4,4'-METHYLENEBIS(2-CHLOROANILINE)

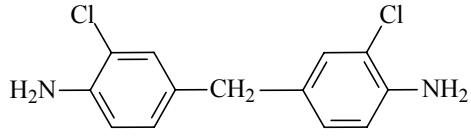
1. Exposure Data

1.1 Chemical and Physical Data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 101-14-4
CAS Name: 4,4'-Methylenebis(2-chlorobenzenamine)
Synonyms: Bis(4-amino-3-chlorophenyl)methane; bis(3-chloro-4-aminophenyl)methane; 4,4'-diamino-3,3′-dichlorodiphenylmethane; di(4-amino-3-chlorophenyl)methane; di(3-chloro-4-aminophenyl)methane; 3,3′-dichloro-4,4'-diaminodiphenylmethane; 2,2'-dichloro-4,4'-methylenedianiline; 4,4'-methylenebis(2-chloroaniline); 4,4'-methylenebis(*ortho*-chloroaniline); methylenebis(chloraniline); methylenebis(*ortho*-chloroaniline); methylenebis(3-chloro-4-aminobenzene); MOCA; MBOCA

1.1.2 *Structural formula, molecular formula, and relative molecular mass*



$C_{13}H_{12}Cl_2N_2$ Rel. mol. mass: 267.15

1.1.3 *Chemical and physical properties of the pure substance (OSHA, 2009)*

Description: Colourless to yellow or light brown crystalline solid with a faint amine-like odor
Melting-point: 110 °C

Solubility: Slightly soluble in water; soluble in diluted acids, diethyl ether, benzene, ethanol, and soluble to varying degrees in most organic solvents

1.1.4 *Technical products and impurities*

Pure 4,4'-methylenebis(2-chloroaniline) (MOCA) is a colourless crystalline solid. Historically, the technical grade of MOCA that is available in the United States came mainly from Japan in the form of tan/yellow fused prills or pastilles. The diamine purity is 99.8%, typically with 0.2% free *ortho*-chloroaniline (monomer) (ATSDR, 1994).

Trade names for 4,4'-methylenebis(2-chloroaniline) include: Bisamine A; Bisamine S; CPP 100; Cuamine M; Cuamine MT; Curene 442; Diamet Kh; Ihara Cuamine MT; Isocross SM; Millionate M; Pandex E; Pandex M 3202; Quodorole; SL 4037.

1.1.5 *Analysis*

Analyses of 4,4'-methylenebis(2-chloroaniline) were first reported in the 1970s. Two interesting recent studies have involved the use of gas chromatography/mass spectrometry to analyse water samples for the presence of 20 carcinogenic amines, and high-performance liquid chromatography in conjunction with UV detection to determine amine levels in extracts from toys, at ppm–ppb (10^{-6}–10^{-9}) concentrations (Garrigós *et al*., 2002; Doherty, 2005). Table 1.1 presents selected recent studies of the analysis of 4,4'-methylenebis(2-chloroaniline) in various matrices.

1.2 Production and use

1.2.1 *Production*

MOCA is produced commercially by reacting formaldehyde with *ortho*-chloroaniline. By-products such as trimers and tetramers—diamines with three- and four-ring structures joined by methylene groups—constitute up to 8–10% of commercial MOCA. 4,4'-Methylenebis(2-chloroaniline) comprises up to 90–92% of the commercial MOCA produced for coatings and cast polyurethanes. There is no commercial use for pure 4,4'-methylenebis(2-chloroaniline) other than for laboratory work (ATSDR, 1994).

Reports in early 1983 indicated that US manufacturers no longer produced MOCA and that any MOCA used in the USA is imported (NTP, 2005). Since the production of MOCA in the US ceased in 1982, the amount of MOCA imported into the US has increased from 1.5 million pounds in 1983 to approximately 2.0 million pounds in 1991. Most of the MOCA used in the US is manufactured in Taiwan, China, where the annual production is about 5000 to 10 000 tonnes (Chen *et al.*, 2005).

Although production of MOCA ceased in the United Kingdom in 1987, the amount imported has increased from 90–120 tonnes in 1995 to more than 200 tonnes in 2006 (Cocker *et al.*, 2009).

