol/water partition coefficient, Log $P_{oct/w}$	:	3.91
Vapour pressure (60°C)	:	1.3 x 10^{-5} mmHg (1.7 mPa)
Relative vapour density (air = 1)	:	No data
Flash point	:	No data
Odour threshold	:	No data
Conversion factor (25 °C, 101.3 kPa, mg/m^3 = molecular weight / 24.45 * ppm)	:	1 mg/m^3 = 0.090 ppm 1 ppm = 10.9 mg/m^3
EU Harmonised Classification & Labelling (EC No 1272/2008 of 16 December 2008)	:	Acute Tox 4: H302; Carc. 1B: H350

2.2 Classification as a carcinogenic substance

The European Commission has classified 4,4'-methylene bis (2-chloroaniline) as a Category 1B carcinogen (presumed to have carcinogenic potential for humans). In the Netherlands, 4,4'-methylene bis (2-chloroaniline) is included on the SZW-list of carcinogenic substances and processes, which involves substances classified as category 1A or 1B carcinogen and substances considered as carcinogenic by the Health Council of the Netherlands. IARC has classified 4,4'-methylene bis (2-chloroaniline) as a Group 1 carcinogen (Carcinogenic to humans).[7]

2.3 Toxicity profile

2.3.1 Humans

Information on acute toxicity in humans was available from two cases of accidental occupational exposure. A worker which was accidentally sprayed with hot liquid 4,4’-methylene bis (2-chloroaniline) over the face (with some of the substance entering his mouth) complained about burning of the eyes and face and feeling ill in the stomach, and he was diagnosed with conjunctivitis in both eyes. Urinalysis suggested decreased renal tubular protein reabsorption, and possible transient damage to the renal tubules.[12] Another worker was sprayed accidentally with molten 4,4’-methylene bis (2-chloroaniline) on his chest, abdomen and extremities. He showed slight erythema and complained of a burning sensation of the affected skin.[13] The level of exposure in these two cases was not known.

2.3.2 Animals

Information on acute toxicity, skin and eye irritation/corrosion, skin sensitisation, sub-chronic repeated-dose toxicity and reproduction toxicity was obtained from the disseminated registration dossier of 4,4’-methylene bis (2-chloroaniline) on the ECHA website.[10] These recent studies (reported in 2005 or 2010) were conducted according to OECD and/or EU guidelines and Good Laboratory Practice (GLP). The reviews of ATSDR and IARC provided limited information on non-neoplastic toxic effects observed in carcinogenicity studies.

Acute toxicity

Following a single oral dose of 2,000 mg/kg bw, one out of three female Sprague-Dawley rats died two days after dosing. Clinical symptoms included red discolouration of ear auricles and limbs, and changes in movement and respiration. These symptoms disappeared within two days. Necropsy of the dead animal revealed white foci in the liver, dark red adrenals, dark red foci in the stomach, and dark red contents in the small intestines. At 300 and 2,000 mg/kg bw, reduced growth or weight loss occurred during the first few days after dosing. Wistar rats (5/sex) were exposed dermally to 4,4’-methylene bis (2-chloroaniline) at 2,000 mg/kg bw for 24 hours (occlusive application of a 20% w/w formulation in propylene glycol at the back; area approximately 25 and 18 cm^2 in males and females, respectively). All rats survived and showed normal growth during the 14-day observation period. These two studies indicate low acute toxicity of 4,4’-methylene bis (2-chloroaniline) (LD_{50} approximately 2,000 mg/kg bw for oral exposure and higher than 2,000 mg/kg bw for dermal exposure).

Skin and eye irritation and corrosion

In vitro, in a human epidermis model (EU guideline B. 46) 4,4’-methylene bis (2-chloroaniline) (10 mg, ground and moistened with 5 µL water) was

non-irritant to skin. The results of a bovine corneal opacity and permeability test (OECD guideline 437) showed that 4,4’-methylene bis (2-chloroaniline) (applied as a 20% w/w formulation in physiological saline, 357-381 mg per cornea) was not severely irritant or corrosive to the eye.

Skin sensitisation

4,4’-Methylene bis (2-chloroaniline) was negative in a mouse local lymph node assay (OECD guideline 429) in which the substance was tested at concentrations of 10, 25 and 50% in dimethylformamide.

Repeated-dose toxicity

The toxicity of 4,4’-methylene bis (2-chloroaniline) after sub-chronic repeated exposure was examined in a combined repeated dose toxicity study with reproduction/ developmental toxicity screening test (OECD guideline 422). Sprague-Dawley rats (12/sex/dose) were treated with 0, 0.4, 2, 10 or 50 mg/kg bw of 4,4’-methylene bis (2-chloroaniline) dissolved in olive oil, once daily during 42 days (males) or up to 4 days after delivery (females) by oral gavage. No adverse health effects were observed up to a dose of 2 mg/kg bw. Adverse effects at 10 mg/kg bw included: decreased plasma concentrations of total protein and albumin in female rats; increased relative kidney weight in female rats; and slight histopathological alterations in the kidneys (increased incidence and severity of basophilic change of renal tubules) and spleen (increased severity, from mild to moderate, of hemosiderin deposits) in male rats. At 50 mg/kg bw, the female rats had decreased weight gain during gestation with decreased body weights on gestation days 14 and 20. Haematology showed decreased haemoglobin concentration and haematocrit value and increased reticulocyte and platelet count in male rats, increased Heinz bodies-containing erythrocytes in female rats, and decreased erythrocyte count and methaemoglobin in both sexes at 50 mg/kg bw. Clinical chemistry changes at 50 mg/kg bw included: decreased total protein and albumin in both sexes; increased total cholesterol, triglycerides and inorganic phosphorus in male rats; and increased lactate dehydrogenase and gamma-glutamyltransferase activity in both sexes, most pronounced in females. Organ weight changes at 50 mg/kg bw consisted of increased liver and spleen weights in both sexes, and increased kidney and thyroid weights in females. Treatment-related histopathological changes at 50 mg/kg bw occurred in the liver, kidneys and spleen. Hepatic changes consisted of swelling and fatty degeneration of hepatocytes in rats of both sexes, and single cell necrosis of hepatocytes in male rats. In the kidneys basophilic change of renal tubules occurred at increased incidence and severity in male rats. Changes in the spleen (red pulp) consisted of increases in the severity (from mild to moderate) of hemosiderin deposits in both sexes, and extramedullary haematopoiesis in female rats.

Information on non-cancer effects upon chronic exposure to 4,4’-methylene bis (2-chloroaniline) is available from the carcinogenicity studies evaluated by the Committee (see Chapter 3 and Table in Annex

C). In several of these studies 4,4’-methylene bis (2-chloroaniline) adversely affected survival, growth and liver. In a feeding study by Kommineni et al. (1979)[14], rats were exposed to approximately 40 mg/kg bw/day for 18 months. Compared to controls, they gained markedly less weight and had slightly lower levels of haemoglobin and haematocrit. In a lifespan study by Russfield *et al*. (1975)[15], rats given 4,4’-methylene bis (2-chloroaniline) via their diet had approximately 6% (20 mg/kg bw/day) or 13% (40 mg/kg bw/day) lower mean body weights than controls at the end of the 18-month treatment period. These differences persisted to the time of necropsy. Liver changes were observed by Stula *et al.* (1975)[16] in rats fed 4,4’-methylene bis (2-chloroaniline) at 40 mg/kg bw/day for 2 years, and included hepatocytomegaly, fatty change, necrosis, fibrosis and bile duct proliferation (incidences of these findings were not reported). Liver changes were also seen in dogs treated orally (capsules) with approximately 10 mg/kg bw/day for up to 9 years. Histopathologic examination revealed nodular hepatic hyperplasia (distorted liver architecture; sharp demarcation of nodule from surrounding liver) in three of the six treated dogs, but not in controls. Clinical chemistry revealed statistically significantly higher activity of glutamic pyruvic transaminase in blood of treated dogs compared with controls. In addition, treated dogs had increased numbers of erythrocytes, leukocytes and epithelial cells in the urine sediment (some epithelial cells showed changes suggestive of neoplasia in the genitourinary tract).

