Social Europe

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

SCOEL/SUM/174

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European Commission

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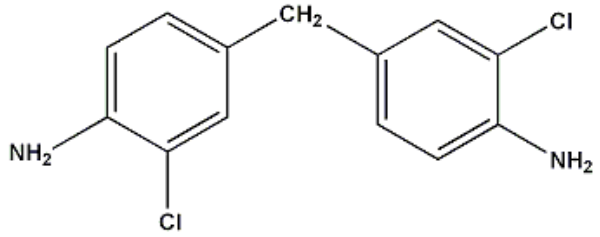
8-hour TWA	:	not feasible to derive a health-based limit (see Recommendation)
STEL (15 min)	:	not feasible to derive a health-based limit (see Recommendation)
Additional classification	:	"Skin" notation
SCOEL carcinogen group	:	A (non-threshold genotoxic carcinogen)
Biological monitoring	:	See Recommendation

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-amino-benzene), 4,4'-methylene-bis-(o-chloro-aniline).

Structural formula:



$C_{13}H_{12}Cl_2N_2$

EU classification: Carc. Cat. 2; R45 May cause cancer
Xn; R22 Harmful if swallowed.
N; R50-53 Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

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/m³; 1 mg/m³ = 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).

Inactivation of MOCA occurs through glucuronide- and acetyl conjugation. It is excreted in the urine as a free compound or as glucuronide or acetyl metabolites, the main individual metabolite in the urine being the N-glucuronide of MOCA. This N-glucoronide of MOCA has been found to be 2-3 times higher than free MOCA in the urine of exposed workers (Robert et al. 1999, I and II). The level of N-acetyl-MOCA in urine was less than 10% of the level of MOCA recovered in urine of exposed workers (Ducos et al. 1985). After an acute high-level (including dermal) 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 in the urine (Osorio et al. 1990). This suggests the rapid excretion of MOCA after acute dermal and/or inhalation exposure.

