Health Council of the Netherlands

# 4,4-Methylenedianiline

Health-based calculated occupational cancer risk values

2015/28

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**Gezondheidsraad**
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *4,4'-Methylenedianiline*
Uw kenmerk : DGV/BMO/U-932542
Ons kenmerk : U-847210/BvdV/cn/459-B72
Bijlagen : 1
Datum : 17 november 2015

Geachte minister,

Graag bied ik u hierbij aan het advies over de gevolgen van beroepsmatige blootstelling aan 4,4'-methyleendianiline.

Dit advies maakt deel uit van een uitgebreide reeks, waarin concentratieniveaus in lucht worden afgeleid die samenhangen met een extra kans op kanker van 4 per 1.000 en 4 per 100.000 door beroepsmatige blootstelling. De conclusies van het genoemde advies zijn opgesteld door de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS) van de Gezondheidsraad en beoordeeld door de Beraadsgroep Volksgezondheid.

In dit advies concludeert de commissie dat 4,4'-methyleendianiline een carcinogene stof is met een stochastisch genotoxisch werkingsmechanisme. Gebaseerd op dierexperimentele gegevens schat de commissie de extra kans op kanker voor 4,4'-methyleendianiline op:
- 4 per 100.000 bij 40 jaar beroepsmatige blootstelling aan 16 $\mu g/m^3$
- en 4 per 1.000 bij 40 jaar beroepsmatige blootstelling aan 1,6 $mg/m^3$.

Ik onderschrijf de aanbevelingen en het advies van de commissie.

Ik heb dit advies vandaag ter kennisname toegezonden aan de staatssecretaris van Infrastructuur en Milieu en aan de minister van Volksgezondheid, Welzijn en Sport.

Met vriendelijke groet,

prof. dr. J.L. Severens,
vicevoorzitter

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# 4,4’-Methylenedianiline

Health-based calculated occupational cancer risk values

Dutch Expert Committee on Occupational Safety (DECOS),
a Committee of the Health Council of the Netherlands

to:

the Minister of Social Affairs and Employment

No. 2015/28, The Hague, November 17, 2015

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect to public health issues and health (services) research..." (Section 22, Health Act).

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# Contents

# Samenvatting

Op verzoek van de Minister van Sociale zaken en Werkgelegenheid, leidt de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS) van de Gezondheidsraad, de concentraties van een stof in de lucht af die samenhangen met een vooraf vastgesteld extra risico op kanker (4 per 1.000 en 4 per 100.000 individuen) door beroepsmatige blootstelling gedurende het arbeidzame leven. Het gaat om kankerverwekkende stoffen die door de Gezondheidsraad of de Europese Unie geclassificeerd zijn in categorie 1A of 1B en die kankerverwekkend zijn via een stochastisch genotoxisch mechanisme. Voor de schatting maakt de commissie gebruik van de *Leidraad Berekening Risicogetallen voor kankerverwekkende stoffen* van de Gezondheidsraad.[1] In dit advies onderzoekt de commissie de mogelijkheid om zo'n schatting te maken voor 4,4'-methyleendianiline. 4,4'-Methyleendianiline is een intermediair produkt dat gebruikt wordt bij de productie van polyurethanen en epoxyharsen.

De commissie concludeert dat 4,4'-methyleendianiline een carcinogene stof is met een stochastisch genotoxisch werkingsmechanisme. Gebaseerd op dierexperimentele gegevens schat de commissie de extra kans op kanker voor 4,4'-methyleendianiline op:

- $4 \times 10^{-5}$ bij 40 jaar beroepsmatige blootstelling aan 16 µg/m$^3$
- en $4 \times 10^{-3}$ bij 40 jaar beroepsmatige blootstelling aan 1,6 mg/m$^3$.

# Executive summary

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, derives so-called health-based calculated occupational cancer risk values (HBC-OCRVs) associated with excess cancer levels of 4 per 1,000 and 4 per 100,000 as a result of working life exposure to substances. It concerns substances which are classified by the Health Council or the European Union in category 1A or 1B, and which are considered stochastic genotoxic carcinogens. For the estimation, the Committee uses the *Guideline for calculating carcinogenic risks* of the Health Council.[1] In this report the Committee evaluates the possibility to establish such estimates for 4,4'-methylenedianiline. 4,4'-Methylenedianiline is a chemical intermediate used in the production of polyurethanes and epoxyresins.

In this report, the Committee concludes that 4,4'-methylenedianiline is a carcinogenic substance with a stochastic genotoxic mechanism. Based on animal data the Committee estimates that the additional lifetime cancer risk for 4,4'-methylenedianiline amounts to:

- $4 \times 10^{-5}$ for 40 years of occupational exposure to 16 µg/m$^3$
- and $4 \times 10^{-3}$ for 40 years of occupational exposure to 1.6 mg/m$^3$.

Chapter 1

# Scope

## 1.1 Background

At the request of the Minister of Social Affairs and Employment (Annex A), the Dutch Expert Committee on Occupational Safety (DECOS), a Committee of the Health Council of the Netherlands, performs scientific evaluations of the toxicity of substances to which man can be exposed at the workplace. The purpose of these evaluations is to recommend a health-based recommended occupational exposure limit (HBROEL) or health-based calculated occupational cancer risk value (HBC-OCRV) for the concentration of the substance in air, provided the database allows the derivation of such value. These recommendations serve as a basis in setting legally binding limit values by the minister. As a preference, the minister has requested the Health Council to align, if possible, with the evaluations of other European organizations.