Reproduction toxicity

4,4’-Methylene bis (2-chloroaniline) is not classified for reproduction toxicity. The results of the above noted combined repeated dose toxicity study with reproduction/developmental toxicity screening test in rats did not show changes indicative of reproduction or developmental toxicity.

2.3.3 Genotoxicity studies

Studies investigating the genotoxicity of 4,4’-methylene bis (2-chloroaniline) have been summarised by the International Agency for Research on Cancer (IARC)[5,6], the Agency for Toxic Substances and Disease Registry (ATSDR)[8] and by McQueen et al. (1990)[17]. A short compilation of their reviews is given below.

The reviews show that 4,4’-methylene bis (2-chloroaniline) is mutagenic in the *Salmonella typhimurium* strains TA98 and TA100, and at the *Tk* locus in mouse lymphoma L5178Y cells, after metabolic activation. Furthermore, unscheduled DNA synthesis was induced in HeLa cells[18] and in primary hepatocytes from rats, mice, hamsters, and rabbits[19]. In vivo, the substance-induced sister chromatid exchange in rat lymphocytes[20], mutations in Drosophila melanogaster[21], and micronuclei in the bone marrow of mice[22]. Increased frequencies of sister chromatid exchanges in peripheral lymphocytes[20], and of micronuclei in peripheral lymphocytes and exfoliated urothelial cells[23,24], were observed in small groups of workers exposed to 4,4’-methylene bis (2-chloroaniline).

4,4'-Methylene bis (2-chloroaniline) induced DNA adducts in the liver, lung and kidney of rats following a single oral dose[25], in the liver and bladder epithelium of dogs after a single or multiple doses[26], and in cultured canine and human urinary bladder cells[27]. DNA adducts were also detected in exfoliated urothelial cells present in urine samples obtained from a worker between 4 and 98 hours after his accidental exposure to 4,4'-methylene bis (2-chloroaniline).[13,28]

The Committee noticed that the above genotoxicity data are from studies published in the open literature and predate modern test guidelines and GLP. There are no publicly available genotoxicity studies conducted according to OECD guidelines. Based on the available data and in consultation with the Health Council's Subcommittee on the Classification of carcinogenic substances, the Committee considers 4,4'-methylene bis (2-chloroaniline) to be a carcinogen acting by a stochastic genotoxic mechanism.

The SCOEL considers 4,4'-methylene bis (2-chloroaniline) as a Group A carcinogen (non-threshold genotoxic carcinogen).[4]

2.4 Existing occupational exposure limits

Table 1 summarizes the occupational exposure limits established by national and international regulatory authorities.

Table 1. Occupational exposure limits of 4,4'-methylene bis (2-chloroaniline)[a]

Country (Organisation)	OEL (ppm)	OEL (mg/m3)	Time-weighted average	Type of exposure limita	Skin notation
The Netherlands	-	0.02	8h	OEL	yes
European Commission	-	-	-	-	yes
Germany (DFG)	-	-	-	-	yes
Germany (AGS)	-	-	-	-	-
UK (HSE)	-	0.005	8h	WEL	yes
Denmark	0.01	0.11	8h	OEL	yes
Sweden	-	-	-	-	-
USA (NIOSH)	-	0.003	-	REL	yes

Abbreviations: OEL, occupational exposure limit; WEL, workplace exposure limit; REL, recommended exposure limit.

[a] Sources: SER OEL database (https://www.ser.nl/en/oel_database.aspx); GESTIS International Limit Values (http://limitvalue.ifa.dguv.de/) [accessed October, 2018]

The SCOEL evaluated the carcinogenicity of 4,4'-methylene bis (2-chloroaniline) and concluded that this substance is a genotoxic carcinogen to which a threshold cannot be assigned. Therefore, the SCOEL did not assign a health-based occupational exposure limit to 4,4'-methylene bis (2-chloroaniline).[4]

03
carcinogenicity studies

3.1 Human studies

The Committee has identified four epidemiological studies with 4,4’-methylene bis (2-chloroaniline). In Annex A these studies have been summarised.

In a retrospective cohort study, Dost *et a*l. (2009)[29] compared the mortality and cancer incidence in 308 male production workers from seven factories, manufacturing polyurethane elastomers using 4,4’-methylene bis (2-chloroaniline), with expected values based on national rates. All workers were exposed to 4,4’-methylene bis (2-chloroaniline) for at least 12 months. Overall cancer mortality and incidence were below average but there was a single death from bladder cancer, and a non-significant excess of malignant bladder cancer based on two cases only.

In a retrospective cohort study by Chen *et al*. (2005)[30], 76 workers potentially exposed to 4,4’-methylene bis (2-chloroaniline) and another 92 non-exposed workers of four Taiwanese factories were recruited for bladder cancer screening. Among the 70 exposed workers who participated in the screening program, one proven bladder carcinoma was observed. In addition, one worker with suspected malignant cells on urine cytology (suspected bladder cancer), and one worker with atypical cytology combined with gross haematuria were identified. Both workers refused additional cystoscopic examination.

Ward *et al*. (1988, 1990)[31,32] conducted a bladder cancer incidence study (retrospective cohort, no control group) among approximately 540 workers exposed to 4,4’-methylene bis (2-chloroaniline). Three cases with non-invasive papillary tumours of the bladder were identified, two in non-smoking men aged 28-29 years and one in a 44-year old ex-smoker.

In a retrospective cohort study by Linch *et al*. (1971)[33], 31 workers who had been exposed to 4,4’-methylene bis (2-chloroaniline) for between 6 months and 16 years and 31 controls were recruited for urine cytology. No deaths and no malignancies were observed in exposed and non-exposed individuals.

The studies described above have significant limitations in design and or reporting (see Annex A), including: lack of information on exposure levels and/or duration; lack of control group; small number of participants; possible exposure to other carcinogens. Therefore, the Committee concludes that these studies are inadequate to evaluate the relationship between human cancer and exposure to 4,4’-methylene bis (2-chloroaniline).

3.2 Animal experiments

Annex B summarises the available carcinogenicity studies in animals. These studies comprise eight oral studies: six performed with rats, one with mice, and one with dogs. Furthermore, one study in rats with subcutaneous injection is available. No long-term inhalation or dermal studies were available. In three of the oral rat studies, a protein-deficient diet was used. Grundmann and Steinhoff (1970)[34] used a protein-deficient diet on account of findings in older studies, which showed delay or prevention of the appearance of liver tumours in rats fed azo-compounds

in protein-adequate diet compared with rats fed diet deficient in protein and other nutrients. Thereafter, Kommineni *et al*. (1979)[14] and Stula *et al*. (1975)[16] examined the carcinogenicity of 4,4’-methylene bis (2-chloroaniline) in rats kept on either protein-deficient diet or normal diet adequate in protein. The studies in rats administered 4,4’-methylene bis (2-chloroaniline) via protein-deficient diet or subcutaneously are not relevant for derivation of occupational cancer risk values due to the unphysiological testing conditions or use of an irrelevant exposure route. The five remaining oral studies are described below.