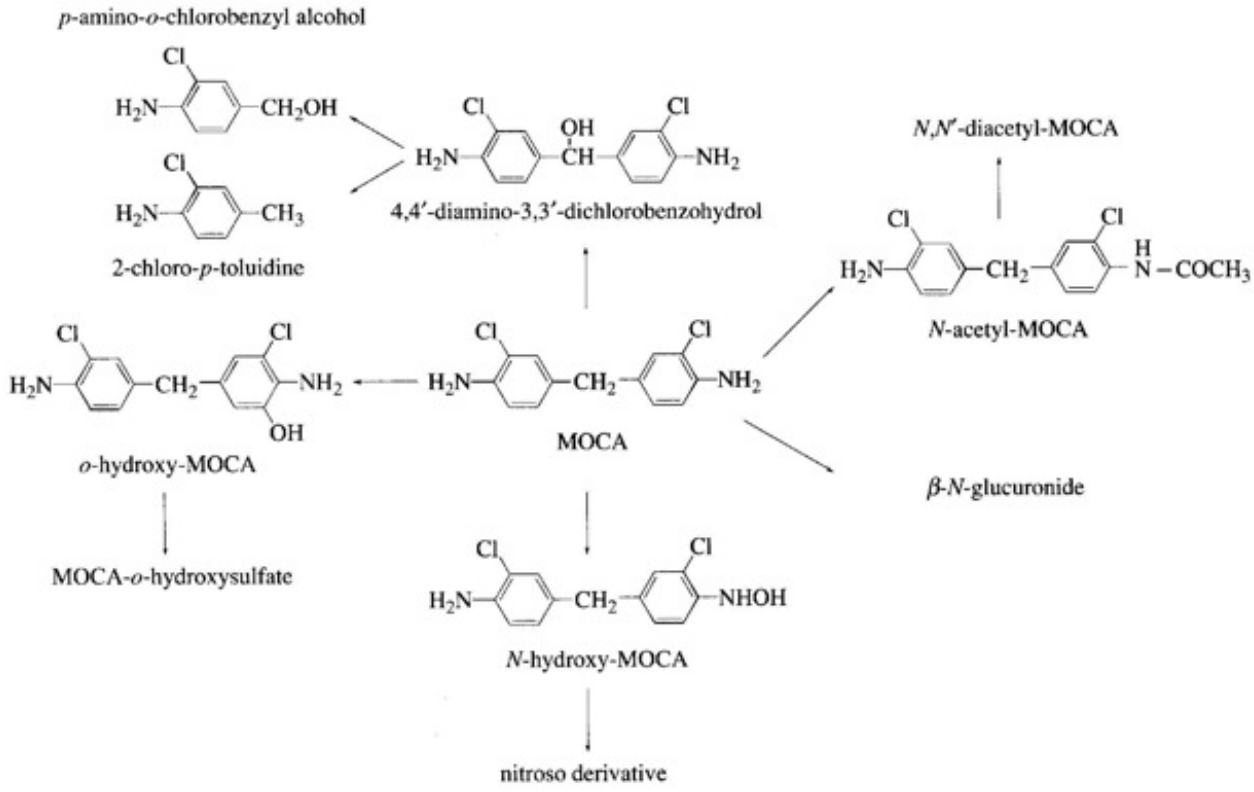


Figure 1: Formation of metabolites from MOCA, based on experimental data, according to DFG (1996).

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. 1999, 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 exposure MOCA 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).

In *Finland*, the Finnish Institute of Occupational Health (FIOH) publishes yearly data on monitoring of the Finnish industry. The total number of MOCA measurements during the years 2000-2008 was 49 (FIOH 2008a). Most of the samples were from workers involved in the manufacturing of polyurethane coatings. MOCA was measured as total MOCA in urine using alkaline hydrolysis. Most of the values were <5 µmol/mol creatinine, the range being <LOD to 10 µmol/mol creatinine (FIOH 2008a). The 95th percentile of these measurements (n=49) was 3.4 µmol/mol creatinine (FIOH, unpublished data). Based on these data, there was a FIOH proposal in 2008 of a "biological action limit value" of 5 µmol/mol creatinine for total MOCA (FIOH 2008 b).

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).

Since MOCA has been shown to bind covalently to haemoglobin, haemoglobin adduct analysis has been also suggested for the biomonitoring of MOCA (Bailey et al. 1993, Vaughan and Kenyon 1996). The advantage of this method is that it reflects 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-postlabeling analysis in exfoliated urothelial cells has been described (Kaderlik et al. 1993).

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 an 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 an 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).

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, Chen 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 an 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 an 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 25 males and 25 females were administered 0 (control) or 1000 ppm MOCA (about 95% pure) in a low-protein diet (7%) for 16 months. Six animals from each group were sacrificed at one year for interim evaluation. 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, a 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 90th 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. In 2008, the Finnish Institute of Occupational Health (FIOH 2008 b) derived a "biological action limit value" of 5 µmol/mol creatinine for total MOCA.

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.

References

ACGIH (2010) 2010 TLVs and BEIs based on the documentation of the Threshold Limit Values for chemical substances and physical agents & Biological Exposure Indices. American Conference of Governmental Industrial Hygienists, Cincinnati, OH, USA.

Bailey E, Brooks AG, Farmer PB, Street B (1993) Monitoring exposure to 4,4'-methylene-bis(2-chloroaniline) through the gas chromatography-mass sprectrometry measurement of adducts to hemoglobin. Environ Health Perspect 99: 175-177.

Cartwright RA (1983) Historical and modern epidemiological studies on populations exposed to N-substituted aryl compounds. Environ. Health Perspectives, 49: 13-19.

Clapp DE, Piacitelli GM, Zaebst DD, Ward E (1991) Assessing exposure to 4,4'-methylene bis(2-chloroaniline) (MBOCA) in the workplace Appl Occup Environ Hyg 6: 125-130.

Cocker J, Nutley BP, Wilson HK (1996) Methylene bis(2-chloroanline) (MbOCA): towards a biological monitoring guidance value. Biomarkers 1: 185-189.

Cocker J, Jones J, Morton J, Mason HJ (2007) Biomonitoring at the UK Health and Safety Laboratory. Int J Hyg Environ Health 210: 383-386.

Cocker J, Cain JR, Baldwin P, McNally K, Jones K (2009) A survey of occupational exposure to 4,4'-methylene-bis(2-chloroaniline) (MbOCA) in the UK. Ann Occup Hyg 53: 499-507.