In 2000, DECOS published an advice on the toxicity of 4,4'-methylenedianiline (MDA).[2] Several years later, the German Committee on Hazardous Substances (AGS) and the European Scientific Committee on Occupational Exposure Limits (SCOEL) published an evaluation on the toxicity of MDA as well.[3,4] The risk evaluation of the SCOEL was qualitative. The AGS applied a quantitative method of linear extrapolation which leads to cancer risk values.

In the present advice, the Committee reconsiders the former health based calculated occupational cancer risk values for MDA based on the previous report of the Committee (2000)[2], the advice of the AGS published in 2010[3], the advice of the SCOEL published in 2012[4], and additional published studies till June 2015. The Committee decides to perform a quantitative risk assessment of MDA according to its own guidelines and methodology.

## 1.2 Committee and method of work

The present document contains the re-assessment of the toxicity and carcinogenicity of MDA by DECOS. The members of the DECOS are listed in Annex B. The submission letter (in English) to the Minister can be found in Annex C. In July 2015, the DECOS released a draft version of the report for public review. The individuals and organisations that commented on the draft are listed in Annex D. DECOS has taken these comments into account in finalising its report.

## 1.3 Data

In Chapter 2 of the present document, the Committee evaluates the toxicity and carcinogenicity of MDA and recommends, if possible, a health-based occupational cancer risk value for MDA. This evaluation is based on the data described in Annex G of the present report.

Annex G of the present document constitutes an update of a Health Council report, issued in 2000. For the present report, relevant data were extracted from the more recent reports on MDA from the AGS and the SCOEL published in 2010 and 2012 respectively. Additional data were searched from the literature published using the online databases Toxline, Medline and Chemical Abstracts (CAPlus), using “4,4-methylenedianiline” and CAS no 101-77-9 as keywords. The last search was performed in June 2015.

Chapter 2

# Hazard assessment

This chapter contains a short summary of the relevant data on the effects of exposure to MDA, based mainly on the data summarized in Annex G of the present report. In addition, the Committee evaluates the carcinogenicity of MDA and recommends health-based calculated occupational cancer risk values for MDA.

## 2.1 Hazard identification

The Committee evaluated both the human and animal studies on MDA and observed that in the period following the publication of the previous DECOS report (2000) hardly any new human or animal data have been published.

### *2.1.1 Observations in humans*

Gastrointestinal complaints and in particular liver damage were reported among humans after occupational exposure and short-term intoxication by contaminated food (icterus, cellular infiltration, bile duct inflammations, cholestasis, hyperbilirubinaemia and increased serum transaminase levels).[3-9] No reliable information is available about concentrations or doses. Various studies definitely demonstrated sensitisation to MDA in humans.[3,4,10,11]

No information was found to evaluate the possible carcinogenicity in humans. The validity of the available human data on occupational exposure to MDA is generally limited because of methodological shortcomings (such as small cohorts, no determination of exposure, confounding factors not taken into account and mixed exposure). These studies suggest an association between MDA exposure at the workplace and an increased incidence of bladder cancer.[3,4,12,13]

### 2.1.2 *Animal data*

The thyroid and liver were the most relevant target organs after repeated administration, rats being more sensitive than mice.[3,4,14-16] Follicular hyperplasia and/or hypertrophy were observed in the thyroid of rats in several studies.[3,4,14-16] Hepatotoxic effects included increased serum levels of liver-specific transaminases, hepatocellular degeneration as well as necrotic alterations and bile duct hyperplasia.[3,4,17-20] Haematotoxicity was another relevant end point in animal studies (anaemia and extramedullary haematopoiesis).[3,21] MDA was slightly irritating to the skin and eyes of animals.[3,21] No reliable animal studies or any human data are available for reproductive toxicity.[3,21]

The only studies suitable for quantitative risk assessment of the carcinogenic potential are the long-term studies performed in the framework of the National Toxicology Program (NTP) in mice and rats.[14-16] The other studies reviewed (two subcutaneous and three gavage studies in rats, and one oral study in dogs) are suitable neither to assess the carcinogenic potentential nor for quantitative risk assessment, due to poor study design, reporting etc.[22-25]

In the NTP studies MDA was administered in drinking water for 103 weeks followed by one week without treatment to groups of fifty male and fifty female mice (B6C3Fa) and rats (F344) at concentration levels of 0, 0.015 or 0.03% (0, 150 or 300 mg/L).[14-16]

In rats given MDA there was good survival at both 78- and 105-weeks with no significant differences between the males and females. In MDA-exposed mice high-dose males showed significantly reduced survival when compared to the low-dose and control groups.

Treatment-related increases in the incidences 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.

The tumour incidences in rats and mice are listed in Table 1.

*Table 1* Tumour incidences in rats and mice treated with MDA (NTP 1984; WGD 2000; Weisburger 1984; Lamb 1986).[2,14-16]

| rats | % MDA in drinking water | | | | | |
|---|---|---|---|---|---|---|
| | males | | | females | | |
| | 0 | 0.015 | 0.03 | 0 | 0.015 | 0.03 |
| MDA, mg/kg bw/day | | | | | | |
| calculated | 0 | 7.5 | 15 | 0 | 8.6 | 17.1 |
| measured* | 0 | 9 | 16 | 0 | 10 | 19 |
| | | | | | | |
| thyroid gland | n=49 | 47 | 48 | 47 | 47 | 48 |
| follic. cell adenoma | 1 | 4 | 3 | 0 | 2 | 17 |
| follic. cell carcinoma | 0 | 0 | 7 | 0 | 2 | 2 |
| C-cell adenoma | 1 | 2 | 1 | 0 | 3 | 6 |
| C-cell carcinoma | 2 | | 1 | 1 | 2 | 1 |
| | | | | | | |
| liver | n=50 | 50 | 50 | 50 | 50 | 50 |
| hepatocellular adenoma | 1 | 12 | 25 | 4 | 8 | 8 |
| carcinoma | 0 | 1 | 1 | 0 | 0 | 0 |