In a well-performed study by Kommineni *et al*. (1979)[14], male Sprague-Dawley rats received protein-adequate diet containing 4,4’-methylene bis (2-chloroaniline) at 0, 250, 500 or 1,000 mg/kg diet (100, 100, 75 and 50 rats per group, respectively). These dietary concentrations were equivalent to 10, 20 and 40 mg/kg bw/day, respectively (based on a default food intake value of 40 g/kg bw/day). The rats were fed the substance for 18 months, after which they were maintained on their respective diet without the substance for 6 months. Survival was statistically significantly lower at 20 and 40 mg/kg bw/day compared with controls. Body weight gain was markedly lower at 40 mg/kg bw/day compared with controls. 4,4’-methylene bis (2-chloroaniline) induced lung adenocarcinomas, mammary adenocarcinomas, Zymbal gland carcinomas and hepatocellular carcinomas. The incidences of the lung tumours were statistically significantly increased at 10, 20 and 40 mg/kg bw/day and showed a dose-related response. The tumours in the other organs occurred at lower incidences than those in the lung.

In a well-performed study by Stula *et al*. (1971, 1975)[16,35], male and female Charles River CD (SD) rats (50/sex/group) received normal diet (23% protein) containing 0 or 1,000 mg of 4,4’-methylene bis (2-chloroaniline) per kg diet for up to two years. These dietary concentrations were equivalent to 40 and 50 mg/kg bw/day in male and female rats, respectively (based on default food intake values of 40 and 50 g/kg body weight/day in male and female rats, respectively). Lifespan was shortened in the rats exposed to 4,4’-methylene bis (2-chloroaniline). The substance-induced lung adenomatosis (preneoplastic lesion progressing to adenocarcinomas) and lung adenocarcinomas in many male and female rats. The incidences of these lung tumours in treated male and female rats were statistically significantly higher compared with controls. Squamous-cell carcinoma of the lung, pleural biphasic tumours, hepatocellular adenomas and hepatocellular carcinomas were observed in one or a few treated male and female rats but not in controls.

In a supportive study by Russfield *et al*. (1975)[15], male Charles River CD-1 rats (25/group) received normal diet containing the hydrochloride salt of 4,4’-methylene bis (2-chloroaniline) at 500 or 1,000 mg/kg diet for 18 months, and then kept untreated for 6 months. These dietary concentrations were equivalent to 20 and 40 mg/kg bw/day, respectively (based on a default food intake value of 40 g/kg bw/day). Survival of treated rats did not differ greatly from that of controls. Mean body weights

were slightly lower compared with controls (on average up to 13% at 40 mg/kg bw/day at 18 months and thereafter). Hepatomas (without malignancy features) and lung adenomatosis were seen in several treated rats, most frequently at the highest dose level, but in none of the controls (differences were not statistically significant).

Stula *et al*. (1978)[36] treated female beagle dogs (six/dose) with 0 or 100 mg of 4,4’-methylene bis (2-chloroaniline) by capsule (orally), 3 or 5 days/ week, for up to nine years (average dose 8-15 mg/kg bw/day). The substance-induced urinary bladder papillary transitional cell carcinoma in four of the six treated dogs. Another treated dog developed a composite tumour (transitional cell carcinoma/adenocarcinoma) in the urethra. The sixth treated dog had no tumours (this dog died after 3.4 years due to causes not related to treatment with 4,4’-methylene bis (2-chloroaniline)).

In a study by Russfield *et al*. (1975)[15], HaM/ICR mice (25/sex/group) were exposed to the hydrochloride salt of 4,4’-methylene bis (2-chloroaniline) at 1,000 or 2,000 mg/kg diet for 18 months, and then kept untreated for 6 months. These dietary concentrations were equivalent to 120 and 240 mg/kg bw/day in males and 130 and 260 mg/kg bw/day in females (based on default food intake values of 120 and 130 g/kg bw/day in male and female mice, respectively). Compared with controls, the incidence of hepatomas was statistically significantly increased in female mice at 130 and 260 mg/kg bw/day. Malignancy features were seen in hepatomas of some treated mice. Additionally, the incidence of vascular tumours (generally subcutaneous haemangiomas and haemangiosarcomas) was higher (not statistically significantly) in treated male and female mice than in concurrent controls. As the incidence of vascular tumours in treated mice was in the historical control range, the occurrence of these tumours cannot be attributed unequivocally to the treatment with 4,4’-methylene bis (2-chloroaniline).

In conclusion, of the eight available oral carcinogenicity studies in animals, three were considered not relevant for derivation of occupational cancer risk values due to unphysiological testing conditions (protein-deficient diet). The well-performed rat feeding study by Kommineni et al. (1979)[14] was considered most suitable for assessment of cancer risk (see next section). The other oral studies were less adequate for this purpose due to limitations in design (only one dose level tested and/or low number of animals), but provided supportive evidence that 4,4’-methylene bis (2-chloroaniline) is carcinogenic in experimental animals.

3.3 Selection of the suitable study for risk estimation in the occupational situation

The Committee considers the study by Kommineni et al. (1979)[14] in male Sprague-Dawley rats, kept on a normal protein-adequate diet, to be the most suitable study available for estimation of the potential cancer risk in humans under occupational exposure conditions. The study is well performed, the exposure period covered the largest part of the standard lifespan of the experimental animals, group sizes are adequate, and

sufficient dose levels have been tested. The study is adequate to use the benchmark dose method for estimation of a starting point for quantitative risk estimation. Limitations of this study are that individual animal data are not reported and only one sex has been tested. The incidence of lung adenocarcinomas was selected as basis for cancer risk derivation since this type of tumour is relevant for humans, and its incidence was dose-dependently and statistically significantly increased from the lowest dose level tested (250 mg/kg diet, equivalent to 10 mg/kg bw/day). The incidences of the other malignant tumours induced by 4,4’-methylene bis (2-chloroaniline) in this study were increased to a lesser extent and did not reach statistical significance at all dose levels tested.

In its previous evaluation of 4,4’-methylene bis (2-chloroaniline) (published in 2000)[3], the Committee calculated cancer risk values (0.02 and 2 mg/m^3 for an extra cancer risk of 4 per 100,000 and 4 per 1,000, respectively) on the basis of the incidence of the number of rats (males and females combined) with malignant tumours in the oral carcinogenicity study of Grundmann and Steinhoff (1970)[34]. This study was selected because it yielded the highest tumour incidence per unit daily dose (calculations were also made for three other studies that were considered suitable, namely: the rat studies of Stula et al. (1975)[16] and Kommineni et al.(1979)[14], and the mouse study of Russfield et al. (1975)[15]).

In the present re-evaluation, the Committee selected the study of Kommineni et al. (1979)[14] for derivation of cancer risk values because this study is adequate to use the benchmark dose method for estimation of a starting point for quantitative hazard assessment. The study of Grundmann and Steinhoff (1970)[37] included only one (high) dose level and is, therefore, not adequate for the benchmark dose method. Moreover, a protein-deficient diet was used in the study of Grundmann and Steinhoff (1970)[37]. Such unphysiological testing conditions render the study less suitable for derivation of occupational cancer risk values.

As concluded at the end of section 2.3, the Committee considers 4,4’-methylene bis (2-chloroaniline) to be a carcinogen acting by a stochastic genotoxic mechanism.

3.4 Calculation of the health-based occupational cancer risk values

3.4.1 Carcinogenic activity in experimental animals, lifetime exposure

To calculate the carcinogenic activity expressed as the incidence per unit daily dose (mg per kg bw per day) of 4,4’-methylene bis (2-chloroaniline), the number of male rats with lung adenocarcinomas was used as starting point. Since the actual values for daily food or 4,4’-methylene bis (2-chloroaniline) intake per kg body weight are not given in the available publication, the standard value for daily food intake per kg body weight (40 g/kg bw/day for male rats) mentioned in the report of the Health Council of the Netherlands on calculating cancer risk[1] was used to calculate the daily dose of 4,4’-methylene bis (2-chloroaniline). Hence, the dietary levels of

250, 500 and 1,000 mg/kg diet were calculated to provide 10, 20 and 40 mg/kg bw/day, respectively. The Committee prefers the benchmark dose (BMD) method for estimation of the starting point for calculation of the carcinogenic activity. Until recently, the Committee used the BMDS software by U.S. EPA. Here, the Committee decided to switch to the PROAST software, which is developed by the RIVM and made available by EFSA. PROAST provides model averaged BMDL and BMDU values, taking into account various models, from which a weighted BMD can be derived. This analysis takes into account all possible values of the true BMD based on the available data, and is therefore used for calculation of the HB-OCRV. The results of these BMD-analyses and the criteria for model acceptance are given in Annex C.

