# Committee for Risk Assessment
# RAC

# Opinion on

# 4,4’-methylene-bis-[2-chloroaniline] (MOCA)

## EC number: 202-918-9
## CAS number: 101-14-4

**ECHA/RAC/A77-O-0000001412-86-147/F**

**Adopted**

**29 May 2017**

**29 May 2017**

**ECHA/RAC/A77-O-0000001412-86-147/F**

**OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON THE EVALUATION OF THE OCCUPATIONAL EXPOSURE LIMITS (OELs) FOR 4,4'-METHYLENE-BIS [2-CHLOROANILINE] (MOCA)"**

Pursuant to Article 77(3)(c) of Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (the REACH Regulation), the Committee for Risk Assessment (RAC) has adopted an opinion on the evaluation of the scientific relevance of occupational exposure limits (OELs) for 4,4'-Methylene-bis-[2-chloroaniline] (MOCA).

**Commission request**

The Commission, in view of the preparation of the third and fourth proposal for an amendment of Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens or mutagens at work (CMD), and in line with the 2017 Commission Communication 'Safer and Healthier Work for All' - Modernisation of the EU Occupational Safety and Health Legislation and Policy'[1], has decided to ask the advice of RAC to assess the scientific relevance of occupational exposure limits for some carcinogenic chemical substances.

Therefore, the Commission has made a request (8 March 2017)[2] in accordance with Article 77 (3)(c) of the REACH Regulation, to evaluate, in accordance with Directive 98/24/EC on the protection of the health and safety of workers from the risks related to chemical agents at work (CAD) and/or Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens or mutagens at work (CMD), the following chemical compounds: 4,4'-methylene-bis[2-chloroaniline] (MOCA), arsenic acid and its inorganic salts, nickel and its compounds, acrylonitrile and benzene.

## I PROCESS FOR ADOPTION OF THE OPINION

Following a request from the European Commission, in the mandate of 15 March 2017[3] the Executive Director of ECHA asked RAC to draw up an opinion on the evaluation of the scientific relevance of occupational exposure limits (OELs) for 4,4'-Methylene-bis-[2-chloroaniline] (MOCA).

In particular, the opinion on MOCA should be based on the current SCOEL- and RAC opinions on the development of occupational exposure limits and the dose-response function of the substance, attached as Appendix 1 and Appendix 2, respectively.

The aim of the recommendation is to support the Commission, by providing scientific advice, to take action on the Proposal to amend Directive 2004/37/EC (3rd wave of amendment). This advice must include a recommendation to be given to the Advisory Committee on Safety and Health at Work (ACSH) in line with the OSH legislative procedures and with the format used by SCOEL in drafting its opinion.

An initial proposal was prepared by the European Chemicals Agency for the consideration by RAC. The current opinion was reviewed by RAC in a written commenting round from 04 May 2017– to 23

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[1] http://ec.europa.eu/social/main.jsp?langId=en&catId=148&newsId=2709&furtherNews=yes

2 https://echa.europa.eu/documents/10162/13641/ec_note_to_echa_oels_en.pdf/f72342ef-7361-0d7c-70a1-e77243bdc5c1

3 https://echa.europa.eu/documents/10162/13641/rac_mandate_oels_en.pdf/9f9b7fb9-545a-214c-69f0-dff5f5092174

May 2017 and at the RAC-41 meeting. Due to the imposed time constraints, the opinion was not subject to a Public Consultation.

## II ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by the RAC: **Tiina Santonen**.

The RAC opinion was adopted by consensus on **29 May 2017.**

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# Assessment of the Scientific Relevance of OELs for 4,4'-Methylene-Bis-[2-Chloroaniline] (MOCA)

## RECOMMENDATION

The opinion of RAC of the assessment of the scientific relevance of OELs for 4,4'-Methylene-bis-[2-chloroaniline] (MOCA) is set out in the table below and in the following summary of the evaluation.

### SUMMARY TABLE

The table summarises the outcome of the evaluation to derive limit values for the inhalation route and the evaluation for dermal exposure and a skin notation. The table also includes carcinogenicity classifications.

**Derived Limit Values**

| | |
|---|---|
| OEL | not established |
| 8-hour TWA : | not derived |
| STEL (15 min) : | not derived |
| BLV | not derived |
| BGV | LoD of biomonitoring method (e.g. ≤ 0.5 µmol/mol creatinine, post shift sample end of the working week ) Using modern analytical methods, the limit of detection is usually 0.5 µmol/mol creatinine or below (See Annex to SCOEL/SUM/174 (2013): Recommendation for a Biological Guidance Value). |

**Carcinogenicity Classification**

| | |
|---|---|
| CLP -Harmonised classification for carcinogenicity | Carc 1B: H350 |
| IARC : | Group 1 –carcinogenic to humans[4] |
| SCOEL Classification of carcinogens scheme[5] | Group A: non-threshold genotoxic carcinogen |

**Notations**

| | |
|---|---|
| Notation[6] | 'Skin' notation |

[4] http://monographs.iarc.fr/ENG/Classification/latest_classif.php; NB: Overall evaluation upgraded to Group 1 based on mechanistic and other relevant data

[5] SCOEL 'Methodology for the Derivation of Occupational Exposure Limits' (SCOEL, 2013; version 7) https://circabc.europa.eu/sd/a/1bd6666f-5c8c-4d13-83c2-18a73dbebb67/SCOEL%20methodology%202013.pdf.

[6] SCOEL 'Methodology for the Derivation of Occupational Exposure Limits' (SCOEL, 2013; version 7)

## SUMMARY


### Background

This opinion concerns the evaluation on 4,4'-methylenebis[2-chloroaniline] (MOCA) and is based on the agreed and published current SCOEL and RAC opinions on the development of occupational exposure limits and the dose-response function of the substance (Appendices 1 and 2), respectively.

The aim of the recommendation is to provide scientific advice on the relevance of OELs for 4,4'-Methylene-bis-[2-chloroaniline] (MOCA) particularly with reference to its carcinogenicity.

### Key conclusions of the evaluation

- MOCA has a harmonised classification as Carc. 1B (H350) according to CLP;
- The critical endpoint for establishing an OEL is carcinogenicity. However, a health based OEL cannot be assigned to MOCA because it is considered a non-threshold genotoxic carcinogen with respect to risk characterisation;
- The major exposure route for MOCA is the dermal route. Therefore, MOCA residues in urinary samples of workers are more appropriate than concentrations in air only, to indicate and assess exposure. However, biomonitoring should be complemented with air monitoring and, when appropriate, measurements of skin and surface contamination in order to identify exposure sources.
- As exposure via the dermal route makes a substantial contribution to body burden, a skin notation is warranted.

### Derived Limit Values and carcinogenicity risk assessment

In their published opinions, SCOEL and RAC described the human and animal evidence on the carcinogenicity of MOCA and its mode of action.

SCOEL did not calculate dose-responses for the carcinogenicity of MOCA in its toxicological evaluation. In the Annex to SCOEL/SUM/174 (2013) (see Appendix 1) it refers to unit cancer risk estimates derived by DECOS (2000) and calculates cancer risk for different urinary MOCA levels. SCOEL gave these estimates for information only, and did not set any limit values based on these calculations

RAC in its toxicological evaluation established a dose-response for MOCA, based on the frequency of combined lung tumours observed in rat oral long-term study of Kommineni *et al*. (1979). MOCA is included in Annex XIV of the REACH Regulation (EC) 1907/2006. The purpose of the toxicological cancer risk evaluation by RAC was to provide a 'reference' dose response relationship for MOCA prior to receiving applications for authorisation.

From these evaluations the following recommendations were given by the respective Committees:

SCOEL Recommendation (See full details in Appendix 1):

*"MOCA is categorized into the SCOEL carcinogen group A as a genotoxic carcinogen to which a threshold cannot be assigned. Hence, a health-based OEL cannot be assigned to MOCA.*

*MOCA is easily absorbed via the skin. Therefore a "skin" notation is warranted. This underlines the relevance of biological monitoring. For biological monitoring, the measurement of total (mostly conjugated) MOCA in post-shift urine appears as a means of choice. As MOCA is not a ubiquitous environmental contaminant or natural body constituent, any noticeable excretion above the detection limit points to occupational sources."*

In the Annex to SCOEL/SUM/174 (2013) it further says *"Since the general population is not exposed to MOCA, MOCA is not detected in the urine of occupationally non-exposed people. This means that urinary levels of occupationally non-exposed stay below the detection limit of the method, which typically lay around 1–1.5 µg/l (3.7–5 nmol/l, ~ 0.37–0.5 µmol/mol creatinine) with commonly used analytical methods, some methods reported to reach the detection limit of 0.1µg/l.*

*Thus, the Biological Guidance Value (BGV) for MOCA corresponds to the detection limit of the biomonitoring method'.*

SCOEL concluded that in occupationally exposed populations, urinary MOCA levels (total MOCA in the urine) below 5 µmol/mol creatinine can be reached using good working practices at the workplace. By referring to the cancer risk estimation by DECOS using linear extrapolations from animal testing, this urinary MOCA level corresponds to a cancer risk of 3–4 × $10^{-6}$."

RAC Dose Response Relationship for Carcinogenicity of MOCA (See full details in Appendix 2):

RAC has established a reference dose response relationship for the carcinogenicity of MOCA. MOCA has caused tumours in several organs in animal tests when exposed daily via the oral route. MOCA is an aromatic amine of the kind usually expected to result in bladder cancer rather than lung cancer. However, in the case of MOCA, no convincing causal association between bladder tumors and exposure to MOCA has been found. Although in some studies liver and bladder cancers have also been seen, lung tumors are most frequently observed in animal studies and lung cancer incidence in Kommineni study (1979) is giving the most complete dose-response data. Data on carcinogenicity in humans was limited and not suitable for deriving dose-response relationships. A dose response relationship for carcinogenicity was therefore derived by linear extrapolation from oral rat study by Kommineni showing increased lung cancer incidence. This can be considered to result in a conservative estimate of risks especially at low exposure levels.

The unit risk for workers' exposure by the inhalation route as calculated by RAC is:

**$9.65 \times 10^{-6}$ per µg/$m^3$**

*RAC has also calculated the unit risk for workers' exposure by the dermal route:*

***$3.38 \times 10^{-5}$ per µg/kg bw/day***

RAC additionally recommended a biomonitoring approach (see Appendix 2):

*"Cancer risks have been calculated for workers' total exposure via different routes of exposure, which can be measured as urinary MOCA levels:*

*Since 1 µg/$m^3$ exposure (which corresponds to a daily dose of 10 µg in occupational exposure) represents a cancer risk of 9.65 x $10^{-6}$.*

***5 µmol/mol creatinine in a Friday afternoon sample (corresponding to a daily dose of 17 µg) corresponds to a risk of 16.4 x $10^{-6}$.***

***0.5 µmol/mol creatinine (detection limit of current analytical techniques) corresponds to cancer risk of 1.64 x $10^{-6}$.***

*While these calculations to estimate daily dose are not precise and include some assumptions, biomonitoring is currently the best method to estimate the total exposure to MOCA in occupational settings. Therefore when biomonitoring data are available, these can be used to estimate cancer risks for occupational exposure".*

Overall conclusion

Based on an evaluation of the published SCOEL and RAC opinions, RAC reconfirms its earlier recommendations as to the cancer risk estimations for MOCA (see above).

**Biological Monitoring**

According to the SCOEL recommendation-Annex to SCOEL/SUM/174 (2013), since MOCA is a genotoxic carcinogen, no health based biological limit value (BLV) can be recommended (SCOEL carcinogen group A). Since the general population is not exposed to MOCA, MOCA is not detected in the urine of occupationally non-exposed people.

This means that urinary levels of occupationally non-exposed stay below the detection limit of the method, which typically lay around 1–1.5 µg/l (3.7–5 nmol/l, ~0.37–0.5 µmol/mol creatinine) with commonly used analytical methods, some methods reported to reach the detection limit of 0.1

µg/l. hence, the Biological Guidance Value (BGV) for MOCA corresponds to the detection limit of the biomonitoring method.

RAC agrees with SCOEL that biomonitoring is currently the best method to estimate the total exposure to MOCA in occupational settings. However, biomonitoring should be complemented with air monitoring and, when appropriate, measurements of skin and surface contamination in order to identify exposure sources.

**Notations**

According to the SCOEL recommendation and the SCOEL methodology[7], a skin notation should be applied if skin uptake is likely to result in substantial contribution (of the order of 10% or more) to the total body burden.

MOCA is easily absorbed via the skin and the skin is the major route of exposure in occupational settings. Therefore, a "skin notation is warranted". There are no reports suggesting that MOCA is a sensitizing substance. No SEN notation is needed".

RAC agrees that dermal exposure is a major route and therefore supports this skin notation.