| mice | % MDA in drinking water | | | | | |
|---|---|---|---|---|---|---|
| | males | | | females | | |
| | 0 | 0.015 | 0.03 | 0 | 0.015 | 0.03 |
| MDA, mg/kg bw/day | | | | | | |
| calculated | 0 | 25 | 50 | 0 | 30 | 60 |
| measured* | 0 | 25 | 57 | 0 | 19 | 43 |
| | | | | | | |
| thyroid gland | n=47 | 49 | 49 | 50 | 47 | 50 |
| follic. cell adenoma | 0 | 3 | 16 | 0 | 1 | 13 |
| follic. cell carcinoma | 0 | 0 | 0 | 0 | 0 | 2 |
| | | | | | | |
| liver | n=49 | 50 | 50 | 50 | 50 | 50 |
| hepatocellular adenoma | 7 | 10 | 8 | 3 | 9 | 12 |
| carcinoma | 10 | 33 | 29 | 1 | 6 | 11 |

* MDA intake was calculated from measured water intake.

### 2.1.3 *Carcinogenic classification and genotoxic mechanism*

DECOS consulted its Subcommittee on the Classification of carcinogenic substances to evaluate the carcinogenic classification and the underlying genotoxic mechanism of MDA. From the available in vitro and in vivo data on genotoxicity, the Subcommittee concludes that MDA is a presumed human carcinogen, and recommends classifying the compound in category 1B *(presumed to be carcinogenic to man)*.[26] This is in agreement with the EU classification.[27] In addition, the Subcommittee is of the opinion that MDA can damage the DNA directly and that a stochastic genotoxic mode of action plays the predominant role in the development of the carcinogenicity (see Annex E and F for further details on the Subcommittee's opinion).

For stochastic genotoxic substances a risk to develop cancer exists at all concentration levels while no safe level (threshold) exists below which no cancer is to be expected. Therefore DECOS calculates cancer risk values for these substances associated with an excess cancer risk of 4 per 1,000 and 4 per 100,000 caused by occupational exposure.[1] These risk values serve as a basis in setting legally binding limit values by the minister.

## 2.2 Risk assessment

The Committee is of the opinion that the epidemiological studies on MDA do not provide a reliable starting point for quantitative risk assessment. There is insufficient epidemiological evidence that MDA is carcinogenic for humans; the majority of the studies does not show increased risks, while in those studies with increased risks there is no statistical significance. Based on the currently available human data no reliable calculation can be made.* Therefore the Committee based the risk assessment on animal data.

The Committee is aware of the uncertainties associated with a risk assessment based on animal data. However, the Committee emphasises that the tumours found in experimental animals only are specifically attributed to exposure to MDA. Therefore the Committee prefers to derive health-based calculated

* The Committee confirms the conclusions regarding the epidemiological studies in its own earlier report (2000) and in those of the SCOEL (2010) and the AGS (2012) in that these studies are not suitable for quantitative risk assessment.[2-4]

occupational cancer risk values from animal data and considers the oral NTP (1984) study as the most suitable study for an estimation of the cancer risk values after exposure by inhalation during a lifetime.

In its previous report (2000), the Committee used the same NTP study to calculate cancer risk values based on a combination of liver- and thyroid tumours in male rats in the highest dose group (0.03%=300 mg/L). The Committee realizes that combining different tumours is no longer conforming to its current guideline and scientific insights. Therefore the Committee now performs a quantitative risk assessment based solely on liver tumours.

First the Committee explores the possibility to derive a bench mark dose (BMD) and applies the EPA software (2.6) to establish the best fitting dose-response. After testing various descriptive models the Committee concludes that the animal data from the NTP study do not provide a reliable derivation of a dose-response relationship and a BMD(L)10 as starting point for quantitative risk assessment.

Next, according to its guideline, the Committee resorts to making a representative point estimate of the tumour incidence based on the animal data from the lowest dose group providing significant differences in tumour incidence (this concerns the dose of 0.015%=150 mg/L and the increase of liver tumours from 1/50 in the controls to 13/50 in the exposed male rats).

From these data[14-16], the incidence per unit dose (mg/kg bw/day) was calculated ($I_{dose}$).

$$I_{dose} = \frac{I_e - I_c}{D \times (X_{po}/L) \times (X_{pe}/L) \times (\text{exposure hours per day}/24) \times (\text{exposure days per week}/7)} =$$

$$= \frac{(13/50) - (1/50)}{(9 \text{ mg/kg bw per day}) \times (721/1000) \times (728/1000)} = 5.08 \times 10^{-2} \text{ [mg/kg bw per day]}^{-1}$$

Where:

- D is the administered daily dose, generally expressed in mg per kg of body weight
- $I_{dose}$ is the carcinogenic activity attributable to the exposure to the substance per unit daily dose under lifespan conditions, assuming a linear dose response relationship, usually expressed per mg/m$^3$ or per mg/kg bw per day

- $I_e$ and $I_c$ are the tumour incidences representing the exposed and control animals respectively
- $X_{po}$ and $X_{pe}$ are the exposure and experimental periods, respectively
- L is the standard lifespan for the animals in question (L rat is assumed to be 1000 days).