The cancer incidence per unit daily dose (mg/kg bw/day) (lifespan conditions, assuming a linear concentration-response relationship) is calculated as follows:

$$I_{dose} = \frac{BMR}{BMD \times \frac{Xpo}{L} \times \frac{Xpe}{L} \times \frac{\text{exposure days per week}}{7}} =$$

$$\frac{0.1}{8.3 \times \frac{546}{1000} \times \frac{728}{1000} \times \frac{7}{7}} = 3.0 \times 10^{-2} \text{ per } \frac{mg}{kg\ bw}$$

Where:

- I_{dose}= the carcinogenic activity attributable to the exposure to the substance per unit daily dose expressed per mg/kg bw/day.
- BMR = benchmark response, expressed as an increase in tumour incidence of 10%.
- BMD = benchmark dose (estimate of the daily dose expected to yield the BMR).
- X_{po} and X_{pe} are the exposure and experimental periods, respectively.
- L = standard lifespan for the animals in question (L rat is assumed to be 1,000 days).

3.4.2 Health risk to humans, exposure under occupational conditions

To estimate the additional lifetime risk of cancer in humans on the basis of results in animal experiments, it is assumed that no difference exists between experimental animals and man with respect to toxicokinetics, mechanism of tumour induction, target, susceptibility etc. Furthermore, it is assumed that the average man lives 75 years, weighs 70 kg and is exposed 24 hours per day, 7 days per week, 52 weeks per year for lifetime. To estimate the additional lifetime risk of cancer in humans under workplace exposure conditions it is further assumed that the average worker is exposed 8 hours per day, 5 days per week, 48 weeks per year for 40 years and inhales 10 m^3 air per 8-hour-working day.

Using as starting point the estimated incidence of 3.0 x 10^{-2} per mg/kg bw, the additional lifetime cancer risk per mg/m^3 under occupational exposure conditions (= HBC-OCRV) amounts to:

$$\text{HBC} - \text{OCRV} = 3.0 \times 10^{-2} \times \frac{40\text{y}}{75\text{y}} \times \frac{48\text{w}}{52\text{w}} \times \frac{5\text{d}}{7\text{d}} \times \frac{10\text{m}^3}{70\ \text{kg}} = 1.5 \times 10^{-3}\ \text{per mg/m}^3$$

3.4.3 Occupational cancer risk values

Based on the HBC-OCRV of 1.5 x 10^{-3} per mg/m^3 the Committee estimated that the concentration of 4,4’-methylene bis (2-chloroaniline) in the air, which corresponds to an excess cancer risk of:

- 4 per 1,000 (4x10^{-3}), for 40 years of occupational exposure, equals to 2.6 mg/m^3
- 4 per 100,000 (4x10^{-5}), for 40 years of occupational exposure, equals to 0.026 mg/m^3.

The toxicity data as summarised in this report allows the Committee to conclude that no adverse health effects other than carcinogenicity at the concentration levels associated with the referential cancer risk levels are expected.

3.5 Skin notation

The Committee assessed whether for 4,4’-methylene bis (2-chloroaniline) a skin notation is needed. The purpose of a skin notation is to indicate the need to prevent skin contamination when systemic effects may result from percutaneous absorption of a substance as a gas, a solid, or a liquid. To determine whether a skin notation needs to be applied, the Committee uses the ECETOC document “Strategy for assigning a skin notation”.[38] According to the guidance, a skin notation is warranted when human experience indicates the importance of skin penetration. A skin notation should be applied when exposure of 2,000 cm^2 of skin (both hands and forearms) to 4,4’-methylene bis (2-chloroaniline) during one hour could result in an absorbed amount exceeding 10% of the amount that can be absorbed via the lungs on exposure for eight hours to the HBC-OCRV. Absorption through the skin is considered the major route of uptake of 4,4’-methylene bis (2-chloroaniline) at the workplace.[4] There are no skin absorption studies with 4,4’-methylene bis (2-chloroaniline) that have been conducted according to modern test guidelines. Data on absorption of 4,4’-methylene bis (2-chloroaniline) through human skin is available from two older in vitro studies. These studies used different testing conditions which do not meet modern guideline criteria. Particularly, these studies used aqueous receptor fluids without protein, which in vivo would drive absorption through protein binding. The Committee estimated potentially absorbed amounts of 4,4’-methylene bis (2-chloroaniline) using absorption rates from both studies.

Chin et al (1983) exposed ten fresh, full-thickness human neonatal foreskins to dry 4,4’-methylene bis (2-chloroaniline) (on a coverglass) for four hours, in a static system.[39] He measured penetration rates between

0.27 and 1.29 µg/cm^2 in 4 hours (corresponding to 0.068 and 0.32 µg/cm^2/hour), based on the recovery of radioactivity on the supporting membrane filter below the skin. Recovery in the receptor fluid was negligible. The distribution of radioactivity in the skin and membrane was between 45-73% and 26-53%, respectively, when expressed as proportion of the amount of radioactivity recovered from the skin, membrane plus medium. The latter amount showed large inter-individual variation: values ranged from about 15% to 90% of total recovery (from coverglass, skin, membrane and medium). Based on the absorption rates measured in this study, the uptake of 4,4’-methylene bis (2-chloroaniline) on 2,000 cm^2 per hour ranges between 135 - 645 µg.

Hotchkiss et al. (1993) exposed fresh, full-thickness human breast skin to a solution of 4,4’-methylene bis (2-chloroaniline) in ethanol for 72 hours in flow-through diffusion cells, on four occasions.[40] The absorption rates through unoccluded skin ranged from 0.0031 to 0.041 µg/cm^2/hour, based on the recovery of radioactivity in the receptor fluid at 72 hours. The percentage of absorption through the skin was low. At 72 hours, 1.4-4.7% of the applied dose was recovered from the receptor fluid. Considerable amounts of residual radioactivity remained within the skin (or adhered to it) and on the skin surface (on average 31% and 42%, respectively). Based on the absorption rates measured in this study, the uptake of 4,4’-methylene bis (2-chloroaniline) on 2,000 cm^2 per hour ranges between 6-82 µg.

In both studies, a significant amount of 4,4’-methylene bis (2-chloroaniline) was retained in the skin, which was not accounted for in the skin absorption rate. As 4,4’-methylene bis (2-chloroaniline) is a lipophilic substance, artificial retention of this substance in the skin may occur when using receptor fluids in which it dissolves poorly. In such situations, the OECD guidance recommends to determine total skin absorption based on both skin and receptor fluid levels of the test substance. Neither of the above studies provided data on the solubility of 4,4’-methylene bis (2-chloroaniline) in the receptor fluid. As aqueous receptor fluids were used, and absorption was calculated from receptor fluid or supporting membrane data alone, the absorption rates from these studies may underestimate in vivo absorption.

Assuming that a volume of 10 m^3 is inhaled in eight hours and that a fraction (by default assumed to be 0.5 by ECETOC) of the atmospheric 4,4’-methylene bis (2-chloroaniline) is absorbed by inhalation, the maximum uptake by inhalation upon exposure for eight hours at the HBC-OCRV is

- 2.6 mg/m^3 (HBC-OCRV, 4x10^{-3}) x 10 m^3 x 0.5 = 13 mg (10% hereof is 1,300 µg)
- 0.026 mg/m^3 (HBC-OCRV, 4x10^{-5}) x 10 m^3 x 0.5= 0.13 mg (10% hereof is 13 µg).