DFG [Deutsche Forschungsgemeinschaft] (1996) 4,4'-Methylenebis(2-chloroaniline). In Occupational Toxicants (Ed. Greim H), Volume 7, pp. 193-218. VCH, Weinheim, Germany.

Ducos P, Maire C, Gaudin R (1985) Assessment of occupational exposure to 4,4'-methylene-bis-(2-chloroaniline) "MOCA" by a new sensitive method for biological monitoring. Int Arch Occup Environ Health 55: 159-167.

Edwards JW, Priestly BG (1992) Biological and biological-effect monitoring of workers exposed to 4,4'-methylene-bis(2-chloroaniline). Hum exp Toxicol 11: 229-236.

Fairfax R, Porter E (2006) Evaluation of worker exposure to TDI, MOCA, and methylene chloride. J Occup Environ Hyg 3: D-50-D53.

FIOH (2008 a) *Biologinen monitorointi vuositilasto [Annual Report on Biological Monitoring, reports from years 2000-2008]* in *Työympäristötutkimuksen raporttisarja*. 2000-2008, Finnish Institute of Occupational Health: Helsinki.

FIOH (2008 b) *4,4'-Metyleenibis(2-kloorianiliini), MOCA. Perustelumuistio MOCAn biologisen altistumisindikaattorin raja-arvolle. [4,4'-Methylenebis(2-chloroaniline), MOCA: Background documentation for MOCA biological limit value]*. 2008, Finnish Institute of Occupational Health: Helsinki.

Grundmann E, Steinhoff D (1970) Liver and lung tumours after administration of 3,3'-dichloro-4,4' -diaminodiphenylmethane to rats. Z Krebsforsch 74: 28-39.

Hosein HR, Van Roosmalen PB (1978) Acute exposure to methylene-bis-ortho-chloroaniline (MOCA). Am ind Hyg Assoc J 39: 496-497.

HSE [UK Health and Safety Executive] (2009) Statement on occupational cancer for MbOCA (updated 9.6.2009). http://www.hse.gov.uk/rubber/mboca.htm (accessed 1.6.2010).

IARC [International Agency for Research on Cancer] (1993) 4,4'-Methylenebis(2-chloroaniline) (MOCA). IARC Monogr Carcinog Risks Hum 57: 271-303.

IARC [International Agency for Research on Cancer] (2010) 4,4'-Methylenebis(2-chloroaniline) (MOCA). IARC Monogr Carcinog Risks Hum 97 (in press)

Kaderlik KR, Talaska G, DeBord DG, Osorio AM, Kadlubar FF (1993) 4,4'-Methylenebis(2-chloroaniline)-DNA adduct analysis in human exfoliated urothelial cells by 32P-postlabelling. Cancer Epidemiol Biomarkers Prev 2: 63-69.

Kommineni C, Groth DH, Frockt IJ, Voelker RW, Stanovick RP (1979) Determination of the tumorigenic potential of methylene-bis-orthochloroaniline. J environ Pathol Toxicol 2: 149-171.

Kuslikis BI, Trosko JE, Braselton WE Jr (1991) Mutagenicity and effect on gap-junctional intercellular communication of 4,4'-methylenebis(2-chloroaniline) and its oxidized metabolites. Mutagenesis 6: 19-24.

Linch AL, O'Connor GB, Barnes JR, Killian AS Jr, Neeld WE Jr (1971) Methylene-bis-ortho-chloroaniline (MOCA): evaluation of hazards and exposure control. Am ind Hyg Assoc J 32: 802-819.

Liu CS, Liou SH, Lioh CH, Yu YC, Uang SN, Shih TS, Chen HI (2005) Occupational bladder cancer in a 4,4'-methylenebis(2-chloroaniline) (MBOCA)-exposed worker. Environ Health Perspect 113: 771-774.

Lowry LK, Clapp DE (1992) Urinary 4,4'-methylenebis(2-chloroaniline) (MBOCA): a case study for biological monitoring. Appl occup environ Hyg 7: 1-6.

Lu CS, Liou SH, Lioh CH, Yu YC, Uang SN, Yu YC, Shih TS (2005) Bladder cancer screening and monitoring of 4,4'-methylenebis(2-chloroaniline) exposure among workers in Taiwan. Urology 66: 305-310.