Subsequently the extra cancer risk per unit concentration (HBC-OCRV) was calculated for humans occupationally exposed during a working life.

$$HBC\text{-}OCRV = I_{dose} \times \frac{40 \text{ years}}{75 \text{ years}} \times \frac{48 \text{ weeks}}{52 \text{ weeks}} \times \frac{5 \text{ days}}{7 \text{ days}} \times \frac{10 \text{ m}^3}{70 \text{ kg}} = 2.55 \times 10^{-3} \text{ [mg/m}^3\text{]}^{-1}$$

Where it is assumed:
- that biological availability of 4,4'-methylenedianiline is 100% both after oral and inhalatory dosing and that targets for tumour induction are similar for both routes;
- that no difference exists between experimental animals and man with respect to toxicokinetics, mechanism of tumour induction, target, susceptibility etc., unless specific information is available which justifies a different approach;
- 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;
- that the average man is occupationally exposed for 40 years, 48 weeks per year, 5 days per week, 8 hours per day, and inhales 10 $m^3$ air per 8-hour-working day.

The Committee estimated that the concentration of 4,4'-methylenedianiline in the air, which corresponds to an excess cancer risk of
- 4 per 1,000 ($4 \times 10^{-3}$), for 40 years of occupational exposure, equals to 1,569 µg/$m^3$
- and 4 per 100,000 ($4 \times 10^{-5}$), for 40 years of occupational exposure, equals to 16 µg/$m^3$.*

* The SCOEL did not perform a quantitative risk assessment.[4] The AGS however, performed a quantitative risk assessment based on animal data from the NTP study.[3] Just like DECOS the AGS based its calculation on the liver tumours in male rats in the lowest dose group, applied a method of linear extrapolation, but chose another starting point (T25) This resulted in exposure of 7 and 731 µg/$m^3$ at an excess cancer risk of 4 per 100,000 and 4 per 1,000).

## 2.3 Groups at extra risk

No groups at extra risk could be identified.

## 2.4 Health-based recommended occupational cancer risk values (HBC-OCRV)

The Committee concludes that since the publication of its previous report in 2000 no new useful data have been published for quantitative risk assessment. The Committee uses animal data based on the same study as chosen in her previous report but applies a procedure that reflects its current guideline and scientific insights.

The Committee estimates that the concentration of 4,4'-dimethylaniline 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 1.6 mg/m$^3$
- and 4 per 100,000 ($4x10^{-5}$), for 40 years of occupational exposure, equals to 16 µg/m$^3$.

The Committee recommends to use this outcome as a basis in setting legally binding limit values by the minister.

## 2.5 Biological monitoring

The Committee is aware that in industrial practice exposure assessment of workers to MDA is routinely carried out by biological monitoring rather than by ambient monitoring. MDA has a very low vapour pressure and the main route of systemic exposure is dermal penetration. As a consequence, it is generally recognized that occupational exposure is best controlled by biological monitoring of MDA in urine.[28,29] A health-based biological limit value (BLV) corresponding with the above proposed value (as OEL) of 1.6 mg/m$^3$ for an additional life time risk of cancer of $4 \times 10^{-3}$ for 40 years of occupational exposure to MDA was calculated applying the general formula for urinary excretion of a substance in urine:

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

Where:

- U is the cumulated amount of substance excreted into the urine at time t
- f is the fraction of absorbed amount that is excreted into the urine; the value applied was that for the rat (5%) since the human value has not been determined
- F is the biological availability (by inhalation); the value applied was that for aniline (90%) since the human value 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-h working day, the totally inhaled volume per day is 10 $m^3$ and the average amount of creatinine excreted during the day in the urine is denoted by cr, a BLV (in mg/g creatinine) can be calculated from the following formula:

$$BLV = [(f \times F)/cr] \times \{(10 \times OEL) - [[(10 \times OEL)/(8 \times k)] \times (1 - e^{(-8 \times k)})]\}$$ *or, considering k equals* $(\ln 2)/t_{½}$,

$$BLV = [(f \times F)/cr] \times (10 \times OEL) \times (1 - \{[(\ln 2)/(8 \times t_{½})] \times (1 - e^{[(-8 \times (\ln 2))/t_{½}]})\},$$ *with ln 2 = 0.693 and cr = 1.5 g,*

$$BLV = 6.7 \times f \times F \times OEL \times \{1 - 0.18 \times t_{½} \times (1 - e^{(-5.54/t_{½})})\}$$

Using an urinary half-life of MDA of approximately 13 h, and a value for OEL of 1.6 mg/$m^3$ this leads to the following value for the BLV:

$$BLV = 6.7 \times 0.05 \times 0.9 \times 1.6 \times \{1 - 0.18 \times 13 \times (1 - e^{(-5.54/13)})\} = 0.092$$ *mg/g creatinine.*

The value of 92 µg/g creatinine is equivalent to 53 µmol/mol creatinine. The UK HSE as well as the Finnish Institute of Occupational Health have set biological limit values of 50 µmol/mol creatinine.[30]

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# Annexes

Annex **A**

# Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request for advice.
- If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of genotoxic carcinogens, a 'exposure versus tumour incidence range' and a calculated concentration in air corresponding with reference tumour incidences of $10^{-4}$ and $10^{-6}$ per year.
- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the Committee is given in Annex B.