Based on the above calculations, the Committee concludes that dermal exposure can considerably contribute to the systemic exposure to 4,4’-methylene bis (2-chloroaniline) and that a skin notation for 4,4’-methylene bis (2-chloroaniline) is required.

3.6 Health-based biological limit value

The Committee is aware that in industrial practice exposure assessment of workers to 4,4’-methylene bis (2-chloroaniline) is routinely carried out by biological monitoring (post-shift measurement in urine) rather than by ambient air monitoring. 4,4’-Methylene bis (2-chloroaniline) has a low vapour pressure and the main route of systemic exposure is dermal penetration.[4,7]

Several regulatory authorities assigned a skin notation to their occupational exposure limit for 4,4’-methylene bis (2-chloroaniline) (see section 2.4). Exposure of workers manufacturing[33] or using[41] 4,4’-methylene bis (2-chloroaniline) was monitored by measuring both personal air exposure levels and urinary excretion. The measured urinary excretion of 4,4’-methylene bis (2-chloroaniline) was much higher than the urinary excretion estimated from the personal air exposure levels, suggesting that a significant amount of 4,4’-methylene bis (2-chloroaniline) is absorbed by other routes than inhalation. This implies that measurement of airborne 4,4’-methylene bis (2-chloroaniline) alone is likely to underestimate systemic exposure to this substance.

Different countries have established biological monitoring guidance values for urinary 4,4’-methylene bis (2-chloroaniline) based on values that can be reached using good working practises at the workplace, but not on health effects (the values below refer to urinary concentrations of total 4,4’-methylene bis (2-chloroaniline) unless indicated otherwise):

- 15 µmol/mol creatinine in Great Britain (Health and Safety Executive)[42]
- 5 µmol/mol creatinine in Finland (Finnish Institute of Occupational Health)[43]
- 100 µg free 4,4’-methylene bis (2-chloroaniline)/L in California (California Occupational Safety and Health Administration)[44]
- 15 µmol/mol creatinine in Australia (Workcover NSW Biological Occupational Exposure Limit (BOEL) Committee)[45]
- 50 µg/g creatinine (about 20 µmol/mol creatinine) in Japan (Japan Society for Occupational Health)[46]
- 5 µmol/mol creatinine recommended by SCOEL[4].

In France, Robert et al. (1999) proposed a biological guiding value of 20 µg sulfamic acid-labile 4,4’-methylene bis (2-chloroaniline)/L (about 30 µg total 4,4’-methylene bis (2-chloroaniline)/L).[47] The ACGIH has listed total 4,4’-methylene bis (2-chloroaniline) in urine as adopted biological exposure determinant, but has refrained from providing a numerical Biological Exposure index due to insufficient data.[4]

Considering the relevance of systemic exposure to 4,4’-methylene bis (2-chloroaniline) via absorption through the skin and the current industrial

practice of worker exposure assessment by biomonitoring, the Committee calculated a health-based biological limit value (BLV) corresponding with the HBC-OCRV of 2.6 mg/m^3 for an additional life time cancer risk of 4x10^{-3} for 40 years of occupational exposure to 4,4’-methylene bis (2-chloroaniline).

Applying the general formula for urinary excretion of a substance in the urine, the cumulative amount of 4,4’-methylene bis (2-chloroaniline), including parent compound and metabolites, excreted at time t is calculated as:

$$U = f \times F \times \{(r \times t) - [(r/k) \times (1-e^{-k \times t})]\}$$

Where:

- U is the cumulative amount of substance excreted into the urine at time t.
- *f* is the fraction of the absorbed amount of total 4,4’-methylene bis (2-chloroaniline) that is excreted into the urine. The value applied was 1.4%, based on the urinary excretion of unchanged 4,4’-methylene bis (2-chloroaniline) in dogs and the ratio unchanged/total 4,4’-methylene bis (2-chloroaniline) in the urine of humans. In dogs 0.54% of systemically available 4,4’-methylene bis (2-chloroaniline) was excreted unchanged into the urine in 24 hours.[48] This value closely resembles that in rats.[49] In workers potentially exposed to 4,4’-methylene bis (2-chloroaniline), the geometric mean urinary concentration of the unchanged compound was 39% of that of total urinary 4,4’-methylene bis (2-chloroaniline).[47] Dividing the dog value of 0.54% for excretion of the unchanged compound by 39% yields a value of 1.4% for the excretion of total 4,4’-methylene bis (2-chloroaniline).
- *F* is the biological availability (by inhalation). A default value of 50% was applied since a value in humans or animals has not been determined.
- *r* is the maximum absorption, which equals the ventilation rate multiplied by the exposure concentration.
- *k* is the elimination constant.
- *t* is the time.

Assuming an hourly ventilation rate of 1.25 m^3 for an operator and an 8-hour working day, the totally inhaled volume per day is 10 m^3. With the average amount of creatinine excreted during the day in the urine denoted by “cr”, A BLV (in mg/g creatinine) can be calculated from the following formula:

BLV = [(f x F)/cr] x {(10 x OEL) – [[(10 x OEL)/(8 x k)] x (1 – e(-8 x k))]} or, considering k equals (ln 2)/t½,

BLV = [(f x F)/cr] x (10 x OEL) x (1 – {[(ln2)/(8 x t½)] x (1 – e[(-8 x (ln2))/ t½])}, with ln 2 = 0.693 and cr = 1.5 g,

BLV = 6.7 x f x F x OEL x {1 – 0.18 x t½ x (1 – e(-5.54/t½))}

Using a urinary half-life of 4,4’-methylene bis (2-chloroaniline) of approximately 23 hours[13], and an OEL of 2.6 mg/m^3, this leads to the following value for the BLV:

BLV = 6.7 x 0.014 x 0.5 x 2.6 x {1 – 0.18 x 23 x (1 - e(-5.54/23))} = 0.014 mg/g creatinine.

The value of 14 µg/g creatinine is equivalent to 6 µmol/mol creatinine (based on molecular weights of 267.15 for 4,4’-methylene bis (2-chloroaniline) and of 113.12 for creatinine.

The calculated BLV of 6 µmol/mol creatinine (corresponding to the HBC-OCRV of 2.6 mg/m^3 for an excess cancer risk of 4 per 1,000) is close to the value of 5 µmol/mol creatinine noted by SCOEL[4] and the Finnish Institute of Occupational Health[43], and somewhat lower than the values established in Great Britain[42], Australia[45] and Japan[46] (15-20 µmol/mol).

3.7 Groups with increased risk

No groups with increased risk were identified by the Committee.

3.8 Conclusions and recommendation

The Committee considers 4,4’-methylene bis (2-chloroaniline) to be a carcinogenic substance acting by a stochastic genotoxic mechanism.
The Committee considers the increased incidence of lung adenocarcinomas in male rats, observed in the study by Kommineni et al. (1979)[14], as the critical effect for cancer hazard quantification.

The Committee estimated that the concentration of 4,4’-methylene bis (2-chloroaniline) in the air, which corresponds to an excess cancer risk of:

- 4 per 1,000 (4x10^{-3}), for 40 years of occupational exposure, equals to 2.6 mg/m^3
- 4 per 100,000 (4x10^{-5}), for 40 years of occupational exposure, equals to 0.026 mg/m^3.