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[7] https://circabc.europa.eu/sd/a/1bd6666f-5c8c-4d13-83c2-18a73dbebb67/SCOEL%20methodology%202013.pdf

# RECOMMENDATION REPORT

## 1. Chemical Agent Identification and Physico-Chemical Properties

*Substance identification:*

Table 1 presents the substance identification of 4,4'-Methylene-bis-[2-chloroaniline] (MOCA)

**Table 1: Substance identification**

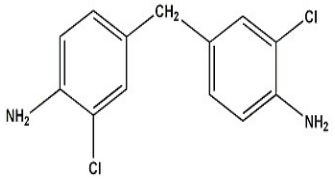
| Substance | CAS No, | EINECS No. | Structural formula | Molecular formula | Molar mass (g/mol) |
|---|---|---|---|---|---|
| **4,4'-Methylene-bis-(2-chloroaniline) [MOCA]** | 101-14-4 | 202-918-9 |  | $C_{13}H_{12}Cl_2N_2$ | 267.16 |

**Table 2: Physical-chemical properties of MOCA**

| Property | Value | References |
|---|---|---|
| Physical state at 20°C and 101.3 kPa | Light yellow granular solid | Registration data (2017) |
| | Solid colourless crystals | Bornscheuer, U.& Roempp, 2008 in ECHA A.XV report (2011) |
| | - Pure substance is colourless crystalline solid<br>- Commonly used forms (industry grade) are tan-coloured pellets or flakes | OECD (2013) |
| Melting point | 101.3 °C | Registration data (2017) |
| | 110 °C | Bornscheuer, U.& Roempp, 2008 in ECHA A.XV report (2011); OECD (2013) |
| Boiling point | Decomposes prior boiling at 370 °C | D. S. Brassington, 2010 in ECHA A.XV report (2011); |
| | Decomposes prior boiling above 277 °C | OECD (2013) |
| Vapour pressure | < 1. 5 x 10-3 Pa at 20 °C | Registration data (2017) |
| | 5.2 × 10-7 Pa at 25 °C | OECD (2013) |
| | 0. 17 Pa at 60 °C | Bornscheuer, U.& Roempp, 2008 in ECHA A.XV report (2011) |
| Density | 1.44 g/cm³ at 20 °C | Registration data (2017) |
| | 1.44 g/cm3 at 24 °C | Bornscheuer, U.& Roempp, 2008 in ECHA A.XV report (2011); |
| Water solubility | 13.8 mg/l at 20 °C, pH 7.6 | Registration data (2017); Baltussen, 2010 in ECHA A.XV report (2011) |
| | 0.509 mg/L at 20 °C | OECD (2013) |
| Partition coefficient n-octanol/water (log value) | 2.50 at neutral pH | Registration data (2017); Baltussen, 2010 in ECHA A.XV report (2011) |

| | | |
|---|---|---|
| | 3.66 at 25 °C | OECD (2013) |

## 2. EU Harmonised Classification and Labelling

The classification of MOCA based on EC Regulation 1272/2008 on classification, labelling and packaging of substances and mixtures is presented in Table 3. No concentration limits are specified for MOCA.

**Table 3: EU classification: Annex VI of Regulation (EC) No 1272/2008 (CLP Regulation) of MOCA**

| Index No. | Annex VI of CLH<br>Hazard class and category | Hazard statement code |
|---|---|---|
| **612-078-00-9** | Carc. 1B | H350 (May cause cancer) |
| | Aquatic Acute 1 | H400 (Very toxic to aquatic life) |
| | Aquatic Chronic 1 | H410 (Very toxic to aquatic life with long lasting effects) |
| | Acute Tox. 4* | H302 (Harmful if swallowed) |

## 3. Chemical Agent and Scope of Legislation

MOCA is a hazardous chemical agent in accordance with Article 2 (b) of Directive 98/24/EC and falls within the scope of this legislation. MOCA is also a carcinogen or mutagen for humans in accordance with Article 2(a) and (b) of Directive 2004/37/EC and falls within the scope of this legislation."

Due to its carcinogenic properties, as classified under the EU CLP Regulation (No 1272/2008), MOCA is included in Annex XIV of the REACH Regulation (EC) 1907/2006 as a substance of very high concern. This means that the substance cannot be used in the EU after the so-called 'sunset date' of 22 November 2017, without an authorisation from the European Commission. However, if the application is submitted before the 'latest application date' (22 May 2016), the applicant can continue to use the substance after the 'sunset date', while waiting for the Commission decision.

## 4. Existing Occupational Exposure Limits

In table 4 an overview on existing international limit values for MOCA is given. The data are taken from the GESTIS database on International limit values for chemical agents[8]. This is a curated database gathering entries from 30 countries.

[8] http://limitvalue.ifa.dguv.de/

**Table 4: Existing Occupational Exposure Limits (OELs) for MOCA (data from GESTIS International Limit Values (2017))**

| Country/ Organisation | Limit value –eight hours (ppm) | Limit value –eight hours (mg/m³) | Limit value short term (ppm) | Limit value –Short term (mg/m³) |
|---|---|---|---|---|
| Australia | 0,02 | 0,22 | | |
| Austria | | 0,02 | | 0,08* |
| Belgium | 0,01 | 0,11 | | |
| Canada - Ontario | 0,0005 | 0,005 | | |
| Canada - Québec | 0,02 | 0,22 | | |
| Denmark | 0,01 | 0,11 | 0,02 | 0,22 |
| Finland | 0,01 | 0,11 | | |
| France | 0,02 | 0,22 | | |
| Ireland | | 0,005 | | |
| Japan | | 0,005 | | |
| New Zealand | | 0,005 | | |
| Singapore | 0,01 | 0,11 | | |
| South Korea | 0,01 | 0,11 | | |
| Spain | 0,01 | 0,1 | | |
| Switzerland | | 0,02 | | |
| The Netherlands | | 0,02 | | |
| USA - NIOSH | | 0,003 | | |
| United Kingdom | | 0,005 | | |

* Technical Reference Concentration-(TRK) value (based on technical feasibility)

A biological limit value for total MOCA in urine (free and conjugated MOCA measured after hydrolysis) has been set in Finland (The Ministry of Social Affairs and Health[9], 2014) to be 5 µmol/mol creatinine.

## 5. Occurrence, Use and Occupational Exposure

MOCA is used primarily to produce polyurethane articles. Polyurethanes are produced by the reaction of a liquid isocyanate with a blend of liquid polyols, catalysts and other additives. MOCA is used as an additive in the polyol blend with the purpose to give the resulting polymer specific properties. Depending on the function MOCA has within the polymer, four uses can be differentiated: curing agent, cross-linker, chain extender and pre-polymer. The only further registered use is as laboratory chemical[10].

ECHA received one full, joint registration for MOCA, indicating use of 1000-10000 tonnes per year. There are no registrations as an on-site isolated intermediate which would indicate other uses.

As noted in Section 3, MOCA is subject to Authorisation under REACH. To date, ECHA received only one application[11] for authorisation for MOCA covering its industrial use as a curing agent/chain extender in cast polyurethane elastomer production, using a total of ca. 500 tonnes per year, at ca. 89 potential sites in the EU (manufacturing of MOCA is reportedly outside the EU) and the total number of potentially exposed workers estimated by the applicant is ca. 200. MOCA is consumed during the polymerisation reaction and is therefore unlikely to be contained in finished articles (see

[9] http://www.julkari.fi/bitstream/handle/10024/116148/URN_ISBN_978-952-00-3479-5.pdf

[10] https://echa.europa.eu/documents/10162/13641/report_moca_mda_edc_diglyme_en.pdf/74327376-608c-4bca-bed8-70ca7ec85cb3

[11] https://echa.europa.eu/documents/10162/8cd19123-4fbe-4676-a2a3-791c63c56b1d

further below). RAC proposed a stringent set of conditions in case the authorisation would be granted. These conditions aim for a higher degree of automation and containment of the process, better extraction of process emissions, improved cleaning and maintenance procedures and improved overall occupational hygiene measures. Furthermore proper training and supervision of the workers needs to be ensured. In order to improve the exposure assessment and ensure the success of the previous conditions twice yearly biomonitoring programmes must be in place accompanied by testing for possible surface contamination.

Similar information, as listed in the SCOEL recommendation (2010), exists about uses outside the EU. According to the National Toxicology Program (NTP) 2002 MOCA is used primarily as a curing agent for polyurethane pre-polymers in the manufacture of castable urethane rubber products, such as absorption pads and conveyor belts. In the Far East it is used as a curing agent in roofing and wood sealing (IARC 1993). There are recent reports on exposures of polyurethane production workers to MOCA (Fairfax and Porter 2006, Cocker et al. 2009).

The major exposure route for MOCA is the dermal route. Therefore, MOCA residues in urinary samples of workers are more appropriate than concentrations in air only, to indicate and assess exposure. However, biomonitoring should be complemented with air monitoring and, when appropriate, measurements of skin and surface contamination in order to identify exposure sources. . Some monitoring studies focusing on residues at the workplace and in worker's urine have been carried out in the polyurethane sector, in which the substance is mainly used. Those studies have shown a potential for significant occupational exposure. Monitoring information provided during the public consultation on ECHA's draft Annex XIV recommendation (2012) shows that proper handling of MOCA and effective implementation of risk management measures is essential to reduce releases and occupational exposure

At industrial sites, usually technical means (e.g. stoichiometric relation between curing agent and monomers) are in place that ensure that content of unreacted MOCA is minimised (<< 0.1 %). However, where such measures are not taken, the content of unreacted MOCA increases quickly and free MOCA might be present in final articles above amounts of 0.1 % by weight (ECHA background document 2012[12]).

It is also noted that when heated MOCA emits toxic fumes of hydrochloric acid and other chlorinated compounds, as well as nitrogen oxides (NTP 2002 in SCOEL Recommendation 2010).

## 6. Monitoring Exposure

Analytical methods for the determination of MOCA in workplace air are described by HSE and the United States Department of Labor.

The procedure for determination of MOCA and other aromatic amines as described by HSE[13] ' Aromatic amines in air and on surfaces' laboratory method using pumped acid coated filters moistened swabs and HPLC' is based on the sampling of the inhalable dust fraction in the workplace air and subsequent HPLC with a reported limit of detection of 0.2 µg/m³. According to the method by the United States Department of Labor[14] (OSHA method ORG-71- July 1989) a closed-face three-piece cassette is employed for the sampling of the workplace air with subsequent derivatisation to form the heptafluorobutyric acid anhydride derivate of MOCA followed by gas chromatography with an electron capture detector. The limit of quantification is reported to be 0.44 µg/m³.

The biological limit value as set by Finland refers to the method of HSL[15] as outlined by Cocker et al. (1996). From post shift urine samples MOCA is extracted by means of either solid-phase

[12] https://echa.europa.eu/documents/10162/13640/backgroundoc_moca_en.pdf/540e5add-dd8c-46dd-bfe1-4879967b33af

[13] http://www.hse.gov.uk/pubns/mdhs/pdfs/mdhs75-2.pdf

[14] https://www.osha.gov/dts/sltc/methods/organic/org071/org071.html

[15] http://www.hsl.gov.uk/media/66145/mboca_urine.pdf

extraction or solvent extraction and subsequent analysis by HPLC with an electrochemical detection. Further information on biomonitoring methods can be found from Annex to SCOEL/SUM/174 (2013).

## 7. Health Effects

For the evaluation of health effects please see the SCOEL Recommendation in Appendix 1 – "Recommendation from the Scientific Committee on Occupational Exposure Limits for 4,4'-Methylene-bis-[2-chloroaniline] (MOCA) _ SCOEL/SUM/174 June 2010/Annex March 2013".

RAC concludes that the critical endpoint for establishing an OEL is carcinogenicity. However, it is considered a non-threshold genotoxic carcinogen with respect to risk characterisation

## 8. Cancer Risk Assessment

For the cancer risk assessment, the evaluation is based on the RAC Dose Response report (See Appendix 2 – "RAC Dose Response Relationship for Carcinogenicity of MOCA").

Although SCOEL describes the human and animal evidence on the carcinogenicity of MOCA, it does not calculate dose-responses for the carcinogenicity of MOCA. In its biomonitoring annex it refers to unit cancer risk estimates derived by DECOS (2000)[16] and calculates cancer risk for different urinary MOCA levels. SCOEL gave these estimates for information only, and do not further discuss or take a stand on the appropriateness of the DECOS cancer risk evaluation (See Appendix 1 –_ SCOEL/SUM/174 June 2010/Annex March 2013).

RAC reconfirms its earlier recommendations as to the cancer risk estimations for MOCA.