Table 1.1. Selected methods of analysis of 4,4'-Methylene-bis(2-chloroaniline) (MOCA) in various matrices

Sample matrix	Sample preparation	Assay method	Detection limit	Reference
Urine	Ion-paired solid-phase extraction on a disposable octadecylsilica column with an acidic methanol solution containing 1-heptane-sulfonic acid	HPLC-ECD	1 µg/L	Okayama *et al.* (1988)
Toy products	Supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), or Soxhlet extraction with methanol	HPLC-UV	< 0.2 µg/g	Garrigós *et al.* (2002)
Water	A mixture of 20 amines is dissolved in methanol, diluted to different concentrations for analysis. Other solvents are dichloromethane, ethyl acetate, and methanol/dichloromethane (50:50).	GC-MS	5 ng/mL	Doherty (2005)
Textiles	Extract fabric with citrate buffer; decolorize extract with hydrosulfite; extract with *t*-butylmethyl ether; concentrate, and dilute with methanol	LC-MS/MS	14.1 µg/L	Sutthivaiyakit *et al.* (2005)

ECD, electro-chemical detection; GC, gas chromatography; HPLC, high-performance liquid chromatography; LC, liquid chromatography; MS, mass spectrometry; UV, ultraviolet

The USEPA (2003, 2007) Inventory Update Rule regulation requires manufacturers and importers of certain chemical substances listed in the TSCA Chemical Substance Inventory to report manufacturing information (aggregate production volumes) for chemicals manufactured (including imported) in amounts of 10 000 pounds or greater (in 1986) or 25 000 pounds or greater (in 2003) at a single site. Table 1.2 presents the aggregate production volumes that were reported for MOCA.

Available information indicates that 4,4'-methylenebis(2-chloroaniline) was produced and/or supplied in the following countries: Germany; Hong Kong Special Administrative Region; Japan; the People's Republic of China; South Africa; Switzerland; Taiwan, China; and the USA (Chemical Sources International, 2008).

Table 1.2. 4,4'-methylenebis(2-chloroaniline) (MOCA) production volumes

Year	Volume (in millions of pounds)
1986	>1–10
1990	>1–10
1994	>1–10
1998	>1–10
2002	>1–10
2006	0.5–<1

1.2.2 *Use*

4,4'-Methylenebis(2-chloroaniline) (MOCA) is an aromatic amine used for curing epoxy resins. It is mixed with diisocyanate-based pre-polymer resins to produce tough, resistant polyurethane products (Cocker *et al.*, 2009). The polyurethane prepolymers are used in the manufacture of castable urethane rubber products such as shock-absorption pads and conveyor belting (IARC, 1993). In the laboratory, MOCA is used as a model compound for studying carcinogens (NTP, 2005; O'Neil, 2006).

1.3 Occurrence and exposure

1.3.1 *Natural occurrence*

MOCA is not known to occur as a natural product.

In MOCA-contaminated soil, the compound is rapidly adsorbed to the soil matrix and probably exists largely in a covalently bound state (Voorman & Penner, 1986).

1.3.2 *Occupational exposure*

Occupational exposure to MOCA can occur during its production and during its use in the polyurethane industry. Workers can be exposed to MOCA in the form of a liquid emulsion, solid pellets with dust, or solid pellets without dust. In most cases, dermal absorption after contact with contaminated surfaces is the most important occupational exposure route, with inhalation and ingestion representing minor routes of exposure (IARC, 1993).

According to Rappaport and Morales (1979), in 1972 some 10 000 persons in industrialized countries were exposed occupationally to MOCA in the context of manufacturing and processing (Will *et al.*, 1981).

The Health and Safety Executive (HSE) in the United Kingdom estimates that in 2005–2006, 300 workers in the United Kingdom were directly exposed to MOCA during polyurethane-elastomer production, and over 1000 workers such as office staff were indirectly exposed (HSE, 2007a).

Estimates of the number of workers in the USA potentially exposed to MOCA in 1977 ranged from 2100 to 33 000. In 1979 an estimated 1400 workers in the USA were directly exposed and 7400 indirectly exposed while working in polyurethane manufacturing processes involving MOCA (Ward *et al.*, 1987). In 1982, the EPA estimated that 1400–2720 workers were directly exposed and 7600–15200 were indirectly exposed.

(a) Exposure measurements

Concentrations of MOCA in air, blood and urine and in surface-wipe samples have been reported for workers employed in the production and use of MOCA from a range of countries, including Australia, France, Germany, Japan, Taiwan (China), the United Kingdom, and the USA. Different analytical methods have been applied, which can complicate comparisons of reported MOCA levels (see Section 1.1.5).