The Committee concludes that applying these exposure values provides sufficient protection against the non-carcinogenic effects of 4,4’-methylene bis (2-chloroaniline). Taking into account an exposure level of 2.6 mg/m^3, the Committee considers a skin notation warranted because dermal penetration may contribute significantly to the body burden of 4,4’-methylene bis (2-chloroaniline).
The Committee calculated a health-based biological limit value based on this exposure level of 2.6 mg/m^3. This value, a concentration of total 4,4’-methylene bis (2-chloroaniline) in the urine, corresponds to a value of 14 µg/g creatinine (6 µmol/mol creatinine).

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annexes

A epidemiological studies

Reference	Study design and population	Data on exposure and health assessment	Results	Remarks
Dost et al. (2009)[29]	Type of study: Retrospective cohort study Country: UK Type of industry: Rubber industry Participants: 308 male production workers using 4,4′-methylene bis (2-chloroaniline) in the manufacture of polyurethane elastomers; first employed between 1973-2000 Control: expected values based on national rates	No information on exposure levels. Duration of exposure: at least 12 months. Mortality rates and cancer registrations of the workers for the period 1979-2007 were compared with expected values, and SMR and SRR were calculated by applying sex-, age- and period-specific mortality rates for UK to corresponding person-years-at-risk Appropriate statistical analysis was performed, but work histories and smoking status were not taken into account	Mortality all causes of death combined: Obs 9, SMR 46 (21-88) Mortality all causes excluding neoplasms: Obs 5, SMR 37 (12-87) Mortality neoplasms: Obs 4, SMR 68 (19-174) Mortality bladder cancer: Obs 1, SMR 560 (14-3122) Cancer incidence all neoplasms: Obs 9, SRR 77 (35-147) Cancer incidence bladder cancer: Obs 2, SRR 328 (40-1184)	Well performed, except size of cohort is small, no data on exposure levels are available, follow-up period may be too short, possible co-exposure to other (bladder) carcinogens not addressed
Chen et al. (2005)[30]	Type of study: Retrospective cohort study Country: Taiwan, China Type of industry: 4,4′-methylene bis (2-chloroaniline) manufacturing Participants: 76 4,4′-methylene bis (2-chloroaniline)-exposed workers Control:92 non-exposed workers from the same factory	Exposure measurement: Air concentration were measured and ranged between less than 0.02 and 0.41 mg/m^3 depending on area in the factory Duration of exposure: unknown Type of examination: Urine occult blood, urine cytology, nuclear matrix proteins, and abdominal ultrasonography in all participants. Intravenous urography cystoscopy, and physical examination as follow-up in workers with positive screening result	One case of proved bladder cancer (transitional cell carcinoma; 14 years exposure, non-smoker, had not worn any personal protective equipment). Additionally, one worker with suspected malignant cells on urine cytology (suspected bladder cancer) and one worker with atypical cytology combined with gross hematuria. Both workers refused additional cystoscopic examination Positive rates for occult blood, matrix proteins and atypical cells were similar in exposed and non-exposed groups	Study contains several flaws. No specific data on exposure levels are available, follow-up period may be too short, no information on duration of exposure, number of participants is small, no statistical analysis performed on tumour incidence, no clear defined study population, possible co-exposure to other (bladder) carcinogens not addressed
Ward et al. (1988, 1990)[31,32]	Type of study: Retrospective cohort study Country: USA Type of industry: 4,4′-methylene bis (2-chloroaniline) manufacturing Participants: 540 exposed workers	No information on exposure levels Duration of exposure: median duration of employment being 3.2 months Follow-up period: maximally 11.5 years Type of examination: Urine cytology and dipstick analysis in all participants, cytoscopy in 200 participants	Three cases of bladder cancer (non-invasive papillary tumours) identified (two non-smokers between 28-29 years of age, the third patient was 44 years and ex-smoker; exposure duration: 12, 9 and 1.5 months respectively). None of the cases had abnormal cytology or hematuria	Study contains several flaws. No specific data on exposure levels are available, follow-up period may be too short, no specific information on duration of exposure and probably too short, no control group used, no valid comparison rate available, no clear defined study population, possible exposure to other (bladder) carcinogens not known
Linch et al. (1971)[33]	Type of study: Retrospective cohort study Country: USA Type of industry: 4,4′-methylene bis (2-chloroaniline) manufacturing Participants: 31 exposed workers and 31 controls	No information on exposure levels (exposure was confirmed by urinalysis) Duration of exposure: between 6 months and 16 years Follow-up period: unknown Type of examination: Urine cytology, medical records	No deaths and no malignancies were observed in exposed workers and controls	Study contains several flaws. No specific data on exposure levels are available, no useful information on duration of exposure, no information on follow-up period, number of participants is small, possible exposure to other (bladder) carcinogens not known

Obs: Observed, SMR: Standardized mortality ratio (ratio of observed to expected death), SRR: Standardized registration ratio (ratio of observed to expected cancer morbidity).

B animal studies

Reference	Study design and animal species	Data on exposure and effect endpoints	Results	Remarks
Kommineni et al. (1979)[14]	ChR CD (SD) male rats 100, 100, 75, 50 animals at 0, 250, 500 and 1000 ppm, resp.	Diet (27% protein) 0, 250, 500, 1000 ppm (mg/kg diet), equivalent to 10, 20 and 40 mg/kg body weight/day (based on default food intake of 40 g/kg body weight/day) X_{po} = 18 months X_{pe} = 24 months Statistical analysis: method unknown	Mortality: Average survival 89, 87, 80 ($p<0.01$), 65 ($p<0.001$) for the 0, 250, 500 and 1000 ppm groups, resp. Adverse effects: Mean body weight gain markedly lower in high dose group compared to control. Slight decrease in haemoglobin and haematocrit in high dose group. Tumours: For 0, 250, 500 and 1000 ppm groups, resp. *Lung adenocarcinomas* in 0/100, 14/100 ($p<0.001$), 20/75 ($p<0.001$), 31/50 ($p<0.001$) *Mammary adenocarcinomas* in 1/100, 5/100, 8/75 ($p<0.01$), 14/50 ($p<0.001$). *Zymbal gland carcinomas* in 1/100, 8/100 ($p<0.05$), 5/75, 11/50 ($p<0.001$) *Hepatocellular carcinomas* in 0/100, 3/100, 3/75, 18/50 ($p<0.001$)	Klimisch score: 2 Well-performed study, adequate for carcinogenicity assessment and derivation of cancer risk values. Deficiencies: only one sex used, exposure less than life-span, statistical method used is unknown
Kommineni et al. (1979)[14]	ChR-CD (SD) male rats 100, 100, 75, 50 animals at 0, 125, 250 and 500 ppm, resp.	Diet: low protein (8%) 0, 125, 250, 500 ppm (mg/kg diet), equivalent to 5, 10 and 20 mg/kg body weight/day (based on default food intake of 40 g/kg body weight/day) X_{po} = 18 months X_{pe} = 24 months Statistical analysis: method unknown	Mortality: Average survival 87, 81, 79, 77 ($p<0.05$) for the 0, 125, 250 and 500 ppm groups, resp. Adverse effects: Mean body weight gain markedly lower in high dose group compared to controls. Slight decrease in haemoglobin and haematocrit in high dose group. Tumours: For 0, 125, 250 and 500 ppm groups, resp. *Lung adenocarcinomas* in 0/100, 3/100, 7/75 ($p<0.01$), 8/50 ($p<0.001$) *Mammary adenocarcinomas* in 0/100, 1/100, 3/75, 3/50 ($p<0.05$). *Zymbal gland carcinomas* in 0/100, 0/100, 4/75 ($p<0.05$), 6/50 ($p<0.001$) *Hepatocellular carcinomas* in 0/100, 0/100, 0/75, 9/50 ($p<0.001$) *Hemangiosarcomas* in 1/100, 2/100, 4/75, 4/50 ($p<0.05$)	Klimisch score: 2 Well-performed study, adequate for carcinogenicity assessment. Deficiencies: statistical method used is unknown, protein level in diet is considerably below the required amount for rats, only one sex used, exposure less than life-span
Stula et al. (1971, 1975)[16,35]	ChR-CD (SD) rats (50/sex/group) Sacrificed for interim evaluation at 1 year: 6/sex/group	Diet (23% protein): 0, 1000 ppm (mg/kg diet), equivalent to 40 (males) and 50 (females) mg/kg body weight/day (based on default food intake of 40 and 50 g/kg body weight/day in males and females, respectively) X_{po}: 2 years X_{pe}: 2 years Statistical analysis: Chi-square	Mortality: Average survival (range) 564 (63-731), 628 (306-733), 560 (152-733), 548 (224-719) days for control male, control female, exposed male and exposed female rats, resp. Adverse effects: liver changes including hepatocytomegaly, fatty change, necrosis, bile duct proliferation, fibrosis Tumours: (for control male, control female, exposed male and exposed female rats, resp.) *Lung*: Adenomatosis (preneoplastic lesion) in 1/44, 1/44, 14/44 ($p<0.05$), 11/44 ($p<0.05$) and adenocarcinoma in 0/44, 0/44, 21/44 ($p<0.05$), 27/44 ($p<0.05$). Squamous cell carcinoma in one exposed male and one exposed female. *Pleural biphasic tumour*: 0/44, 0/44, 4/44, 2/44. *Liver*: hepatocellular adenoma: 0/44, 0/44, 3/44, 2/44, hepatocellular carcinoma: 0/44, 0/44, 3/44, 3/44 *Mammary adenocarcinoma*: 0/44, 3/44, 3/44, 5/44 The following malignant tumours were observed in a single treated animal and were absent in controls: vagina fibrosarcoma, kidney adenocarcinoma, peritoneal mesothelioma, duodenum adenocarcinoma	Klimisch score: 2 Well-performed study, adequate for carcinogenicity assessment. Deficiencies: limited (only liver) information on non-cancer effects, only one dose tested