## 9. Groups at Extra Risk

None identified

[16] https://www.gezondheidsraad.nl/sites/default/files/0009osh.pdf

# Appendix 1. Recommendation from the Scientific Committee on Occupational Exposure Limits for 4,4'-Methylene-bis-[2-chloroaniline] (MOCA)[17]

| | | |
|---|---|---|
| 8-hour TWA | : | not feasible to derive a health-based limit (see Recommendation) |
| STEL (15 min) | : | not feasible to derive a health-based limit (see Recommendation) |
| Additional classification | : | "Skin" notation |
| SCOEL carcinogen group | : | A (non-threshold genotoxic carcinogen) |
| Biological monitoring | : | See Recommendation [a] |

[a] See also Recommendation for a Biological Guidance Value (Annex March 2013).

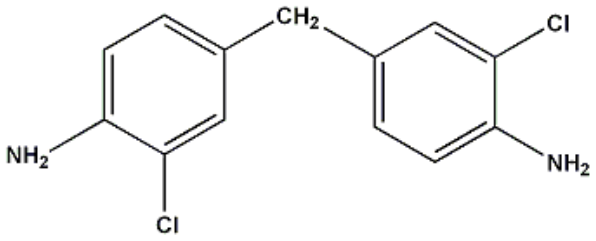
*Substance identification:*

4,4'-Methylene-bis-[2-chloroaniline]

*Synonyms:*

MOCA, MBOCA, bis-(4-amino-3-chlorophenyl) methane, bis-(3-chloro-4-aminophenyl)-methane, 3,3'-dichloro-4,4'-diaminodiphenylmethane, methylene-bis-(3-chloro-4-aminobenzene), 4,4'-methylene-bis-(o-chloro-aniline).

*Structural formula:*




$C_{13}H_{12}Cl_2N_2$

*EU classification:* Carc. Cat. 2; R45May cause cancer

Xn; R22 Harmful if swallowed.
N; R50-53Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

[17] SCOEL/SUM/174 June 2010, with Annex March 2013: ec.europa.eu/social/BlobServlet?docId=6929&langId=en

CAS No.: 101-14-4

Molecular weight: 267.16

Melting point: 100-110°C

Boiling point: not specified (decomposition)

Conversion factor: 1 ppm = 10.9 mg/m$^3$; 1 mg/m$^3$ = 0.090 ppm

This summary document is based on documentations of IARC (1993), DFG (1996) and NTP (2002), supplemented by a recent literature search of SCOEL.

**1. Occurrence, use and occupational exposure**

4,4'-Methylene-bis-(2chloroaniline) [in this Summary document referred to as MOCA = 4,4'-methylene-bis-(*o*-chloro-aniline)] is used primarily as a curing agent for polyurethane pre-polymers in the manufacture of castable urethane rubber products, such as absorption pads and conveyor belts (NTP 2002). In the Far East, it is used as a curing agent in roofing and wood sealing (IARC 1993). There are recent reports on exposures of polyurethane production workers to MOCA (Fairfax and Porter 2006, Cocker et al. 2009).

MOCA occurs as tan-coloured pellets or flakes with a faint, amine-like odour. It is soluble in alcohol, ether, most organic solvents, and lipids, and barely soluble in water. When heated, it emits toxic fumes of hydrochloric acid and other chlorinated compounds, as well as nitrogen oxides (NTP 2002).

**2. Health significance**

MOCA has been anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals (IARC 1993). As an aromatic amine with structural similarity to benzidine, the likely human target of carcinogenicity is the urothelium, which is underlined by human case studies. The genotoxicity of MOCA is straightforward. Just recently, MOCA has been upgraded by IARC (2010) to be a "Group 1" carcinogen, supported by mechanistic and other relevant data.

**2.1. Toxicokinetics/metabolism**

MOCA is taken up through both the respiratory tract and the skin; most of the absorbed substance is excreted within a few days in the urine and faeces (see also 2.2.1). There has been considerable occupational exposure by cutaneous absorption in early years of use of MOCA, as evidenced by urine analysis (IARC 1993). The rapid skin penetration of MOCA has also been confirmed experimentally with human skin in vitro (Yun et al. 1992). Most authors consider that absorption through the skin is the major route of uptake of the substance at the workplace (Clapp *et al.* 1991, Edwards and Priestly 1992, Linch *et al.* 1971, Lowry and Clapp 1992).

Studies in rats and dogs have demonstrated that MOCA metabolites bind covalently to macromolecules, such as DNA and proteins (see DFG 1996 for details).

The metabolic pathways of MOCA have been well investigated experimentally. These were comprehensively reviewed by IARC (1993) and DFG (1996). *Figure 1* represents a summary scheme of mammalian metabolism, as compiled by DFG (1996).

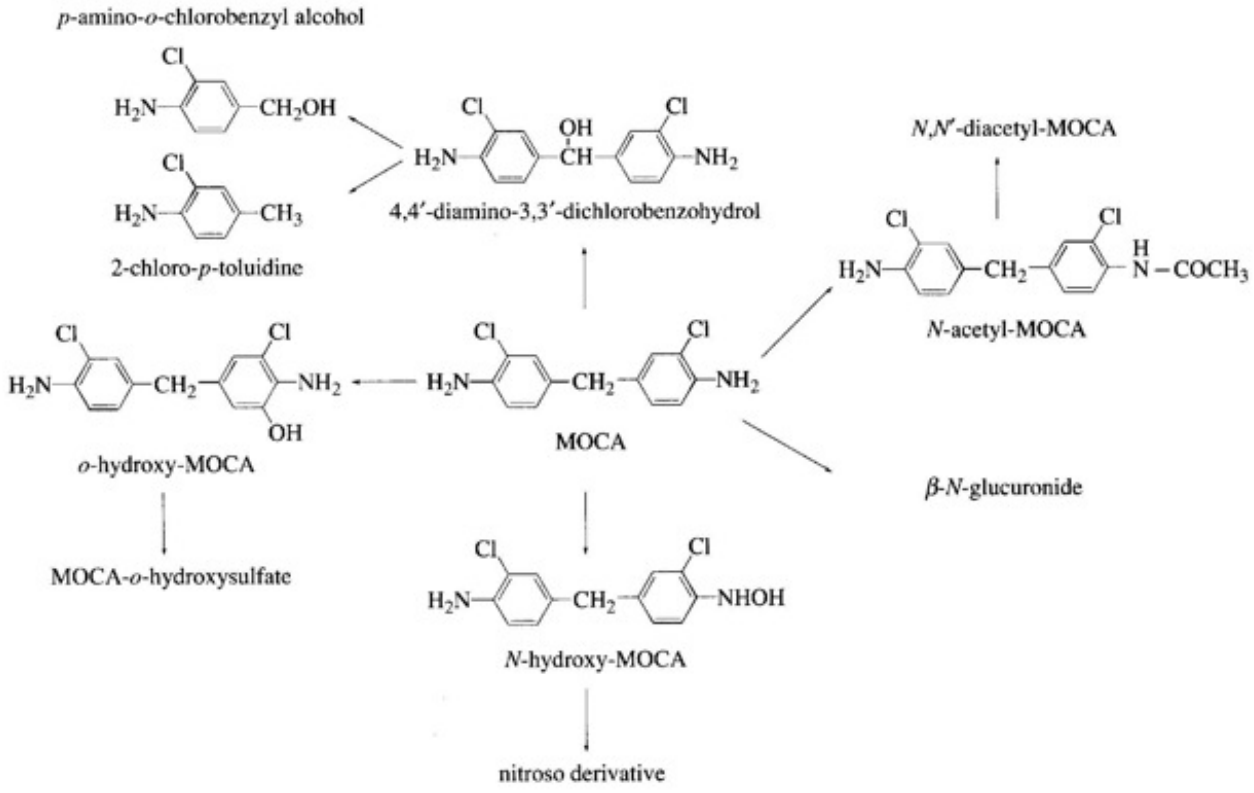



*Figure 1:* Formation of metabolites from MOCA, based on experimental data, according to DFG (1996).

The covalent binding of MOCA to haemoglobin is comparable to that of other bicyclic aromatic amines, such as 4,4'-methylenedianiline, 4,4'-oxydianiline, 4,4'-thiodianiline or benzidine (Sabbioni and Schütze 1998). Analysis of the haemoglobin adducts has been recommended as a means of biological monitoring (Bailey et al. 1993, Vaughan and Kenyon 1996).

Urinary metabolites of MOCA detected in humans include its N-acetyl derivative and its N-glucuronide. Urinary thioethers were not detected (IARC 1993). Recent studies on MOCA-exposed humans are focussed on analysis of free and conjugated MOCA and *N*-acetyl-MOCA in the urine, with a focus on biological monitoring (Wu et al. 1996, Robert et al. 1999a,b, Shih et al. 2007). Methods for the determination of MOCA in human plasma have been described (Vaughan and Kenyon 1996).

### 2.1.1. Biological monitoring

The dose-dependence of haemoglobin adducts of MOCA has been studied experimentally in rats (Bailey et al. 1993, Sabbioni and Schütze 1998); however, there is only very limited data on this parameter in exposed humans (Vaughan and Kenyon 1996).

Most authors reporting on biomonitoring results have studied the urinary excretion of conjugated MOCA (i.e. total MOCA after acid hydrolysis of the conjugates). Reliable analytical methods are available (Wu et al. 1996, Robert et al. 1999a, Shih et al. 2007, Cocker et al. 1996, 2007), and typical detection limits were reported in the order of 1 µg/L (Wu et al. 1996, Robert et al. 1999a).

In a study conducted in *France*, urinary MOCA was measured in samples collected at the end of workshift. Fourty workers from four factories were observed for three consecutive days. For all factories, the postshift urinary MOCA concentrations ranged between 1 µg/L (detection limit) and 570 µg/L; workers handling crystallized MOCA excreted the highest amounts of MOCA in urine. The urinary MOCA concentrations (median) were: 84.0 µg/L (mixer), 15.5 µg/L (moulder), 59.0 µg/L (maintenance) and 3.0 µg/L (others) (Robert et al. 1999b).

A recent study in the *United Kingdom* was designed to gather information about the current controls and levels of MOCA exposure in a representative cross section of workplaces in the manufacture polyurethane elastomers. Urine samples (n = 79) were collected and 49% were below

the detection limit for MOCA; only three samples had levels of MOCA that exceeded the U.K. Biological Monitoring Guidance Value of 15 µmol/mol [35.43 µg/g] creatinine. The highest urinary MOCA concentrations were in samples from workers casting and moulding. The 90th percentile of the urine MOCA results was 8.6 µmol MOCA per mol [20.31 µg/g] creatinine (Cocker et al. 2009).

The levels of MOCA in urine of five individuals who were exposed to MOCA during the manufacture of polyurethane elastomers in *Australia* were determined. The MOCA concentrations in urine ranged from 4.5 to 2390 nmol/L [1.20 – 638 µg/L] (Vaughan and Kenyon 1996).

Urinary MOCA levels were also reported for 54 MOCA-exposed workers in *Taiwan*. The median excretion was reported as 38.6 ng/mL [=µg/L] (Shih et al. 2007).

### 2.2. Acute toxicity

#### 2.2.1. Human data

In an accident in a Canadian factory, hot liquid MOCA was sprayed over the face of a worker and into his mouth. He was wearing safety glasses. In the hospital, conjunctivitis was diagnosed. The man complained of burning in the eyes and face and feeling ill in the stomach. Urine analysis revealed rapid excretion of MOCA during the first 24 hours (Hosein and van Roosmalen 1978).

A 30-year old polyurethane worker was sprayed accidentally with about 12 litres of molten MOCA on his upper body and extremities. He was wearing working trousers, a shirt with rolled-up sleeves, asbestos gloves, safety glasses and respirator. The substance was not swallowed; the exposure period was restricted to the time required to disrobe, shower and gently wash off the residual substance (about 45 minutes). Initially the man complained of a sensation like mild sunburn on the arms. No further symptoms were reported during the 14 day period following the accident. Tests for liver and kidney function yielded normal results. There was no methaemoglobinaemia, haematuria or proteinuria. In the urine collected 4 hours after the accident the highest level of MOCA, 1700 µg/1, was found and levels of 100 µg/l were detected during the subsequent 4 days. The excretion half-time was calculated to be 23 hours (Osorio et al. 1990).

#### 2.2.2. Animal data

Intraperitoneal administration of MOCA at a single dose of 64 mg/kg body weight to B6C3F$_1$ mice killed half of the animals within 7 days; after 85 mg/kg, half of the animals died within 4 days (Salamone 1981).

### 2.3. Irritation and corrosivity

An individual who was sprayed with three gallons of molten MOCA reported an initial „mild sunburn" sensation on the arms, but no further symptom was found in a two-week follow-up period. Renal and liver function tests were normal, and methaemoglobinemia, haematuria and proteinuria were not observed (Osorio et al. 1990). The initial responses in the worker sprayed in the face with MOCA were conjunctivitis, a burning sensation in the eyes and face and nausea (Hosein and van Roosmalen 1978).

### 2.4. Sensitisation

There are no published data on sensitisation.