Mastromatteo E (1965) Recent occupational health experiences in Ontario. J Occup Med. 7: 502.

NTP (2002) 4,4'-Methylenebis(2-chloroaniline), CAS No. 101-14-4. Rep Carcinog 10: 149-151.

Osorio AM, Clapp D, Ward E, Wilson HK, Cocker J (1990) Biological monitoring of aworker acutely exposed to MBOCA. Am J ind Med 18: 577-589.

Robert A, Ducos P, Francin JM (1999) Biological monitoring of workers exposed to 4,4'-methylene-bis-(2-orthochloroaniline) (MOCA). I. A new and easy determination of „free" and „total" MOCA in urine. Int Arch Occup Environ Health 72: 223-228.

Robert A, Ducos P, Francin JM (1999) Biological monitoring of workers exposed to 4,4'-methylene-bis-(2-orthochloroaniline) (MOCA). II. Comparative interest of „free" and

„total" MOCA in the urine of exposed workers. Int Arch Occup Environ Health 72: 229-237.

Russfield AB, Homburger F, Boger E, Van Dongen CG, Weisburger EK, Weisburger JH (1975) The carcinogenic effect of 4,4'-methylene-bis(2-chloroaniline) in mice and rats. Toxicol appl Pharmacol 31: 47-54.

Sabbioni G, Schütze D (1998) Hemoglobin binding of bicyclic aromatic amines. Chem Res Toxicol 11: 471-483.

Salamone ME, Heddle JA, Katz M (1981) Mutagenic activity of 41 compounds in the in vivo micronucleus assay. Prog Mutat Res 1: 686-697.

Savage RE Jr, Weigel WW, Krieg EF Jr (1992) Induction of ornithine decarboxylase activity by 4,4'-methylene bis(2-chloroaniline) in the rat. Cancer Lett 62: 63-68

Shih WC, Chen MF, Huang CC, Uang SN, Shih TS, Liou SH, Wu KY (2007) Simultaeous analysis of urinary 4,4'-methylenebis(2-chloroaniline) and N-acetyl-4,4'-methylenebis(2-chloroaniline) using solid-phase extraction and liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 21: 4071-4078.

Stula EF, Sherman H Zapp JA, Clayton JW (1975) Experimental neoplasia in rats from oral administration of 3,3'-dichlorobenzidine, 4,4'-methylene-bis(2-chloroaniline), and 4,4'-methylene-bis(2-methylaniline). Toxicol Appl Pharmacol 31: 159-176.

Stula EF, Barnes JR, Sherman H, Reinhardt CF, Zapp JA (1977) Urinary bladder tumors in dogs from 4,4'-methylene-bis(2-chloroaniline) (MOCA). J Environ Path Toxicol 1: 31-50.

Vaughan GT, Kenyon RS (1996) Monitoring for occupational exposure to 4,4'-merhylenebis(2-chloroaniline) by gas chromatographic-mass spectrometric analysis of haemoglobin adducts, blood, plasma and urine. J Chromatogr B Biomed Appl 678:197-204.

Ward, E., Halperin, W, Thun, M., Grossman, H.B., Fink, B., Koss, L., Osorio, A.M. & Schulte, P. (1988) Bladder tumors in two young males occupationally exposed to MBOCA. Am. J. ind. Med., 14, 267-272.

Ward E, Halperin W, Thun M, Grossman HB, Fink B, Koss L, Osorio AM, Schulte P (1990) Screening workers exposed to 4,4''-methylene bis(2-chloroaniline) for bladder cancer by cystoscopy. J occup Med 32: 865-868.

Wu K, Leslie CL, Stacey NH (1989) Effects of mutagenic and non-mutagenic aniline derivatives on rat liver drug-metabolizing enzymes. Xenobiotica 19: 1275-1283.

Wu WS, Szkar RS, Smith R (1996) Gas chromatographic determination and negative-ion chemical ionization mass spectr5ometric confirmation of 4,4'-methylenebis(3-chloroaniline) in urine via thin-layer chromatographic separation. Analyst 121: 321-324.

Yun Ch-H, Shimada T, Guengerich FP (1992) Contributions of human liver cytochrome P450 enzymes to the N-oxidation of 4,4'-methylene-bis(2-chloroaniline), Carcinogenesis 13: 217-222.