Reference	Study design and animal species	Data on exposure and effect endpoints	Results	Remarks
Stula et al. (1971, 1975)[16,35]	ChR-CD (SD) rats (25/sex/group) Sacrificed for interim evaluation at 1 year: 4/sex/group	Diet: low protein (7%) 0, 1000 ppm (mg/kg diet), equivalent to 40 (males) and 50 (females) mg/kg body weight/day (based on default food intake of 40 and 50 g/kg body weight/day in males and females, respectively) Xpo: 16 months (study terminated because of reduced survival on protein-deficient diet) Xpe: 16 months Statistical analysis: Chi-square	Mortality: Average survival (range) 384 (44-495), 466 (371-498), 400 194-475), 423 (287-475) days for control male, control female, exposed male and exposed female rats, resp. Adverse effects: liver changes including hepatocytomegaly, fatty change, necrosis, bile duct proliferation, fibrosis Tumours: (control male, control female, exposed male and exposed female rats, resp.) Lung adenomatosis: 1/21, 1/21, 8/21 ($p<0.05$), 14/21 ($p<0.05$) Lung adenocarcinoma: 0/21, 0/21, 5/21 ($p<0.05$), 6/21 ($p<0.05$). Hepatocellular adenoma: 0/21, 0/21, 5/21 ($p<0.05$), 2/21. Hepatocellular carcinoma: 0/21, 0/21, 11/21 ($p<0.05$), 1/21 Mammary fibroadenoma: 0/21, 7/21, 0/21, 1/21 ($p<0.05$) Mammary adenocarcinoma: 0/21, 0/21, 0/21, 6/21 ($p<0.05$) The following malignant tumours were observed in a single treated animal and absent in controls: skin squamous cell carcinoma, malignant lymphoma, ileum adenocarcinoma.	Klimisch score: 3 Supportive study. Deficiencies: limited (only liver) information on non-cancer effects, low number of animals used, exposure period too short, maximum tolerated dose exceeded, protein level in diet is considerably below the required amount for rats, only one dose tested
Russfield et al. (1975)[15]	HaM/ICR mice (25/sex/group)	Diet 0, 1000 and 2000 ppm (mg/kg diet), equivalent to 120 and 240 (males) and 130 and 260 (females) mg/kg body weight/day (based on default food intake of 120 and 130 g/kg body weight/day in males and females, respectively) Xpo = 18 months Xpe = 24 months Statistical analysis: Fisher exact test	Mortality: increased early mortality in female mice of the high dose group. Adverse effects: no effects on growth, no treatment-related non-neoplastic changes Tumours: Hepatomas: male: 3/18, 3/13, 4/20; female: 0/20, 9/21 ($p<0.01$), 7/14 ($p<0.01$) at 0, 1000 and 2000 ppm, resp. (Liver tumours in mice have little relevance to man) Vascular tumours (generally subcutaneous hemangiomas and hemangiosarcomas): male: 0/18, 3/13, 8/20; female: 1/20, 0/21, 6/14 at 0, 1000 and 2000 ppm, resp. (incidence in treated animals comparable to historical control)	Klimisch score: 2 Well-performed study, adequate for carcinogenicity assessment Deficiencies: low number of animals used, exposure less than life-span
Russfield et al. (1975)[15]	ChR CD-1 rats (25 males/ group)	Diet 0, 500 and 1000 ppm (mg/kg diet), equivalent to 20 and 40 mg/kg body weight/day (based on default food intake of 40 g/kg body weight/day) Xpo = 18 months Xpe = 24 months Statistical analysis: Fisher exact test	Mortality: At 18 months: 96, 80% of control and treated animals survived, resp. At 20-22 months: about 55% survival in all groups Adverse effects: Body weight: ca. 780, 730 and 680 g at 0, 500 and 1000 ppm, resp., after 18 months. Differences persisted until necropsy. Tumours: Lung adenomatosis: 0/22, 3/22, 4/19 at 0, 500 and 1000 ppm, resp. Hepatomas (without invasion of hepatoma cells in blood vessel walls): 0/22, 1/22, 4/19 at 0, 500 and 1000 ppm, resp.	Klimisch score: 3 Supportive study. Deficiencies: only one sex used, low number of animals used, exposure less than life-span

Reference	Study design and animal species	Data on exposure and effect endpoints	Results	Remarks
Grundmann and Steinhoff (1970)[34]	Wistar rats (25/sex/group)	Low protein diet (protein level not specified) 0, 0.1% (total 27 g/kg body weight, corresponding to 54 mg/kg body weight/day based on 500-day exposure period) X_{po}: 500 days X_{pe}: lifespan	Mortality: Survival mean 730, 565, and 535 days for control, exposed male and exposed female rats, resp. Adverse effects:Changes in the livers of treated animals that died without visible tumour (fatty liver, necrosis and bleedings in two males; necrosis and fatty liver in five females). Tumours: For control males, control females, treated males and treated female, resp. *Total*: 0/25, 2/25, 23/25, 20/25 *Liver tumours (multilocular hepatomata; metastases in lung of two males and in brain of one male)*: 0/25, 0/25, 22/25, 18/25 *Lung tumours (mainly carcinomata)*: 0/25, 0/25, 8/25, 5/25	Klimisch score: 3 Supportive study. Deficiencies: short exposure period, low number of animals used, limited information on non-cancer effects, histopathology limited to liver and organs showing gross lesion, no statistical analysis performed, protein level in diet is considerably below the required amount for rats, only one dose tested
Stula et al. (1978)[36]	Beagle dogs (6 females/ group)	Oral (capsules) 0 and 100 mg/day, first six weeks: 3 days/week, then 5 days/week (8-15 mg/kg bw/day) X_{po}: 9 years X_{pe}: 9 years Statistical analysis: Fisher exact test, one tail	Mortality: One treated dog died after 3.4 years (death not related to treatment). Other animals were killed after 8.3 (one dog) or 9 years of treatment. Adverse effects: Increased blood glutamic pyruvic transaminase (GPT) activity in treated dogs. Increased numbers of erythrocytes, leukocytes and epithelial cells in urine sediment in treated dogs (some epithelial cells showed changes suggestive of neoplasia in the genitourinary tract). Nodular hyperplasia in the liver in 0/6 control and 3/5 treated dogs Tumours: *Urinary bladder papillary transitional cell carcinoma*: 0/5, 4/5 ($p<0.025$) The fifth treated dog had a combined transitional cell carcinoma / adenocarcinoma in the urethra.	Klimisch score: 3 Supportive study. Deficiencies: only one sex used, low number of animals used, only one dose tested
Steinhoff and Grundmann (1971)[50]	Wistar rats Control and exposed groups: 25 and 17 rats/sex, resp.	Subcutaneous injection 500 or 1000 mg/kg bw, once a week or longer, total dose: 25 g/kg bw X_{po} = 620 days X_{pe} = lifespan	Mortality: Survival mean: 1040 and 778 days control and exposed rats, resp. Tumours: In total 13 and 29 malignant tumours in control and exposed group, resp. *Hepatocellular carcinoma*: 0/50, 9/34 *Lung tumours (adenocarcinomas and carcinomas)*: 1/50, 7/34	Klimisch score: 3 Supportive study. Deficiencies: very limited information on study design and results (reported as short communication), low number of animals used, exposure period too short, route of exposure not relevant, no information on non-cancer effects, no statistical analysis performed