### 2.5. Repeated dose toxicity

There are only limited data on repeated dose toxicity.

#### 2.5.1. Human data

In occupationally MOCA-exposed persons haematuria has occasionally been described (Mastromatteo 1965), but otherwise, even after long-term occupational exposure, no non-neoplastic chronic effects.

#### 2.5.2. Animal data

In a nine-year chronic study in dogs (Stula et al. 1977), elevated levels of plasma glutamic-pyruvic transaminase were noted during the first and last two years of treatment, accompanied by urinary changes indicative of genitourinary cancer after seven years.

MOCA also induces enzymes involved in drug metabolism and cell proliferation. Single intraperitoneal injections of technical-grade MOCA (purity, 90-100%) to male Sprague-Dawley rats at doses of 0.4-100 mg/kg bw in dimethyl sulfoxide resulted in dose-dependent increases in the levels of microsomal epoxide hydratase, ethoxyresorufin O-deethylase, ethoxycoumarin O-deethylase and glutathione S-transferase, but a decrease in aldrin epoxidase activity (Wu et al., 1989). Ornithine decarboxylase, which regulates polyamine synthesis and cell division and is increased by tumour promoters, was strongly induced in male Sprague-Dawley rats 12 h after intraperitoneal injection of 75 mg/kg bw MOCA in corn oil; the level returned to control values after 42 h (Savage et al., 1992).

### 2.6. Genotoxicity

#### 2.6.1. In vitro

The mutagenicity of MOCA has been investigated in numerous short-term tests. The substance has mutagenic activity in the standard Ames test in *Salmonella typhimurium* TA100 and generally also in strain TA98, only in the presence of S9 mix. Numerous tests for DNA damage, sister chromatid exchange and transformation also yielded positive results. The results of the individual studies were tabulated in detail by both IARC (1993) and DFG (1996), to which reference is made.

Of the metabolites of MOCA which have been tested, N-hydroxy-MOCA has been shown to be mutagenic without metabolic activation in the two *S. typhimurium* strains TA100 and TA98; *o*-hydroxy-MOCA and 4,4'-methylene-bis-(2-chloro-nitrosobenzene) had no mutagenic activity and the mono-nitroso metabolite yielded negative results in strain TA100 and weak positive results in TA98 (Kuslikis et al. 1991).

In essence, MOCA has clear genotoxic properties. According to the detailed evaluation of IARC (1993) MOCA induced DNA damage in prokaryotes, cultured mammalian and human cells and in animals treated in vivo. Gene mutation was induced in bacteria and cultured mammalian cells, but not in yeast. Equivocal results for mitotic recombination were obtained in yeasts. Aneuploidy was induced in yeast and sister chromatid exchange, transformation and inhibition of intercellular communication in cultured mammalian cells.

#### 2.6.2. In vivo - Human data

In exfoliated urothelial cells obtained from urine collected at various times (up to 430 hours) after accidental acute dermal exposure of a worker to molten MOCA (see also 2.2.1; Osorio et al. 1990), the MOCA-DNA adduct, *N*-(deoxyadenosin-8-yl)-4-amino-3-chlorobenzyl alcohol, was demonstrated for the first time in man. The adduct was found in the urine samples obtained between 4 and 98 hours after the accident, but not in later samples (Kaderlik et al. 1993).

From a cohort of 11 workers (10 men, 1 woman) divided into three groups according to the level of MOCA exposure, urine and blood samples were collected simultaneously in the middle of the working week, both before and after the shift, to determine the incidence of sister chromatid exchange (SCE). The control group comprised 6 men and 4 women from a works with no MOCA exposure. In the peripheral lymphocytes there was a gradual, apparently exposure-related increase in SCE from the control group to the group of MOCA process workers. In spite of the classification of the workers as smokers and non-smokers, the small numbers involved preclude the drawing of further conclusions (Edwards and Priestly 1992).

In an Australian cohort of MOCA workers, Murray and Edwards (1999, 2005) showed an elevation of micronucleated cells in the exfoliated urothelium, pointing to genotoxicity at the urothelial target site.

#### 2.6.3. In vivo - Animal data

Micronuclei were induced in the bone marrow of mice treated with MOCA in vivo, and sister chromatid exchange (SCE) was induced in the bone marrow of rats treated in vivo (IARC 1993).

### 2.7. Carcinogenicity

### 2.7.1. Human data (DFG 1996)

Systematic clinical and cytological examination of 31 workers who had been exposed to MOCA for between 6 months and 16 years revealed no signs of cancer although occupational exposure at varying levels was confirmed by urine analysis. Likewise, negative results were obtained in a study of medical reports for 178 other workers who had been exposed more than 10 years previously (Linch et al. 1971).

It has been reported in a review that a cohort study in a MOCA production works revealed 13 new cases of bladder cancer in a period of only a few years; this is many more than expected (Cartwright 1983). The details of this study have not been published.

In a systematic examination of 540 workers who worked in a factory producing MOCA between 1968 and 1979 and of 20 other workers employed from 1980-1981, two cases of bladder tumours were found in the years 1986 and 1987; the men were aged 28 and 29 and were non-smokers. The first worker had been employed for 1 year (1978, 8 years before the tumour diagnosis) in the MOCA production plant as a pipefitter and maintenance man. According to his own report, the man worked directly on the MOCA process for about 4 to 6 hours per week and did not always wear gloves. A non-invasive, papillary transitional cell tumour grade 1-2 was diagnosed in the urinarg bladder. The second worker had been employed for 9 months (1976, 11 years before the tumour diagnosis) in MOCA production where he operated the drying oven and packed the substance into barrels. These were the jobs at the plant with the greatest potential MOCA exposure. He reported that he used a respirator and wore gloves and overalls. A papillary urothelial neoplasm, grade 1, was diagnosed. Apart from their exposure in this factory, neither of these workers had been exposed to potential bladder carcinogens. In 1988, a noninvasive papillary transitional cell carcinoma, grade 1 was detected in a third worker (at this time 200 persons from the original cohort had been subjected to cystoscopic examination). The man was 44 years old and an ex-smoker. He had worked for 1.5 months in direct contact with MOCA and following his employment in the MOCA plant had held other jobs in the chemical industry (Ward et al. 1988, 1990).

Two more recent reports from Taiwan describe single cases of urothelial neoplasia in workers exposed to MOCA (Liu et al. 2005, Lu et al. 2005).

### 2.7.2. Animal data (evaluation of IARC 1993)

*Mouse:* Groups of 25 male and 25 female HaM/ICR mice, six to eight weeks old, were fed diets containing 0, 1000 or 2000 mg/kg of diet (ppm) MOCA as the hydrochloride (97% pure) for 18 months. Surviving animals were killed 24 months after the start of the study; about 55% of the control and treated mice were still alive at 20-22 months. The effective numbers of animals at the end of the study were: males-control, 18; low-dose, 13; high-dose, 20; females-control, 20; low-dose, 21; high-dose, 14. Haemangiomas or haemangiosarcomas (mainly subcutaneous) combined occurred in 0/18 control, 3/13 low-dose and 8/20 high-dose male mice. "Hepatomas" occurred in 0/20 control, 9/21 low-dose and 7/14 high-dose female mice ($p < 0.01$, Fisher exact test). The incidence of lymphosarcomas and reticulum-cell sarcomas was decreased in treated females. The authors stated that the incidence of vascular tumours in the high-dose animals was comparable to that in historical controls of the Same strain (Russfield et al. 1975).

*Rat:* Groups of 25 male and 25 female Wistar rats, 100 days [14 weeks] of age, were fed 0 or 1000 mg/kg of diet (ppm) MOCA [purity unspecified] in a protein-deficient diet [not otherwise specified] for 500 days [71 weeks] [total dose, 27 g/kg bw], followed by an observation period with protein-deficient diet. Animals were killed when moribund; mean survival of treated males and females was 565 days [81 weeks] and 535 days [76 weeks], respectively, and mean survival of male and female controls with a similar diet was 730 days [104 weeks]. Of the 25 treated males, 23 died with tumours; "hepatomas" occurred in 22/25 [$p < 0.001$, Fisher exact test], and hing tumours (mainly carcinomas) in 8/25 [$p = 0.002$, Fisher exact test]. Among the treated females, 20 rats died with tumours; "hepatomas" occurred in 18/25 [$p < 0.001$ Fisher exact test], and lung tumours were observed in 5/25 [$p = 0.025$, Fisher exact test]. No "hepatoma" or lung tumour was observed among control animals (Grundmann and Steinhoff 1970).

Groups of 25 male Charles River CD-1 rats, six to eight weeks old, were administered diets containing 0, 500 or 1000 mg/kg of diet (ppm) MOCA as the hydrochloride (97% pure) for 18 months. All surviving animals were killed 24 months after the start of the study; about 55% of the

control and treated animals were still alive at 20-22 months. The effective numbers were: 22 control, 22 low-dose and 19 high-dose animals. `Hepatomas' occurred in 0/22 control, 1/22 low-dose and 4/19 high-dose rats [$p < 0.05$, Cochran-Armitage trend test] (Russfield et al. 1975).

Groups of 50 males and 50 female Charles River CD rats, 36 days [5 weeks] of age were administered 0 (control) or 1000 mg/kg of diet (ppm) MOCA (- 95% pure) in a standard diet (23% protein) for life. The average duration of the experiment was 560 days [80 weeks] for treated males, 548 days [78 weeks] for treated females, 564 days [80 weeks] for male controls and 628 days [89 weeks] for female controls. Six animals from each group were sacrificed at one year for interim evaluation. Lung adenocarcinomas occurred in 21/44 ($p < 0.05$, chi-square test) treated males and 27/44 ($p < 0.05$, chi-square test) treated females. An additional squamous-cell carcinoma of the lung was observed in one treated male and one treated female. No lung tumour was observed among control animals. Lung adenomatosis, considered to be a preneoplastic lesion, developed in 14/44 treated males and 11/44 treated females and in 1/44 male controls and 1/44 female controls ($p < 0.05$). Pleural mesotheliomas occurred in 4/44 treated males and 2/44 treated females; no such tumour was observed among controls.

Hepatocellular adenomas and hepatocellular carcinomas occurred in 3/44 and 3/44 treated males and in 2/44 and 3/44 treated females, respectively, but not in controls. Ingestion of MOCA resulted in a lower incidence of pituitary tumours in treated females than in controls (1/44 versus 12/44) (Stula et al. 1975).

In the same study, another 21 males and 21 females were administered 0 (control) or 1000 ppm MOCA (about 95% pure) in a low-protein diet (7%) for 16 months. The average duration of the experiment was 400 days [57 weeks] for treated males, 423 days [60 weeks] for treated females, 384 days [55 weeks] for control males and 466 days [66 weeks] for control females. Lung adenocarcinomas occurred in 5/21 treated males ($p < 0.05$, chi-square test) and 6/21 females ($p < 0.05$, chi-square test); no such tumour developed in 21 untreated male or female controls. Hepatocellular adenomas occurred in 5/21 treated males ($p < 0.05$, chi-square test) and 2/21 treated females; hepatocellular carcinomas were observed in 11/21 treated males ($p < 0.05$, chi-square test) and 1/21 treated females; no hepatocellular tumour was observed among 21 untreated males or females. Fibroadenomas of the mammary gland occurred in 1/21 treated and 7/21 control female rats ($p < 0.05$). Mammary gland adenocarcinomas developed in 6/21 treated female rats and in 0/21 untreated females ($p < 0.05$, chi-square test) (Stula et a1. 1975).

Groups of 100, 100, 75 and 50 male Charles River CD rats, 35 days [5 weeks] of age, were fed either a "protein-adequate" (27%) diet containing 0, 250, 500 or 1000 mg/kg of diet (ppm) MOCA (purity unspecified) or a "protein-deficient" (8%) diet containing 0, 125, 250 and 500 ppm MOCA for 18 months followed by a 32-week observation period. Animals were sacrificed at 104 weeks. Administration of MOCA was associated with decreased survival in both groups: mean survival time (weeks) was: "protein-adequate" diet: control, 89; low-dose, 87; mid-dose, 80 ($p < 0.01$); high-dose, 65 ($p < 0.001$); "protein-deficient" diet: control, 87; low-dose, 81; mid-dose, 79; high-dose, 77 ($p < 0.05$). The numbers of rats on the "protein-adequate" diet still alive at week 104 were: control, 20/100; low-dose, 14/100; mid-dose, 10/75; and high-dose, 0/50 (at 84 weeks, there were six surviving rats). The numbers of animals on the "protein-deficient" diet still alive at week 104 were: control, 34/100; low-dose, 22/100; mid-dose, 14/75; and high-dose, 5/50. MOCA induced several tumour types in both groups. Dose-related increases in the incidences of lung tumours, mammary adenocarcinomas, Zymbal gland carcinomas and hepatocellular carcinomas were observed in both experiments. The highest tumour incidence was observed in the lung. An increased incidence of haemangiosarcomas was observed only in the group on the "protein-deficient" diet. In groups given 500 ppm MOCA, tumour incidence was generally lower in those fed "protein-deficient" diet, but hepatocellular carcinomas and Zymbal gland carcinomas occurred at a higher incidence in this group (18 and 12%) than in the `protein-adequate' group (4 and 7%). The incidence of pituitary adenomas decreased with increasing concentration of MOCA in the "protein-adequate" diet, perhaps because of decreased survival in the treated groups (Kommineni et al. 1979).