Annex **B**

# The Committee

- R.A. Woutersen, *chairman*
  Toxicologic Pathologist, TNO Innovation for Life, and Professor of Translational Toxicology, Wageningen University and Research Centre, Wageningen
- P.J. Boogaard
  Toxicologist, Shell International BV, The Hague
- D.J.J. Heederik
  Professor of Risk Assessment in Occupational Epidemiology, Institute for Risk Assessment Sciences, Utrecht University, Utrecht
- R. Houba
  Occupational Hygienist, Netherlands Expertise Centre for Occupational Respiratory Disorders (NECORD), Utrecht
- H. van Loveren
  Professor of Immunotoxicology, Maastricht University, Maastricht, and National Institute for Public Health and the Environment, Bilthoven
- A.H. Piersma
  Professor of Reproductive and Developmental Toxicology, Utrecht University, and National Institute for Public Health and the Environment, Bilthoven
- H.P.J. te Riele
  Professor of Molecular Biology, VU University Amsterdam, and Netherlands Cancer Institute, Amsterdam

- I.M.C.M. Rietjens
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- G.B.G.J. van Rooy
  Occupational Physician, Arbo Unie Expert Centre for Chemical Risk Management, and Radboud UMC Outpatient Clinic for Occupational Clinical Toxicology, Nijmegen
- F. Russel
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  Epidemiologist, Maastricht University, Maastricht
- R.C.H. Vermeulen
  Epidemiologist, Institute for Risk Assessment Sciences, Utrecht
- P.B. Wulp
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- B.P.F.D. Hendrikx, *advisor*
  Social and Economic Council, The Hague
- G.B. van der Voet, *scientific secretary*
  Toxicologist, Health Council of the Netherlands, The Hague

### The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the chairperson and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the inaugural meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

# The submission letter (in English)

Subject : Submission of the advisory report *4,4'-Methylenedianiline*
Your Reference: DGV/BMO/U-932542
Our reference : U-847210/BvdV/cn/459-B72
Enclosed : 1
Date : November 17, 2015

Dear Minister,

I hereby submit the advisory report on the effects of occupational exposure to 4,4'-methylenedianiline.

This advisory report is part of an extensive series in which carcinogenic substances are evaluated for the possibility to establish health-based occupational cancer risk values in accordance with European Union guidelines. This involves substances to which people can be exposed under working conditions.

The advisory report was prepared by the Dutch Expert Committee on Occupational Safety (DECOS) of the Health Council. The advisory report has been assessed by the Health Council's Standing Committee on Public Health.

In this report, the Committee concludes that 4,4'-methylenedianiline is a carcinogenic substance with a stochastic genotoxic mechanism. The Committee estimated that the additional lifetime cancer risk for 4,4'-methylenedianiline amounts to:

- $4 \times 10^{-5}$ for 40 years of occupational exposure to 16 µg/m$^3$
- and $4 \times 10^{-3}$ for 40 years of occupational exposure to 1.6 mg/m$^3$.

I have today sent copies of this advisory report to the State Secretary of Infrastructure and the Environment and to the Minister of Health, Welfare and Sport, for their consideration.

Yours sincerely,
(signed)
Professor J.L. Severens
Vice President

Annex **D**

# Comments on the public review draft

A draft of the present report was released in July 2015 for public review. The following organization and persons have commented on the draft document:

- Lentz TJ, B'Hymer C, Reynolds S, National Institute for Occupational Safety and Health (NIOSH), Cincinnati OH, USA.

Annex **E**

# Evaluation of the Subcommittee on Classification of carcinogenic substances

On 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, estimates the additional lifetime cancer risk associated with occupational exposure to substances that have been classified by the European Union or by Health Council in category 1A or 1B, and which are considered 'stochastic genotoxic' carcinogens.

[Previously IARC classified 4,4'methylenedianiline in category 2B ('possibly carcinogenic to humans').[1]]

To date, 4,4'-methylene dianiline has been classified by the European Union in carcinogenicity category 1B ('presumed to be carcinogenic to humans') according to regulation no 1272/2008), and is incorporated in the latest list of carcinogenic substances of the Netherlands' Department of Social Affairs and Employment.[2,3]

More than a decade ago (2000) DECOS quantified the additional lifetime cancer risk for 4.4'-methylenedianiline by calculating health-based occupational cancer risk values (HBC-OCRVs).[4] In its present report DECOS evaluates whether it can concur with, and benefit from, the recently (2010) published risk calculations for carcinogenicity by the German Committee on Hazardous Substances (AGS).[5]

As part of this evaluation DECOS requested its Subcommittee on the Classification of carcinogenic substances to evaluate the specific information regarding the mechanisms of genotoxicity.

## Genotoxic mechanism

Detailed reviews on the genotoxic mechanisms of 4,4'-diaminodiphenylmethane are available (IARC, 1986; McQueen & Williams, 1990; EU-RAR, 2001; AGS, 2010; SCOEL, 2012).[1,5-8] Original studies were consulted when this was considered necessary by the Subcommittee. The main results are summarized below.

## Gene mutation assays

### In vitro

Studies in bacteria (*Salmonella typhimurium*) showed mainly dose-related mutagenic effects after metabolic activation. The results were all negative without activation. More specifically; after metabolic activation, 4,4'-diaminodiphenylmethane is mutagenic in the Ames test in *Salmonella typhimurium* TA100 (Andersen et al., 1980; Cocker et al., 1986; 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 & Shimizu 1978; Tanaka et al., 1985).[9-20] 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 & Shimizu, 1978).[11-13,15-17,19,21]

4,4'-Diaminodiphenylmethane was activated more effectively by rat liver microsomes induced with phenobarbital than by those induced with Aroclor (Rao et al., 1982).[17] 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).[17]

The metabolites, N-acetyl-4,4'-diaminodiphenylmethane and N,N'-diacetyl-4,4'-diaminodiphenylmethane, were not mutagenic in the Ames test system (Cocker et al.,1986; Tanaka et al., 1985) with and without metabolic activation, whereas the metabolites N'-11 nitroso- and N'-hydroxy-N-acetyl MDA were mutagenic both with or without metabolic activation (ATSDR, 1998; BUA, 1994; EU-RAR, 2001; Montelius, 2002; Morgott et al.,1982).[6,10,20,22-25]

### In vivo

No information on in vivo mutagenicity could be retrieved.