X_{po} = duration of exposure; X_{pe} = duration of the experiment.
Klimisch scores were based on Klimisch et al (1997)

C BMD-analysis

Software	Proast, version 65.7
BMR, risk type	10%, extra risk
BMDL	Lowest 95% confidence interval of the BMD
Model fit and averaging	The fit of a model is measured by the comparison with the best fitting model (the one with the lowest AIC (AICmin)). If [AICmodel < AICmin + 2] then both models are similar and the tested model provides a fit comparable with the best fitting model. The weight of a model depends on the fit – models with lower fit are attributed lower weights for model averaging.
Data source	Kommineni C, Groth DH, Frockt IJ, Voelker RW, Stanovick RP. Determination of the tumorigenic potential of methylene-bis-orthochloroaniline. J Environ Pathol Toxicol. 1979;2(5):149-171 Effect data analysed only when statistical differences between exposed and control group was $p \leq 0.05$.
Exposure design	Male ChR CD (SD) rats exposed via the diet (fixed concentrations) for 18 months; experimental period 24 months
Effect parameter	Incidence of lung adenocarcinomas

Data on exposure and response

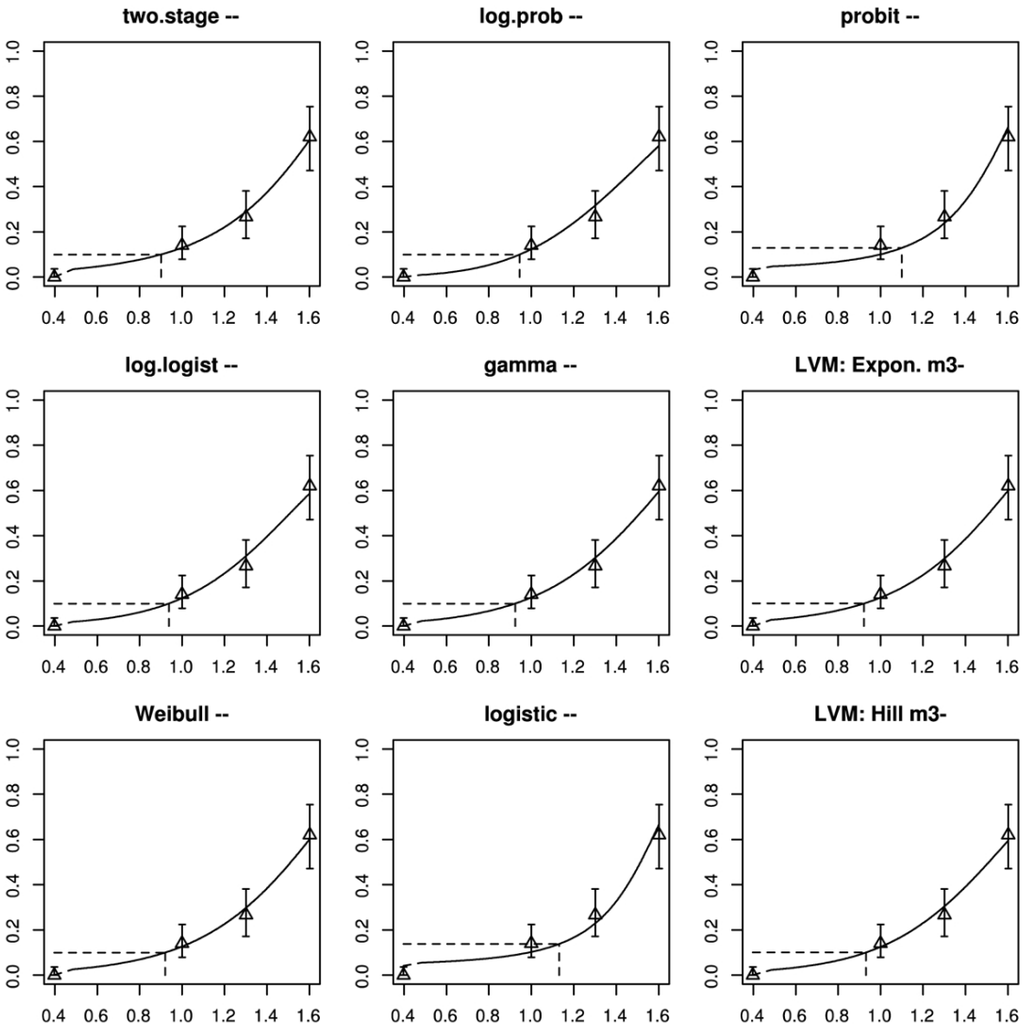
Dose (mg/kg body weight/ day)	Number of male rats per dose	Number of male rats with lung adenocarcinomas
0	100	0
10	100	14
20	75	20
40	50	31

Result of BMD-analysis for male rats

Model	No. Par.	Log-lik	AIC	BMDL	BMDU	BMD	conv	Weight
Null	1	-162.63	327.26	NA	NA	NA	NA	
Full	4	-117.19	242.38	NA	NA	NA	NA	
two stage	3	-117.35	240.7	5.44	10.9	7.95	Yes	0.1802
log.logist	3	-117.75	241.5	5.88	11.1	8.66	Yes	0.1208
Weibull	3	-117.48	240.96	5.44	11	8.32	Yes	0.1582
Log.prob	3	-117.92	241.84	6.18	11.1	8.81	Yes	0.1019
gamma	3	-117.56	241.12	5.36	11	8.39	Yes	0.1460
logistic	2	-122.73	249.46	11.7	15.6	13.5	Yes	0.0023
probit	2	-121.64	247.28	NA	NA	12.6	Yes	0.0067
LVM: Expon. M3-	3	-117.53	241.06	5.42	11.1	8.36	Yes	0.1505
LVM: Expon. M3-	3	-117.65	241.3	5.67	11.1	8.53	yes	0.1335

Final BMDL	Final BMDU	Final BMD#
5.85	11.8	8.3

The model averaged BMD is calculated using the formula: BMD=exp((LN(BMDL)+LN(BMDU))/2)


two.stage --
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gamma --
LVM: Expon. m3-
Weibull --
logistic --
LVM: Hill m3-
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0.4 0.6 0.8 1.0 1.2 1.4 1.6

The Committee

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