*Dog:* A group of six female beagle dogs, approximately one year old, were administered a daily dose of 100 mg MOCA (purity about 90%, 10% polyamines with a three-ring structure and 0.9% o-chloroaniline) by capsule on three days a week for six weeks, then on five days a week for up to nine years. A further group of six females served as untreated controls. One treated dog died

early, at 3.4 years of age, because of intercurrent infection; the other animals were killed between 8.3 and nine years. Transitional-cell carcinomas of the urinary bladder occurred in four of five treated dogs, and a composite tumour (transitional-cell carcinoma/adenocarcinoma) of the urethra developed in one dog. No such tumour was observed among six untreated controls ($p < 0.025$, Fisher exact test) (Stula et al. 1977).

*Subcutaneous administration:* In a study reported as a short communication, groups of 17 male and 17 female Wistar rats [age unspecified] were injected subcutaneously with 500 or 1000 mg/kg bw MOCA (94% pure) as a suspension in saline either once a week or at longer time intervals for 620 days [88 weeks] (total dose, 25 g/kg bw). The rats were fed a laboratory diet with normal protein content. The mean observation period was 778 days [111 weeks]. A total of 22 animals developed 29 malignant tumours. Hepatocellular carcinomas occurred in 9/34 [$p < 0.0042$, Fisher exact test], and malignant lung tumours (six adenocarcinomas, one carcinoma) were observed in 7/34 [$p < 0.016$, Fisher exact test]. A malignant subcutaneous tumour [unspecified] was found in one rat [sex unspecified]. Among 25 male and 25 female untreated controls (mean observation period, 1040 days [148 weeks]), a total of 13 malignant tumours, including one lung tumour, developed; no hepatocellular carcinoma was observed (Steinhoff and Grundmann 1971).

### 2.8. Reproductive toxicity

There are no published data on reproductive toxicity.

### Recommendation

MOCA is a genotoxic carcinogen. Rats, dogs and humans metabolize MOCA to *N*-hydroxy-MOCA by hepatic cytochromes P450; DNA adducts are formed by reaction with *N*-hydroxy-MOCA, and MOCA is genotoxic in bacteria and mammalian cells. The same major MOCA-DNA adduct is formed in the target tissues for carcinogenicity in animals (rat liver and lung; dog urinary bladder) as that found in urothelial cells from a man with known occupational exposure to MOCA (IARC 1993).

MOCA was tested for carcinogenicity by oral administration in the diet in mice in one study, in rats of each sex in two studies, in male rats in a further two studies using normal and low-protein diets and in capsules in female dogs. It was also tested by subcutaneous administration to rats in one study. Oral administration of MOCA increased the incidence of liver tumours in female mice. In a series of experiments in which rats were fed either standard or low-protein diets, it induced liver-cell tumours and malignant lung tumours in males and females in one study, a few liver-cell tumours in male rats in another, lung adenocarcinomas and hepatocellular tumours in males and females in a third and malignant lung tumours, mammary gland adenocarcinomas, Zymbal gland carcinomas and hepatocellular carcinomas in a fourth. Oral administration of MOCA to female Beagle dogs produced transitional-cell carcinomas of the urinary bladder and urethra. Subcutaneous administration to rats produced hepatocellular carcinomas and malignant lung tumours. MOCA has been classified by IARC as a Group 1 carcinogen, taking also into account mechanistic and other relevant data (IARC 2010). As an aromatic amine with (some) structural similarity to benzidine, the reasonable human target of carcinogenicity is the urothelium. This is supported by limited data in humans and by the induction by MOCA of urothelial carcinomas in the dog, which is known from experiments with other aromatic amines, which are clear human carcinogens (benzidine, 2-naphthylamine), to respond in this respect similar to humans.

Based on these data, MOCA is categorized into the *SCOEL carcinogen group A* as a genotoxic carcinogen to which a threshold cannot be assigned. Hence, a health-based OEL cannot be assigned to MOCA.

MOCA is easily absorbed via the skin. Therefore a "skin" notation is warranted. This underlines the relevance of biological monitoring. For biological monitoring, the measurement of total (mostly conjugated) MOCA in post-shift urine appears as a means of choice. As MOCA is not a ubiquitous environmental contaminant or natural body constituent, any noticeable excretion above the detection limit points to occupational sources. In the United States, the ACGIH (2010) has listed total MOCA in urine as adopted biological exposure determinant, but has refrained from providing a numerical Biological Exposure Index "due to insufficient data". Based on national industry exposure data, the U.K. HSE (2009) has recommended that worker's exposure to MOCA should be as low as reasonable practicable, located below an airborne WEL (Working Exposure Limit) of 0.005 mg/m$^3$ MOCA and a BMGV (Biological Monitoring Guidance Value, based on the 90$^{th}$ percentile of data from

workplaces with good control) of 15 µmol MOCA/mol (35 µg/g) creatinine. However, Cocker et al. (2009) have indicated that this value should be further reduced, as it would no longer act as an effective stimulus to reduce exposure.

Reported values for MOCA excretion by exposed workers from different countries are summarized in chapter 2.1.1. This may serve as practical background information for the application of biological monitoring.

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# Annex to SCOEL/SUM/174, March 2013: Recommendation from the Scientific Committee on Occupational Exposure Limits for the Biological Guidance Value for 4,4'-Methylene-bis-(2-chloroaniline) [MOCA]

| | |
|---|---|
| BGV: | Detection limit of the method [LOD] [a] |
| Carcinogenic risk assessment: | See Recommendation section |

[a] See Chapter 4.

The present annex to the Recommendation from SCOEL on 4,4'-methylene-bis-(2-chloroaniline) [MOCA] presents further details on the possibilities of quantitation of exposure by biological monitoring and on associated cancer risk assessment. A Biological Guidance Value (BGV) is recommended.

## 1. Toxicokinetics

The toxicokinetics of MOCA has been described in SCOEL/SUM/174 (Section 2.1). Because of its low vapour pressure and ability to pass through the skin, skin contact to MOCA is often the most significant route of exposure. Therefore, biological monitoring plays an important role in the assessment of exposure to MOCA in occupational settings.

MOCA is activated by the cytochrome P450 system to reactive intermediates including *N*-hydroxy-MOCA, which is the main toxic DNA- and protein-reactive intermediate in MOCA metabolism (Cocker *et al* 1985). *N*-Hydroxy-MOCA has been shown to form adducts in human urothelial cells (Kaderlik *et al* 1993). After an acute high level exposure, DNA adduct levels were increased 4–98 hours after the exposure, the levels being highest at 4 hours (Kaderlik *et al* 1993).

Inactivation of MOCA occurs mainly through glucuronide- and acetyl-conjugation. MOCA is excreted in the urine as a free compound and as glucuronide or acetyl derivatives, the main metabolite in the urine being the *N*-glucuronide of MOCA (Cocker *et al* 1990). In the urine of exposed workers, MOCA-*N*-glucuronide levels 2–3 times higher than those of free MOCA have been found (Cocker *et al* 1990, Robert *et al* 1999a,b). The level of *N*-acetyl MOCA in urine is generally less than 10 % of the level of MOCA recovered in urine of exposed workers (Ducos *et al* 1985). After an acute high-level exposure, the excretion of MOCA in the urine was highest 4 hours after the exposure; 23 hours after the exposure 50 % of the dose was excreted (Osorio *et al* 1990). This suggests a rapid excretion of MOCA after an acute (dermal and/or inhalation) exposure.

## 2. Biological monitoring of MOCA

For biological monitoring of MOCA exposure, total MOCA (free and conjugated MOCA) can be determined in the urine. Analytical methods typically applied include high-performance liquid chromatography (HPLC) coupled with ultraviolet or electrochemical detection, or gas chromatography (GC) connected with mass spectrometric detection (Cocker *et al* 1988, Roberts *et al* 1999a, Vaughan *et al* 1996, Wu *et al* 1996, Okayama *et al* 1988).

Earlier, only the analysis of so-called free MOCA (i.e. MOCA detected without hydrolysis) was used. Later, it was found that MOCA is mostly excreted as labile glucuronide and acetyl conjugates, which can break down forming free MOCA during sample storage, thus affecting the final levels of free MOCA in the sample. Therefore, it was recommended to pre-treat samples to take into account these labile conjugates (Cocker *et al* 1988, Cocker *et al* 1990). There are different methods which have been used for this purpose. The method described by Cocker *et al* (1990) involves heat hydrolysis of labile conjugates followed by solid-phase extraction into 90 % acetonitrile, with separation of MOCA by reverse-phase HPLC and electrochemical detection. The detection limit of this method was reported as 10 nmol/l (~ 3 µg/l) (Cocker *et al* 2009). Also

alkaline hydrolysis has been used for the measurement of total MOCA in urine samples. Robert *et al* used a method involving stabilisation of MOCA by sulphamic acid followed by alkaline hydrolysis at 80 °C, a single isooctane extraction and HPLC analysis, either with UV or electrochemical detection. The detection limit of this method was 1 µg/l (UV detection) and 0.1 µg/l (electrochemical detection) (3.745 nmol/l and 0.37 nmol/l, respectively) (Robert *et al* 1999a).

Robert *et al* (Robert *et al* 1999b) compared the different methods to measure MOCA in urine. The methods tested involved the measurement of 1) "free" MOCA from non acid-stabilised urine, 2) acid-labile MOCA in sulphamic acid-stabilised urine samples without hydrolysis, 3) "heat-labile" MOCA in non-acid-stabilised urines and 4) total MOCA in urines after alkaline hydrolysis. The comparative results showed that the mean sulphamic acid-labile MOCA concentrations were close to the total and heat-labile MOCA concentrations. MOCA measured in sulphamic acid-protected urine samples without hydrolysis could, therefore, be used as a practical and reliable biological marker of exposure to MOCA. According to the correlations observed in this study, values of 100 and 45 µg/l of "free"' MOCA correspond to 130 and 60 µg/l sulphamic acid-labile MOCA, respectively, and values of 90 and 45 µg/l as heat-labile MOCA are equivalent to 60 and 30 µg/l of sulphamic acid-labile MOCA.

Shih *et al* (2007) described a method to assess MOCA and its acetyl metabolite by using a solid-phase extraction and liquid chromatography/tamdem mass spectrometry. The limits of quantification of this method were 1 µg/l for MOCA and 0.03 µg/l for acetyl-MOCA.

Since the half-life of MOCA is 23 hours, urine samples are recommended to be collected at the end of the work-shift.

Since MOCA has been shown to bind to haemoglobin, haemoglobin adduct analysis has also been suggested for the biological monitoring of MOCA (Bailey *et al* 1993, Vaughan and Kenyon 1996). The advantage of this method is that it reflects the levels of biologically active MOCA and integrates exposure over a period of several weeks. However, it is currently not in routine use for the biomonitoring of MOCA in Europe. Also, a method to detect DNA adducts by $^{32}$P-post labelling analysis in exfoliated urothelial cells has been described (Kaderlik *et al* 1993).

**3. Exposure to MOCA**

Usually, MOCA cannot be detected in the urine of occupationally non-exposed people (levels below current detection limits).

In 1996, Cocker *et al* (Cocker *et al* 1996) published results on the biological monitoring of MOCA (free and heat-labile MOCA) in the UK industry during 1977–1994. These results showed a steady decline in urinary MOCA levels during this period. The 90th percentiles declined from 180 µmol/mol creatinine at 1977 to 15 µmol/mol creatinine at 1993–1994. Based on these results the UK HSE proposed a biological guidance value of 15 µmol/mol creatinine for MOCA.

Robert *et al* (Robert *et al* 1999b) published results on the biological monitoring programme of 40 workers in three polyurethane factories in France with potential exposure to MOCA. The results (measurements using sulphamic acid pre-treatment without alkaline hydrolysis, see above) showed levels varying with job categories, with highest levels in mixers and maintenance workers. Also a variation between the factories was seen. Combined results showed a geometric mean of 12.8 µg/l, with a range of 0.5–570 µg/l. There were, however, significant differences between factories with factory B showing the lowest exposure levels (geometric mean 2.9, range 0.5–47 µg/l). Differences were explained by differences in exposure conditions, including use of enclosed MOCA handling systems with hoods, glove boxes and local exhaust ventilation during the loading of MOCA vessels. It was concluded that at the present time (1999), it was possible in France to reach urinary MOCA levels of around 20 µg/l, expressed as sulphamic acid-labile MOCA. Therefore, a guidance value (based on current feasibility) of 20 µg/l was proposed (~30 µg/l of total MOCA, corresponding to 112 nmol/l).