## Cytogenetic assays

### In vitro

In mammalian cells in vitro, induction of chromosome aberrations was detected (CHO cells; positive with metabolic activation; questionable result without activation), and marginally positive results were obtained for the induction of sister chromatid exchanges (SCE) using the same test system (Gulati et al., 1989, McQueen & Williams, 1990).[8,26]

A weakly positive result was obtained in the mouse lymphoma test (tested only 27 without metabolic activation)(McGregor et al., 1988).[27]

Zhong et al. (2001) demonstrated a dose-related induction of micronuclei in V79 cells; the micronuclei were negative for anti-kinetochore antibodies and thus provided evidence of the clastogenic effects of MDA.[28]

Matsuoka et al. (2008) confirmed the chromosome-damaging effect in CHL cells (SCE and, to a lesser extent, chromosome gaps and chromosome breaks), which had already been observed in earlier studies in CHO cells.[29]

Robbiano et al. (1999) observed no DNA breaks or micronuclei formation in primary kidney cells from rats or humans.[30]

Martelli et al. (2002) also reported a negative result in primary cultures of the kidneys, bladder and ovaries of rats and primary human kidney and bladder cells.[31] However, these authors detected DNA breaks in primary rat and human hepatocytes and thyreocytes, which are typical target organs for the carcinogenicity of MDA. In more detail Martelli et al. reported that 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.

In human leukocytes, the findings for chromosome aberrations and SCE were negative both with or without metabolic activation.

### In vivo

In vivo tests in mice for the induction of micronuclei in the bone marrow and peripheral blood cells yielded weakly positive results after i.p. injection (up to 140 mg/kg), (Shelby et al., 1993; Morita et al., 1997).[32,33]

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 (SCE)(Parodi et al., 1983).[34]

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).[35]

Another study on DNA-damaging effects (induction of strand breaks) in various organs of mice after oral exposure (250 mg/kg) reported positive effects in the stomach, liver, kidney, bladder, lung and brain, but not in the colon or bone marrow (Sasaki et al. 1999a,b).[36,37]

Robbiano et al. (1999) observed no DNA fragmentation or micronuclei formation in rat kidney cells (oral exposure; 415 mg/kg once or 277 mg/kg 3 times).[30]

Suzuki et al. (2005) exposed rats once to MDA at doses of up to 400 mg/kg orally or 34 by i.p. injection (route unclear).[38] They investigated the induction of micronuclei in the peripheral blood (negative) and in hepatocytes (first laboratory: negative; second laboratory: positive at the highest dose at an increased mortality; only 2 animals examined).

## Miscellaneous assays

### In vitro

Studies on the induction of unscheduled DNA synthesis (UDS) in rat hepatocytes yielded inconsistent results under comparable test conditions (Mori et al. 1988).[39]

Kenyon et al. (2004) detected DNA adducts after 24-hour exposure of human skin to MDA in vitro. They differentiated three different adducts, but did not characterize them in detail. The number of adducts (total) per $10^6$ nucleotides as well as for one of the adducts were increased in relation to the dose, whereas no dose-response relationship was found for the other two adducts.[40]

With the alkaline elution method it was demonstrated that 4,4'-diamino-diphenylmethane at concentrations of 1 to 3 mM caused DNA strand breaks in Chinese hamster V79 cells (Swenberg, 1981).[41]

Clearly positive results were obtained with 4,4'-diaminodiphenylmethane in one DNA 6 repair test with rat hepatocytes (UDS test) (Mori et al., 1988), negative results in another 7 (Mirsalis et al., 1989).[39,42]

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).[43]

### In vivo

No induction of UDS was observed in rats and mice after oral doses of up to the range of the LD50. DNA breaks were reported after i.p. injection of 74 mg/kg (LD50) in the rat liver (Mirsalis et al., 1989).[42]

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).[16]

A study in rats provided evidence of primary genotoxicity in vivo; Schütze et al. (1996) studied DNA adducts after application (i.p. injection) of radiolabelled 4,4'-diaminodiphenylmethane (up to 23.1 mg/kg to rats) and reported DNA adduct formation in the liver.[44] 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.

In a study by Vock et al. (1996), rats were orally exposed to MDA (3 times up to 50 mg/kg).[45] Dose-related DNA adduct formation was detected in the liver.

## Mechanistic considerations

4,4'-Methylenedianiline is a genotoxic substance which induces genetic damage in bacterial, and mammalian systems in vitro and in vivo.

The Subcommittee is of the opinion that there is sufficient evidence that stochastic genotoxic mechanisms underly carcinogenicity of 4,4'-methylene-aniline.

## Recommendation

The Subcommittee concludes that MDA is a presumed human carcinogen, and recommends classifying the compound in category 1B *(presumed to be carcinogenic to man)*. In addition the Subcommittee concludes that stochastic genotoxic mechanisms underly carcinogenicity.

For regulatory standard setting the Subcommittee recommends health-based occupational cancer risk values (HBC-OCRVs) to be calculated.