Cocker *et al* (2009) published results from an occupational survey of 2 suppliers and 20 workplaces using MOCA in the UK. The survey included an assessment of types of exposure controls and nature of work activities. Collected samples were from workplace air (personal and static), glove samples, surface wipes and urine samples. Urine samples were from workers involved in the weighing, melting and pouring of MOCA and from some indirectly exposed workers. Of 80 personal assessed exposures to MOCA by inhalation, only 16 % were above the detection limit (10 nmol/l)

for MOCA and only two exceeded the UK workplace exposure limit of 5 µg/m$^3$. The mean exposure was 2.4 µg/m$^3$. About 60 % of surface samples had detectable MOCA contamination, ranging from 0.019 to 400 µg/cm$^2$. Contaminations of both inner and outer gloves were also detected (48 % and 90 % had detectable levels, respectively). Urine samples were obtained from 78 workers in 18 companies using MOCA, and from one supplier. Urinary analyses were performed according to the method of Cocker *et al* (Cocker *et al* 1988, Cocker *et al* 1990). MOCA was detected in 51 % of the samples, but only 3 samples exceeded the proposed guidance value of 15 µmol/mol creatinine. The maximum urinary concentration of MOCA was 25 µmol/mol creatinine from a moulder. The 90th percentile of all the urine results was 8.6 µmol/mol creatinine. Among workers directly exposed to MOCA (n = 59) the 90th percentile was 11.7 µmol/mol creatinine and among those (n = 19) who were not directly exposed but who might have been exposed if best practice was not followed the 90th percentile was 2.9 µmol/mol creatinine. Since there was a clear need for improvements in occupational hygiene at these workplaces, it was concluded that a guidance value based on the 90th percentile of data from workplaces with good control should be less than the 90 % value of 8.6 µmol/mol creatinine found in this study. It was also noted that the current UK guidance value of 15 µmol/mol creatinine would no longer be a stimulus to further reduce exposure (Cocker *et al* 2009).

In a follow-up study performed in 2008, a total of 19 polyurethane manufacturing sites were visited and altogether 446 post-shift urine samples were collected from 90 different workers (Keen *et al* 2012). One hundred and seventy samples had no detectable MOCA (LOD 0.4 µmol/mol), the median was 1.4 µmol/mol and the 90 % value was 10 µmol/mol. There was a positive correlation between glove contamination and urinary MOCA levels, but not between surface contamination at the workplace and urinary levels. Marked differences in urinary MOCA values between different workers performing similar tasks were noted. After detailed feedback of monitoring results to the companies involved in the survey, a significant decrease in urinary MOCA levels was seen in routine monitoring, the 90 % value decreasing from 10 µmol/mol to 3 µmol/mol.

The Finnish Institute of Occupational Health (FIOH) publishes yearly results from monitorings of the Finnish industry. The total number of MOCA measurements during the years 2000–2008 was 49 (FIOH 2000-2008). Most of the samples were derived from workers involved in the manufacturing of polyurethane coatings. MOCA was measured as total MOCA using alkaline hydrolysis. Most of the values were < 5 µmol/mol creatinine, the range being between below the LOD (1 µmol/mol creatinine) and 10 µmol/mol creatinine (FIOH 2000-2008). The 95th percentile of these measurements (n = 49) was 3.4 µmol/mol creatinine (FIOH, unpublished data). Based on these data, FIOH proposed in 2008 a "biological action limit" value of 5 µmol/mol creatinine for total MOCA (FIOH 2008). The cancer risk for this exposure level was assessed on the basis of the available information. DECOS has estimated using linear extrapolations from animal testing that the cancer risk of MOCA at a daily dose level of 1 mg/kg is $3.7 \times 10^{-2}$ (DECOS 2000). This corresponds to a total risk of $1.9 \times 10^{-4}$ for a worker weighing 70 kg, with 40 years working life, working 48 weeks/year, 5 days/week and 8 hours/day. Assuming that the half-time is 23 hours (open one-compartment model; steady state after one-week exposure), the average urinary concentration of MOCA at steady state is 2.6 µmol/mol creatinine when the concentration in the Friday afternoon sample is 5 µmol/mol creatinine. Noting that the average daily excretion of creatinine for a 50-year old man of 70 kg is 12 mmol (Moriyama *et al* 1988, Wang *et al* 1996, Welle *et al* 1996, Remer *et al* 2002) and assuming that 50 % of the MOCA absorbed in the body is measured in the urine analysis (hydrolysis of the acetyl, glucuronide and sulphate conjugates), this corresponds to a daily dose of 17 µg (2.6 µmol/mol creatinine × 0.012 mol × 267.17 g/mol × 2). Thus, 5 µmol/mol creatinine corresponds approximately to a cumulative life-time cancer risk of $3 \times 10^{-6}$ (Friday specimen; for a Tuesday specimen with 5 µmol MOCA/mol creatinine, the risk estimate is $4 \times 10^{-6}$.

**4. Recommendation**

Since MOCA is a genotoxic carcinogen, no health based biological limit value can be recommended (SCOEL carcinogen group A). Since the general population is not exposed to MOCA, MOCA is not detected in the urine of occupationally non-exposed people. This means that urinary levels of occupationally non-exposed stay below the detection limit of the method, which typically lay around 1–1.5 µg/l (3.7–5 nmol/l, ~ 0.37–0.5 µmol/mol creatinine) with commonly used analytical

methods, some methods reported to reach the detection limit of 0.1 µg/l. Thus, the Biological Guidance Value (BGV) for MOCA corresponds to the detection limit of the biomonitoring method.

In occupationally exposed populations, urinary MOCA levels (total MOCA in the urine) below 5 µmol/mol creatinine can be reached using good working practises at the workplace. According to the risk assessment presented above, this corresponds to a cancer risk of $3–4 \times 10^{-6}$. Urinary samples should be collected at the end of the work-shift.

The present Annex was adopted by SCOEL on 20 March, 2013.

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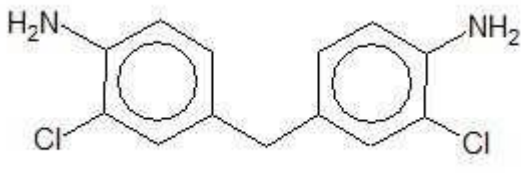
# Appendix 2. RAC Reference Dose Response Relationship for MOCA [18]




**2,2'-Dichloro-4,4'-methylenedianiline (MOCA, CAS RN: 101-14-4; EC Number: 202-918-9) is included in Annex XIV of REACH "List of substances subject to authorisation".**

## Relevance of endpoints

For applicants applying for authorisation under Article 60(2) (adequate control route), in order to conclude whether adequate control is demonstrated, only endpoints (i.e. properties of concern) for which the substance is included in Annex XIV need to be addressed in the hazard assessment[1]. However, information on other endpoints might be necessary for comparing the risks with the alternatives.

For applicants aiming at authorisation based on Article 60(4) (socio-economic analysis route) Article 62(4)(d) also applies and the socio-economic analysis (SEA) route will as a consequence focus on the risks that are related to the intrinsic properties specified in Annex XIV. The SEA should in turn consider the impacts related to such risks. In practice the applicant is expected to provide this information in their Chemical Safety Report (CSR) for which an update may be advisable. However, for an authorisation to be granted, the applicant should also demonstrate that there are no suitable alternatives. In this latter analysis it may be the case that other endpoints than those for which the substance was listed in 'Annex XIV' become relevant in order to demonstrate that no suitable alternative is available.

MOCA was included on Annex XIV due to its carcinogenic properties. The reference dose response relationships proposed in the present document are only based on carcinogenicity arising from MOCA exposure[2].

## Carcinogenicity

Table 1 below provides an overview of expert assessments on the carcinogenic mode of action, the assumed carcinogenic mechanism and the low-dose extrapolation approaches that were used:

[1] Article 60(2) states "…an authorisation shall be granted if the risk to human health or the environment from the use of the substance arising from **intrinsic properties specified in Annex XIV** is adequately controlled."

[2] Endpoints relevant to the authorisation are also discussed in section 5 of the document: "How RAC and SEAC intend to evaluate the applications" (common approach of RAC and SEAC in opinion development on applications for authorisation, agreed RAC-20/SEAC14, 24/03/2012). Link: http://echa.europa.eu/web/guest/applying-for-authorisation/additional-information

[18] RAC/32/2015/10 rev.1, agreed at RAC-32: https://echa.europa.eu/documents/10162/13641/dose-response-carc-moca_en.pdf/b66fb862-351b-492f-aa16-f7a75c71ca23

**Table 1 Overview of the findings of Expert assessments on the carcinogenic mode of action of MOCA**

| Expert evaluation | Primary mechanism | Threshold/non-threshold approach | Studies | Threshold dose |
|---|---|---|---|---|
| **IARC (2010)** | Genotoxic mechanism:<br>• metabolic activation to *N*-hydroxy MOCA | Not addressed | Inadequate evidence in humans of carcinogenicity<br>sufficient evidence in experimental animals of carcinogenicity – the main target tissues:<br>• liver and lungs in rats<br>• urinary bladder in dogs | not addressed |
| **IARC (2012)** | Genotoxic mechanism: metabolic activation<br>*N*-oxidation in the liver<br>*O*-acetylation in the bladder | Not addressed | Inadequate evidence in humans of the carcinogenicity of MOCA sufficient evidence in experimental animals of the carcinogenicity of MOCA | not addressed |
| **ATSDR (1994)** | Not reported | Not addressed | Reported to be a suspected bladder carcinogen considered a probable human carcinogen | not addressed |

| Expert evaluation | Primary mechanism | Threshold/non-threshold approach | Studies | Threshold dose |
|---|---|---|---|---|
| **Chemtura Belgium N.V., 2014; Limburge Urethane Casting N.V., 2010** | Genotoxic mechanism | Non-threshold | Lung, mammary, zymbal gland and liver tumours detected in an 18-month study in male rats (Kommineni *et al*., 1979) | **WORKERS:**<br>**dermal:**<br>BMDL10 = 178 mg/kg bw/day AF = 40 000<br>DMEL = 4.45 x $10^{-3}$ mg/kg bw/day<br>**inhalation:**<br>BMCL10 = 7.76 mg/$m^3$ SF = 10 000<br>DMEL = 7.76 x $10^{-4}$ mg/$m^3$ |

| Expert evaluation | Primary mechanism | Threshold/non-threshold approach | Studies | Threshold dose |
|---|---|---|---|---|
| **Chemtura Belgium N.V., 2014; Limburge Urethane Casting N.V., 2010** | Genotoxic mechanism | non-threshold | lung, mammary, zymbal gland and liver tumours detected in an 18-month study in male rats (Kommineni *et al*., 1979) | **GENERAL POPULATION:**<br>**dermal:**<br>BMDL10 = 178 mg/kg bw/day SF = 40 000<br>DMEL = 4.45 x $10^{-3}$ mg/kg bw/day<br>**inhalation:**<br>BMCL10 = 3.07 mg/$m^3$ SF = 10 000<br>DMEL = 3.07 x $10^{-4}$ mg/$m^3$<br>**oral:**<br>BMDL10 = 4.44 mg/kg bw/day SF = 40 000<br>DMEL = 1.11 x $10^{-4}$ mg/kg bw/day |
| **DECOS, 2000** | Genotoxic mechanism | Non-threshold | DECOS assessed all the studies for additional lifetime cancer risk associated with occupational exposure. Different methodology using malignant tumours to calculate incidence/mg/kg bw/day. | Results varied from 2.2 x $10^{-3}$ to highest incidence 3.7 x $10^{-2}$/mg/kg bw/day (Grundmann and Steinhoff, 1970)<br>Corresponds to additional lifetime cancer risk of 4 x $10^{-5}$ for 40 y exposure to 0.02 mg/$m^3$ |

BMCL10: Lower 95% confidence limit of a benchmark concentration representing a 10% tumour response following lifetime exposure.
BMDL10: Lower 95% confidence limit of a benchmark dose representing a 10% tumour response following lifetime exposure.
DMEL: Derived Minimum Effect Level.
SF: Safety Factor (Assessment Factor)

## Mechanism of action

The precise mechanism of action for carcinogenicity of MOCA is not fully understood; however, MOCA has the potential to form adducts with DNA. (ATSDR, 1994; IARC, 2012). The reactive nitrenium ion was identified as reacting primarily with C8-deoxyadenosine in rats (Beland and Kadlubar, 1990; IARC, 2010; IARC, 2012; Swaminathan *et al.*, 1996). These MOCA-DNA adducts have been reported in urothelial cells of an exposed worker; liver, kidney, lung and bladder of rat and dog in *in vivo* studies (IARC, 2012; Swaminathan *et al.*, 1996).

As well as forming MOCA-DNA adducts, data suggest that MOCA can also react and generate adducts with haemoglobin and serum albumin (Cheever *et al.*, 1988, 1990, 1991; Vaughan and Kenyon, 1996).

## Genotoxicity

IARC (2010, 2012) reported strong evidence of the carcinogenicity of MOCA via a genotoxic mechanism of action. The data suggest that the genotoxic mechanism includes metabolic activation of MOCA to form adducts with DNA, resulting in the induction of mutagenic and clastogenic effects in humans.

The data suggest that MOCA is mutagenic in several strains of *Salmonella typhimurium* tested with metabolic activation in the Ames assay. The genotoxic assays conducted in several strains of *Saccharomyces cerevisiae* indicate that MOCA is not genotoxic in these test systems. MOCA induced chromosomal aberrations (including single DNA strand breaks) either with or without metabolic activation, and unscheduled DNA synthesis without activation. The mouse lymphoma assay identified both positive and negative results with and without metabolic activation, respectively. The available *in vivo* data strongly suggest that MOCA is genotoxic in experimental animals following dermal, inhalation and oral exposure. MOCA induced DNA adduct formation in two species of rat following oral, dermal and intraperitoneal injection. MOCA also induced micronuclei in B6C3F1 mice via intraperitoneal injection; however, it did not induce micronuclei via the same exposure route in CD-1 mice.

**The weight of evidence from the genotoxicity data, particularly the *in vivo* studies, indicates that it should be considered a genotoxic agent.**

## Animal studies

IARC classified MOCA as Group 2B (possibly carcinogenic to humans) because, while there is strong evidence of carcinogenicity in animals, there was no convincing evidence in humans (IARC, 2010, 2012).

There have been a number of carcinogenicity studies with MOCA although they are all rather old and conducted before the modern guidelines and GLP were implemented. These are outlined in Table 2. Although these studies in rats suffer from a limited range of doses and exposure times, and some experienced high mortality rates, they consistently show an increased incidence of lung and liver tumours.

**Table 2 Overview of the chronic carcinogenicity studies of MOCA**

| Reference | Study details | Dose | Findings |
|---|---|---|---|
| **Russfield *et al.* (1975)** | • HaM/ICR mice M/F<br>• 25/sex/dose<br>• 18 months exposure and 6 months observation<br>• oral exposure via diet | • 0,130 or 260 mg/kg bw/day MOCA hydrochloride salt (purity 97%) | • significant increase in incidence of hepatomas in both dose groups of F |
| **Grundmann and Steinhoff (1970)** | • Wistar rats M/F<br>• 25/sex/dose and 50/sex controls<br>• 500 days and observation period (lifetime)<br>• oral exposure via | • 0 or 54 mg/kg bw/day MOCA (purity unspecified) | • significant increase in hepatomas and lung tumours in M & F<br>• high mortality rate in M & F |
| **Russfield *et al.* (1975)** | • Charles River CD-1 rats M<br>• 25/dose<br>• 18 months exposure and 6 months observation<br>• oral exposure via standard protein diet | • 0, 25 or 50 mg/kg bw/day MOCA hydrochloride salt (purity 97%) | • no significant increase in tumours |
| **Stula *et al.* (1975)** | • Charles River CD rats M/F<br>• 50/sex/dose<br>• 2 years<br>• oral exposure via a standard-protein diet (23% protein)<br>• 6/dose sacrificed for a one- year interim | • 0 or 50 mg/kg bw/day MOCA (purity ~95%) | • significant increase in lung adenomatosis (pre-neoplastic lesion) and lung adenomatosis in M & F |
| **Stula *et al.* (1975)** | • Charles River CD rats M/F<br>• 25/sex/dose<br>• 16 months<br>• oral exposure via a low-protein diet (7%)<br>• 6/dose sacrificed for a | • 0 or 50 mg/kg bw/day MOCA (purity ~95%) | • significant increase in lung adenocarcinomas and lung adenomatosis in M & F<br>• significant increase in hepatocellular carcinomas and hepatocellular adenomas in M |

| Reference | Study details | Dose | Findings |
|---|---|---|---|
| | year interim evaluation | | • significant increase in mammary gland adenocarcinomas in F |
| **Kommineni *et al*. (1979)** | • Charles River Sprague-Dawley rats M<br>• 100 rats (control and low-dose group), 75 rats (mid-dose group) and 50 rats (high-dose group)<br>• 18 months exposure and 6 months on diet and 32 weeks observation<br>**Group A:**<br>○ protein-adequate diet (27%)<br>**Group B:**<br>○ a protein-deficient diet (8%) | **Group A (male rats)**:<br>Dietary levels - 0, 250, 500, 1000 ppm (12.5, 25 or 50 mg/kg bw/day) MOCA (industrial grade)<br>**Group B**:<br>Dietary levels – 0, 125, 250, 500 ppm (0, 6.25, 12.5 and 25 mg/kg bw/day) MOCA | • significant increase in lung adenocarcinomas, all lung tumours, mammary gland adenocarcinomas, Zymbal gland carcinomas, hepatocellular carcinomas and haemangiosarcomas |
| **Stula *et al*. (1978)** | • Beagle dogs F<br>• 6/dose<br>• 3 days/week for 6 weeks, then 5 days/week for 9 years<br>• oral exposure | • 100 mg MOCA (~90% purity) in a gelatine capsule (average 10 mg/kg bw/day) | • Urinary bladder transitional cell carcinomas were reported in 4/5 (80%) of the treated female dogs The other treated dog died early, not related to treatment. |
| **Steinhoff and Grundmann (1969)** | • Wistar rats M/F<br>• 17/sex/dose and 25/sex controls<br>• 88 weeks exposure and 23 weeks observation (lifetime) | • 0, 500 or 1000 mg/kg bw MOCA (94% purity) | • significant increase in hepatocellular carcinomas and lung cancers |

F: Female.
M: Male.

There is an oral long-term (up to 9 years) study in Beagle dogs with a single dose in which bladder tumours were observed in 4 out of 5 surviving treated dogs. This result, together with the epidemiological studies, indicates weak evidence that bladder cancer may be associated with MOCA exposure (Stula *et al*., 1978). Due to the limited number of animals the study is not however, suitable for risk assessment. The most-complete dose-response study, although with high mortality, is that of Kommineni *et al.* (1979) in which rats with an adequate protein diet (a further treated group had inadequate protein) were treated orally. The use of T25 in the cancer risk estimates using lower dose tumour incidences counters this higher mortality in the study. This study was used for risk assessment in the Chemical Safety Reports (CSRs: Chemtura, 2014; Limburge Urethane Casting N.V., 2010).

In the Dutch DECOS (2000) assessment of the long-term carcinogenicity studies, the Kommineni *et al*. (1979) study had an incidence of tumours of 3.5 $x10^{-2}$/mg kg bw/day, close to the highest incidence of 3.7 $x10^{-2}$/mg/kg bw/day (Grundmann and Steinhoff, 1970). This assessment, while giving some indication of the comparative sensitivity of the carcinogenicity studies, uses different methodologies to those REACH Guidance methods used in this risk assessment and so the tumour frequencies are not suitable.

The frequency of combined lung tumours observed in the Charles River CD rat oral long-term study of Kommineni *et al.* (1979) will be used in this review to derive lifetime cancer risk estimates. In the part of the study to be used, male rats were exposed to industrial grade MOCA (unspecified purity) in protein-sufficient diets (27% protein; a further group had a protein-restricted diet, 8% protein) at 0, 250, 500 and 1000 ppm for 18 months following by a 6-month recovery period. This corresponded to a received dose of 0, 12.5, 25 and 50 mg/kg bw/day estimated by assuming that a rat consumes 5% of its body weight per day (US EPA, 2006). These doses were expanded to continuous lifetime exposure by multiplying by 18/24 months to give a corrected dose (US EPA, 2006). Tumours were detected in the lung, mammary gland, Zymbal gland and liver. Combined lung tumours (adenomas, epidermoid carcinomas and adenocarcinomas) gave the most complete dose response data, and lung tumours are the most frequently observed tumours seen in the experimental animal long-term studies. The tumour incidence is shown in Table 3 below.

**Table 3 Lung tumour incidence in Male rats (Kommineni et al, 1979)**

| Dietary Dose (ppm) | 0 | 250 | 500 | 1000 |
|---|---|---|---|---|
| Dose/animal (mg/kg bw/day) | **0** | **12.5** | **25** | **50** |
| Corrected Dose (mg/kg bw/day) | **0** | **9.4** | **18.8** | **37.5** |
| Total Tumours/animals | **1/100** | **23/100** | **28/75** | **35/50** |
| Incidence | **0.01** | **0.23** | **0.37** | **0.70** |

## Human studies

Four epidemiological studies were located and these mainly concentrated on the possible increased incidence of bladder cancer (Ward *et al*., 1990; Chen *et al*., 2005; Mason *et al*.,

1990; Dost *et al*., 2009). This was based on the known properties of other similar amine compounds such as benzidine and naphthylamine. There were US, Taiwan and UK studies following workers exposed to MOCA and monitoring urine samples. There were low levels of bladder cancers and abnormalities in cells in urine detected in these studies but the lack of appropriate controls and exposure to a number of other potentially carcinogenic chemicals and other confounders means that there is no convincing evidence of a causal association between MOCA exposure and bladder cancer. IARC (2010, 2012) reported that "no adequate epidemiology studies were available to the Working Group to evaluate an association between MOCA and bladder cancer risk".

## Bioavailability

Data from occupational studies have identified that the most likely routes of exposure to MOCA are from contact with contaminated surfaces i.e. dermal, followed by inhalation and oral pathways.

No specific studies were located on the absorption of MOCA in humans following oral exposure. The results from rats administered a single oral dose of radiolabelled MOCA via oral gavage suggest that MOCA is partially absorbed following oral exposure. 16.5% MOCA was excreted in urine within 72 hours, 13.7% was retained in the tissue, while approximately 60% remained unabsorbed in faeces (Groth *et al*., 1984).

Occupational workers exposed to MOCA during its manufacturing process, which can either exist as a liquid emulsion, solid pellets with dust, or as solid pellets without dust (IARC, 2012). NIOSH (1986) reported the concentrations of MOCA in the urine of exposed workers over a period of 22 months and identified the levels of MOCA from 5.3 to 43.8 µg/l. A detailed review of the data identified that the highest MOCA concentrations in urine detected were in workers in direct daily contact with MOCA i.e. mixers and molders.

One study indirectly evaluated the absorption of MOCA in five male factory workers over a 5-day period. MOCA air concentrations were monitored for each worker over 6-7 hours every other day and urinary MOCA concentrations were obtained over the 5 days. MOCA air concentrations ranged from 0.0002 to 0.0089 mg/m$^3$. The concentration of MOCA detected in urine was greater than the reported air concentrations, identifying that another potential route of MOCA exposure is dermal (Ichikawa *et al*., 1990).

The differences in absorption rates of radiolabelled MOCA ($^{14}$C-MOCA) in Beagle dogs following either dermal or intravenous exposures were reported for 24 hours following MOCA administration. Only 2.4% MOCA was reported to be absorbed via dermal administration (Manis *et al*., 1984). Groth *et al*. (1984) reported 11.5-21.9% of MOCA absorption in Sprague Dawley rats following 72 hours of dermal application to the skin.

The absorption and penetration of radiolabelled MOCA through 7 x 7 mm area of fresh human neonatal foreskin organ cultures was reported over a four-hour period. One hour following dermal application, 46% of the radiolabelled MOCA was reported on the skin, 0.5% was detected on the underlying membrane, while the remaining 53.5% radiolabelled MOCA was unabsorbed. Four hours after the initial radiolabelled MOCA, 61% was detected in the skin, 26% was detected on the underlying membrane and 12% remained unabsorbed. The authors suggested that MOCA was readily absorbed without being metabolised (Chin *et al*., 1983).

No additional studies were located on the direct measurement of MOCA absorption in humans or experimental animals via inhalation exposure.

Therefore for the risk estimations, the following absorption values were used:

**Oral absorption** – no human data and partially absorbed in rats; therefore, an oral absorption of 50% is assumed and when extrapolating from oral to inhalation toxicity a correcting factor of 2 is used according to the REACH Guidance.

**Dermal absorption** – There are no in vivo dermal absorption data in humans, in one study in rats dermal absorption of 11.5-21.9% is observed and human tissue culture study suggests even higher absorption; 50% default value for dermal absorption is used according to the REACH Guidance.

**Inhalation absorption** - No studies located – 100% default value according to the REACH Guidance

## Carcinogenicity risk assessment

### T25 Derivation

The T25 value for MOCA has been derived using information from a long-term study on Charles River CD rats administered MOCA in the diet with adequate protein (Group A) and using the frequency of all lung tumours (adenoma, epidermoid carcinoma and adenocarcinoma) (Table 3; Kommineni *et al*. 1979).