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## The Subcommittee

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- G.B. van der Voet, scientific secretary
  Toxicologist, Health Council of The Netherlands

Date meeting: September 04, 2014

Annex **F**

# Carcinogenic classification of substances by the Committee

The Committee expresses its conclusions in the form of standard phrases:

| Category | Judgement of the Committee ($GR_{GHS}$) | Comparable with EU Category | |
|---|---|---|---|
| | | (before 16 Decemb er 2008) | (as from 16 Decemb er 2008) |
| 1A | The compound is known to be carcinogenic to humans.<br>• It acts by a stochastic genotoxic mechanism.<br>• It acts by a non-stochastic genotoxic mechanism.<br>• It acts by a non-genotoxic mechanism.<br>• Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether the compound is genotoxic. | 1 | 1A |
| 1B | The compound is presumed to be carcinogenic to humans.<br>• It acts by a stochastic genotoxic mechanism.<br>• It acts by a non-stochastic genotoxic mechanism.<br>• It acts by a non-genotoxic mechanism.<br>• Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether the compound is genotoxic. | 2 | 1B |
| 2 | The compound is suspected to be carcinogenic to man. | 3 | 2 |
| (3) | The available data are insufficient to evaluate the carcinogenic properties of the compound. | not applicable | not applicable |
| (4) | The compound is probably not carcinogenic to man. | not applicable | not applicable |

Source: Health Council of the Netherlands. Guideline to the classification of carcinogenic compounds. The Hague: Health Council of the Netherlands, 2010; publication no. A10/07E.[26]

Annex **G**

# Human and animal carcinogenicity data on 4,4'-methylenedianiline

For the present report, DECOS extracted (below) and evaluated relevant data from the more recent reports on MDA from the AGS and the SCOEL published in 2010 and 2012 respectively. Additional data were searched in the published literature up till June 2015.

## Observations in humans

AGS (2010)
The validity of the available human data on occupational exposure to MDA is generally limited because of methodological inadequacies (such as small cohorts, no determination of exposure, confounding not taken into account and mixed exposure). These studies suggest an association between MDA exposure at the workplace and an increased occurrence of bladder cancer, but this would have to be verified by further studies (ATSDR, 1998; EU RAR, 2001; Montelius, 2002).[21,31,32]

SCOEL (2012)
In a cohort of 595 power generator workers potentially exposed to MDA 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 MDA 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).[13]

Between 1967 and 1976, 10 workers at a plant in Ontario that used MDA 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).[12]

### Additional publications

No new publications were identified by the Committee.

## Animal data

### AGS (2010)

*Inhalation*

There are no studies available for inhalation.

*Oral route*

In an NTP study (1983; Weisburger et al., 1984; Lamb et al., 1986), F344 rats and B6C3F1 mice (50 per sex and dose) were exposed to MDA hydrochloride in the drinking water at concentrations of 150 and 300 mg/L (converted to MDA) for 103 weeks.[14-16] The ingested doses were 9 and 16 mg/kg b.w. • d in male rats, 10 and 19 mg/kg b.w. • d in female rats, 25 and 58 mg/kg b.w. • d in male mice and 19 and 43 b.w. mg/kg • d in female mice. Animals given drinking water that had been adjusted with HCl to the same pH as the test substance solution for the exposed animals served as controls.

Drinking water consumption was reduced among the exposed groups with the exception of male mice. At the high dose, the body weight gain of female rats and mice of both sexes was reduced, and survival was lower in male mice of the high dose. No clinical signs occurred during treatment.

Carcinogenicity was observed in the thyroid and liver of both rats and mice.

In male rats, the incidence of follicular carcinomas of the thyroid was significantly increased in the high dose group (control: 0/49; 150 mg/L: 0/47;

300 mg/L: 7/48); corresponding adenomas were significantly elevated in the females (control: 0/47; 150 mg/L: 2/47; 300 mg/L: 17/48). Non-carcinogenic lesions of the thyroid (follicular cysts) were also observed in female rats of the high dose group.

Significant and dose-related increases in the neoplastic nodules of the liver (control: 1/50; 150 mg/L: 12/50; 300 mg/L: 25/50) and one carcinoma per dose group were observed for male rats; in female rats, the incidence of neoplastic nodules of the liver was not clearly related to the dose (control: 4/50; 150 mg/L: 8/50; 300 mg/L: 8/50). No hepatocellular carcinomas were found in female rats.

The incidences of thyroid follicular adenomas were significantly elevated in male mice (control: 0/47; 150 mg/L: 3/49; 300 mg/L: 16/49) and female mice (control: 0/50; 150 mg/L: 1/47; 300 mg/L: 13/50). Significant effects on the liver consisted of increased rates of hepatocellular carcinomas (males: control: 10/49; 150 mg/L: 33/50; 300 mg/L: 29/50; females: control: 1/50; 150 mg/L: 6/50; 300 mg/L: 11/50) and adenomas (females only: control: 3/50; 150 mg/L: 9/50; 300 mg/L: 12/50).

Other statistically significant carcinogenic effects included:

- Phaeochromocytomas in male mice (control: 2/48; 150 mg/L: 12/49; 300 mg/L: 14/49)
- Alveolar/bronchiolar adenomas in female mice (control: 1/50; 150 mg/L: 2/50; 300 mg/L: 6/49)
- Malignant lymphomas in female mice (control: 13/50; 150 mg/L: 28/50; 300 mg/L: 29/50).