- lowest dose with a significantly increased frequency (C) of 9.4 mg/kg bw/day
- Incidence at C, 0.23
- Control incidence, 0.01

T25 is derived using the following calculation:

C x (Reference incidence 0.25)/(incidence at C – control incidence) x (1-control incidence)/1

The lowest $T25_{(oral, rat)}$ = 9.4 x 0.25/0.23-0.01 x 1-0.01/1
= 10.6 mg/kg bw/day.

Therefore **$T25_{(oral, rat)}$ = 10.6 mg/kg bw/day**.

This value is used as the PoD for the derivation of route-specific risk estimates for workers and the general population.

## Workers

### *Workers inhalation risk estimate*

The $T25_{(oral, rat)}$ was corrected for inhalation exposure assuming 100% absorption and correcting for:

- rat oral intake (mg/kg bw/day) to rat inhalation (0.8 l/min/8 h);0.384 $m^3$/kg bw/8 h
- oral absorption rat/inhalation humans (50/100)
- activity driven difference for workers (standard respiratory volume for humans, 6.7/respiratory volume in light work for workers,10 $m^3$)

**$T25_{(inhalation, human)}$ = 10.6 x 1/0.384 x 6.7/10 x 50/100 = 9.25 mg/$m^3$**

Correcting for worker exposure:

- workers exposure is 5 day/week, 48 weeks/year, 40 years in an average lifespan of 75 years
- correction factor for workers' exposure of 7/5 x 52/48 x 75/40 = **2.8**

**$T25_{(inhalation, workers)}$ = 9.25. mg/$m^3$ x 2.8 correction factor = 25.9 mg/$m^3$**

### *Workers dermal risk estimate*

Taking the $T25_{(oral, rat)}$ and correcting for

- dermal default exposure of 50% and oral absorption of 50%
- allometric scaling of 4 from rats to humans:

**$T25_{(dermal, human)}$ = 10.6/(50/50)/4 = 2.65 mg/kg bw/day**

Correcting for workers' exposure as above:

**$T25_{(dermal, workers)}$ = 2.65 x 2.8 = 7.4 mg/kg bw/day**

**General population inhalation risk estimate**

$T25_{(oral, rat)}$ 10.6 mg/kg bw/day corrected for general population inhalation exposure:

- allometric scaling from rats to humans, 4,
- human weight 70 kg
- human general population breathing 20 $m^3$ per person
- default oral absorption (50%) to inhalation absorption (100%).

**$T25_{(inhalation, gen pop)}$ = 10.6/4 x 70/20 x 50/100 = 4.6 mg/$m^3$**

**General population oral risk estimate**

$T25_{(oral, rat)}$ corrected to $T25_{(oral, general pop)}$ by allometric scaling, from rats to humans, 4.

**$T25_{(oral, general pop)}$ = 10.6/4 = 2.65 mg/kg bw/day**

A summary of the cancer risk estimates is shown in Table 4.

**Table 4 Cancer risk estimates for MOCA**

| Route of exposure | Population | T25 Descriptor | Cancer risk for 1 unit amount |
|---|---|---|---|
| **Oral** | General populati | $T25_{(oral, general pop)}$ 2.65 mg/kg bw/day | **$9.43 \times 10^{-5}$ per µg/kg bw/day** |
| **Inhalation** | Workers | $T25_{(inhalation, workers)}$ 25.9 mg/$m^3$ | **$9.65 \times 10^{-6}$ per µg/$m^3$** |
| | General populati | $T25_{(inhalation general pop)}$ 4.6 mg/$m^3$ | **$5.43 \times 10^{-5}$ per µg/$m^3$** |
| **Dermal** | Workers | $T25_{(dermal, human)}$ 7.4 mg/kg bw/day | **$3.38 \times 10^{-5}$ per µg/kg bw/day** |

Assuming linearity of response the cancer risk for lifetime exposure to each unit amount of MOCA will increase in proportion, e.g. for workers' exposure by inhalation

| | |
|---|---|
| **1 µg/m$^3$** | **9.65 x 10$^{-6}$** |
| **2 µg/m$^3$** | **1.93 x 10$^{-5}$** |
| **5 µg/m$^3$** | **4.83 x 10$^{-5}$** |
| **10 µg/m$^3$** | **9.65 x 10$^{-5}$** |

## Biomonitoring approach

An additional approach for assessing the exposure and risk of MOCA is the biomonitoring of occupationally exposed workers. This approach has been summarised by SCOEL particularly in the 2013 Annex to its recommendations on MOCA (SCOEL, 2010/2013).

There have been a number of studies measuring MOCA in urine. MOCA is excreted as 'free' MOCA but also as metabolites, glucuronide-MOCA and acetyl-MOCA. Commonly used methods have been developed to measure total MOCA (free and conjugated MOCA) expressed in µmol/l or µmol/mol creatinine (to correct for urinary creatinine excretion). Detection limits vary between 3.7-5 nmol/l (1-1.5 µg/l), corresponding approximately to 0.35-0.5 µmol/mol creatinine (SCOEL, 2010/2013). In workers not exposed to MOCA, urinary levels are below the detection limits of these modern analytical techniques.

Since MOCA is a genotoxic, non-threshold carcinogen, SCOEL has not set any biological limit value for MOCA, but has derived a Biological Guidance Value which typically represents the 95$^{th}$ percentile of the biomarker levels in occupationally non-exposed populations. In the case of MOCA, this is below the detection limit, and so any concentrations detected suggest occupational exposure.

There are no reliable measured data on correlations between urinary MOCA levels and MOCA air concentrations, so it is not possible to directly calculate urinary levels which correspond to occupational exposure, e.g. 1 or 10 µg/m$^3$.

In SCOEL (2010/2013) an open one-compartment model to calculate the daily dose corresponding to urinary MOCA level of 5 µmol/mol creatinine in the Friday afternoon (end of shift) sample (SCOEL 2010/2013) is described. For a substance following first order elimination kinetics the decrease in urinary level follows the formula

$$Ct = Cp \times e^{-t \times kelim}$$

where Ct = concentration at time point t after the peak concentration; Cp = peak concentration, and $K_{elim}$ = elimination rate constant, = ln2/$T_{1/2}$.

Assuming that the half-time of MOCA is 23 hours and the steady state is reached after one-week exposure, an average urinary concentration of MOCA at steady state is 2.6 µmol/mol creatinine when the concentration in the Friday afternoon sample is 5 µmol/mol creatinine.

Urinary excretion of 5 µmol/mol creatinine in the Friday afternoon can then be calculated to using the formula:

$$D = C_{ss} \times Cr_{24h} \times M/BW \times F_{ue}$$

where D = daily dose (µg), Css = average concentration in the urine, $Cr_{24h}$ = average daily excretion of creatinine for a 50-year old man of 70 kg (12 mmol), Fue = proportion of dose excreted in urine (50% in the case of MOCA).

2.6 µmol/mol creatinine × 0.012 mol × 267.17 g/mol /0.5 = 17 µg

SCOEL then used unit cancer risk estimates derived by DECOS (see Table 1) to calculate cancer risk for different urinary MOCA levels. These risk estimates were derived using a different method from that in the REACH Guidance. It should be noted, that SCOEL gave these risk estimates for information only, and did not set any limit value based on these calculations.

The risk estimates derived above using the REACH Guidance can be used to calculate the risk level for different urinary MOCA levels.

Since 1 µg/$m^3$ exposure (which corresponds to a daily dose of 10 µg in occupational exposure) represents a cancer risk of 9.65 x $10^{-6}$,

**5 µmol/mol creatinine in a Friday afternoon sample (corresponding to a daily dose of 17 µg) corresponds to a risk of 16.4 x 10-6.**

**0.5 µmol/mol creatinine (detection limit of current analytical techniques) corresponds to cancer risk of 1.64 x $10^{-6}$.**

While these calculations to estimate daily dose are not precise and include some assumptions, biomonitoring is currently the best method to estimate the total exposure to MOCA in occupational settings. Therefore when biomonitoring data are available, these can be used to estimate cancer risks for occupational exposure.

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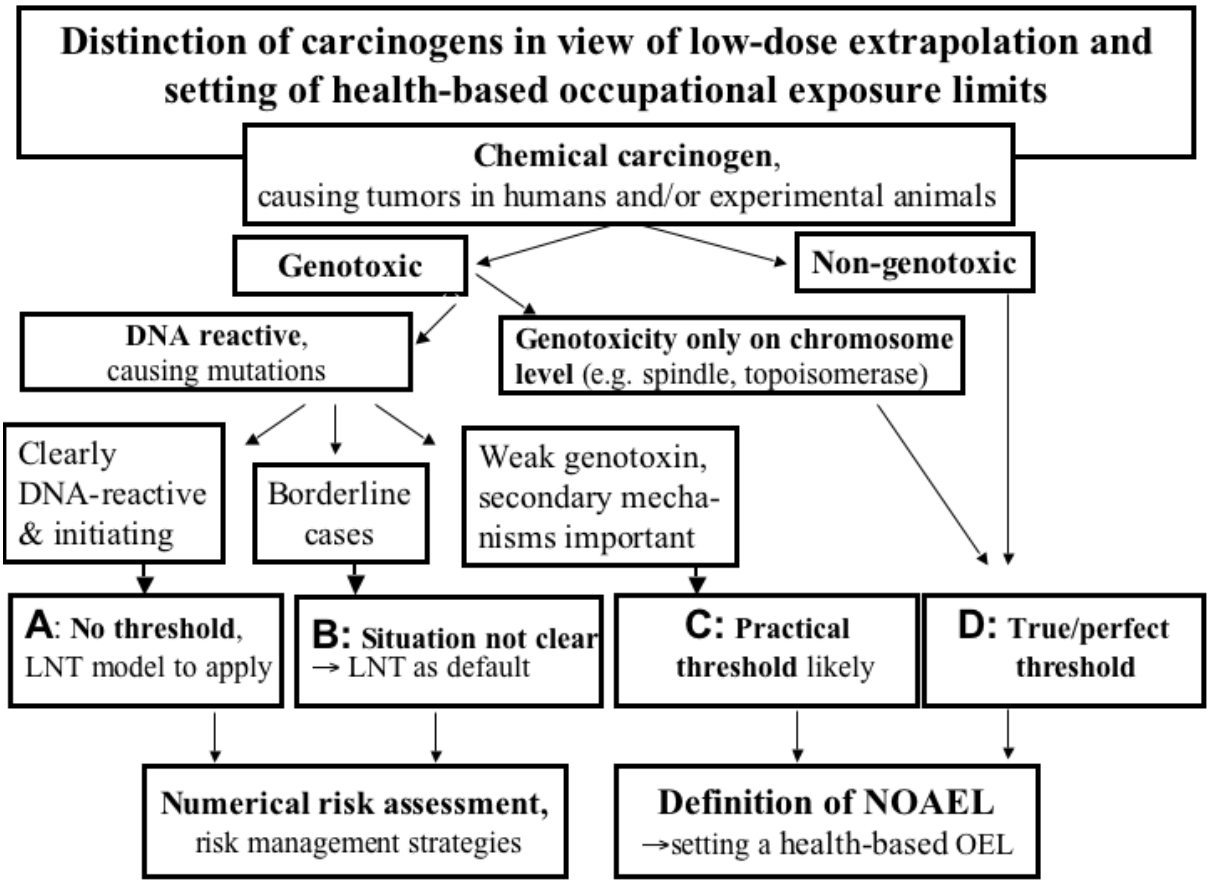
Ward, E., Halperin, W., Thun, M., Grossman, H.B., Fink, B., Koss, L., Osorio, A.M. and Schulte, P. (1990) Screening workers exposed to 4,4'-methylenebis(2-chloroaniline) for bladder cancer by cystoscopy. J. Occup. Med., 32, 865–868.

# Appendix 3 SCOEL classification of carcinogens

Taken from current SCOEL 'Methodology for the Derivation of Occupational Exposure Limits' (SCOEL, 2013; version 7[19]),




**Group A**: Non-threshold genotoxic carcinogens; for risk low-dose assessment the linear non-threshold (LNT) model appears appropriate.

**Group B**: Genotoxic carcinogens, for which the existence of a threshold cannot be sufficiently supported at present. In these cases the LNT model may be used as a default assumption, based on the scientific uncertainty.

**Group C**: Genotoxic carcinogens for which a practical threshold is supported.

**Group D**: Non-genotoxic carcinogens and non-DNA reactive carcinogens; for these compounds a true ("perfect") threshold is associated with a clearly founded NOAEL.

[19] Available on Commission webpage on SCOEL [http://ec.europa.eu/social/main.jsp?catId=148&intPageId=684&langId=en]