Moreover, an uncommon bile duct adenoma was observed in one male rat of the high dose group and three urinary bladder papillomas were found (2 at the low dose and 1 at the high dose).

After initiation with N-bis(2-hydroxypropyl)nitrosamine or different nitrosamines given consecutively, oral exposure of rats to MDA had a tumour-promoting effect on the thyroid but not on the liver, kidney or bladder (in most studies after initiation with different nitrosamines). In addition to these studies, several older studies are available, but these are not considered to be relevant for assessment because of methodological inadequacies or insufficient documentation (ATSDR, 1998; BUA, 1994; ECB, 2001).[21,31,33]

*Dermal route*

A study on the dermal exposure of C3Hf/BD mice reported a dose-related increase in the incidence of liver tumours after 24-month exposure (3 times per

week) to 5.3- 21.3 mg/kg • d. Since this strain is particularly sensitive as regards the formation of liver tumours, the findings need to be verified (ATSDR, 1998).[31]

### SCOEL (2012)

The SCOEL presents a detailed description of the NTP study (1983) as published by Weisburger (1984) and Lamb (1986) similar to the AGS (above, and will not be copied here).[4]

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-dimethyl-benz[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 negativecontrol 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'-diamino-diphenylmethane dihydrochloride-treated (one hyperplasia) animals (Griswold et al., 1968).[23]

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).[24]

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'-diamino-diphenylmethane (50% 4,4'-diaminodiphenylmethane (50% higher molecular weight analogues). Total doses were 5.0- 6.26 g/kg bw 'pure' 4,4'-diamino-diphenylmethane 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'-diaminodiphenyl-

methane 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).[22]

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).[25]

### Additional publications

No new publications were identified by the Committee.

*Table 2* Carcinogenicity studies in experimental animals with 4,4'-methylenedianiline (MDA).

| authors | species | exposure characteristics | findings | remark |
|---|---|---|---|---|
| NTP-Study (1983)[15] ; Weisburger et al. (1984)[16]; Lamb et al. (1986)[14] | rat, F344/N 50/sex/group | p.o. drinking water dose levels: 0, 0.015, 0.03% Xpo: 103 weeks Xpe: 104 weeks | treatment related increases in the incidences of thyroid follicular-cell carcinomas and hepatic nodules in males and thyroid follicular- cell adenomas in females (see text for actual numbers) | |
| NTP-Study (1983)[15]; Weisburger et al. (1984)[16] ; Lamb et al. (1986)[14] | mouse, B6C3F1 50/sex/group | p.o. drinking water dose levels: 0, 0.015, 0.03% Xpo: 103 weeks Xpe: 104 weeks | treatment related increases in the incidences of thyroid follicular- cell adenomas and hepatocellular neoplasms (see text for for exact numbers) | |
| Griswold et al. (1968)[23] | rat, Sprague-Dawley females N = 20 controls: 140 females | p.o. gavage dose: 30 mg every 3 days for 30 days (total dose: 300 mg/rat) Xpo: 30 days Xpe: 9 months Limited histopathology | mammary lesions were found in 5/132 controls and in 1 of 14 MDA-treated animals | no MDA induced tumours were found. Study design focussed on the appearance of mammary tumours in female SD-rats |
| Schoental (1968)[24] | rat, strain not specified N =16 (8/sex) | p.o. gavage dose levels: 4 or 5 x 20 mg/animal Xpo: < 8 months Xpe: lifetime | a hepatoma and a hemangioma-like tumour of the kidney in a male (18 months). Uterus adenocarcinoma in one female (24 months) | study not suitable for evaluation of carcinogenic potential of MDA |

| | | | | |
|---|---|---|---|---|
| Deichmann et al. (1978)[22] | dog, Beagle<br>females<br>N = 5 pure MDA<br>N = 4 “crude” MDA | p.o. gelatineous capsules<br>dose: 70 mg/dog, 3 times a week<br>Xpo: 4 - 7 years<br>no control group included | no tumours of liver and bladder were found.<br>MDA-induced liver damage was seen in all animals<br>pure MDA: one survivor<br>crude MDA: two survivors | study not suitable for assessment of carcinogenic potential of MDA |
| Steinhoff and Grundmann (1975)[25] | rat, Wistar<br>25/sex/group | subcutaneous<br>dose: 30-50 mg/kg bw at one to 3-week intervals (total dose 1.4 g/kg bw)<br>Xpo: 705 days<br>Xpe: 970 - 1,060 days (MDA); 1,007 days (controls) | MDA group: 29 benign tumours, 33 malignant tumours<br>controls: 15 benign tumours, 16 malignant tumours | limited reporting. Study not suitable for assessment of carcinogenic potential of MDA |

Xpo=exposure period, Xpe=experimental period

# Health Council of the Netherlands

## Advisory Reports

The Health Council's task is to advise ministers and parliament on issues in the field of public health. Most of the advisory opinions that the Council produces every year are prepared at the request of one of the ministers.

In addition, the Health Council issues unsolicited advice that has an 'alerting' function. In some cases, such an alerting report leads to a minister requesting further advice on the subject.

## Areas of activity

**Optimum healthcare**
What is the optimum result of cure and care in view of the risks and opportunities?

**Prevention**
Which forms of prevention can help realise significant health benefits?

**Healthy nutrition**
Which foods promote good health and which carry certain health risks?

**Environmental health**
Which environmental influences could have a positive or negative effect on health?

**Healthy working conditions**
How can employees be protected against working conditions that could harm their health?

**Innovation and the knowledge infrastructure**
Before we can harvest knowledge in the field of healthcare, we first need to ensure that the right seeds are sown.