Monitoring of airborne MOCA alone is considered ineffective in the assessment of worker exposure (Robert *et al.*, 1999), and post-shift urine measurement is the most-employed method to assess exposure. Concentrations of MOCA in urine reflect recent exposure, since the biological half-life of this compound is approximately 23 hours (Osorio *et al.*, 1990).

Some studies have determined urinary concentrations of acetyl-MOCA in addition to MOCA, showing that *N*-acetyl-MOCA is a minor urinary metabolite compared with the elimination of the parent amine (Cocker *et al.*, 1988; Shih *et al.*, 2007).

An alternative to measuring MOCA in urine is to determine the concentrations of haemoglobin adducts of MOCA in blood. These adducts are stable for the lifespan of haemoglobin, which in humans is about 120 days (Vaughan & Kenyon, 1996).

(b) MOCA-production workers

Air concentrations of MOCA have been reported from two MOCA-production plants. In a study from the USA (Linch *et al.*, 1971), the airborne concentration of MOCA was below the detection limit (< 0.01 mg/m^3). In a study from Taiwan, China (Chen *et al.*, 2005), the highest concentrations in air were measured during the purification of MOCA (0.41 mg/m^3).

Urinary concentrations of MOCA in production workers have been reported from France, Taiwan (China), and the USA; they are summarized in Table 1.3.

In a full-scale commercial MOCA-manufacturing plant in the USA, urinary concentrations as high as 3000 μg/L were reported for the year 1969 (Linch *et al.*, 1971). The use of gloves and protective equipment such as suits and respirators was shown to lower exposure considerably.

During a biological monitoring programme in a French factory that periodically produces MOCA, urinary concentrations were reported to range from < 0.5μg/L up to 1600 μg/L (Ducos *et al.*, 1985).

In workers from a production plant in the USA, urinary concentrations of MOCA were measured several months after production had ceased. The maximum level measured was 50 000 μg/L (Ward *et al.*, 1990). [The Working Group noted that the high exposure levels reported may be due to continued exposure to MOCA after its production had ceased, through workplace and environmental contamination].

In 10 workers from a MOCA-manufacturing plant in Taiwan, China (Liu *et al.*, 2005), urinary concentrations ranged between 268 and 15701 μg/g creatinine (Table 1.3).

In another study from Taiwan, China (Shih *et al.*, 2007), 54 urine samples were collected from workers in three MOCA-manufacturing factories. MOCA and acetyl-MOCA (*N*-acetyl-4,4'-methylenebis(2-chloroaniline)) were measured, with median values of 38.6 and 1.8 ng/mL, respectively. MOCA concentrations correlated significantly with the corresponding acetyl-MOCA concentrations in urine.

(c) Polyurethane production workers

Concentrations of MOCA have been measured in the urine of polyurethane-production workers from Australia, Canada, France, Germany, Japan, the United Kingdom and the USA; results are summarized in Table 1.4.

Five hours after an accidental spill of hot MOCA onto the face of a worker cleaning out a MOCA-delivery line in 1976, the urinary level of the exposed worker was 1400 μg/g creatinine (Hosein & Van Roosmalen, 1978).

MOCA concentrations were measured in 49 urine specimens from MOCA-exposed personnel of a plastics manufacturing and processing plant in Germany (Will *et al.*, 1981). The concentrations ranged between 15–100 μg/L.

During a biological monitoring programme conducted in 1978–1981 in a factory in the United Kingdom that manufactured polyurethane elastomers by use of MOCA pellets, urinary MOCA concentrations were measured before and after introduction of safety

Table 1.3. Urinary levels of MOCA in MOCA production workers

Reference	Country, year of study	Task	No. of samples	Measurement	Concentration of MOCA	
					µg/L	µg/g creatinine
Linch *et al.* (1971)	USA, 1962	MOCA production	1	Max	25 000	
	USA, 1969–70	Operators	4	Range	40–3800	
		Operators and moulders without gloves	14	Mean (SD)	278 (252)	
		Operators and moulders with gloves	14	Mean (SD)	80 (92)	
		Operators with gloves, suit, respirator	3	Mean (SD)	3.8 (1.9)	
Ducos *et al.* (1985)	France, 1982	Before improvements	12	Mean	600	450
	France, 1983	After improvements	11	Mean	62	63
Ward *et al.* (1990)	USA, 1981	Production workers	385	Max	50 000[a]	
Liu *et al.* (2005)	Taiwan, China, 2002	MOCA purification process	10	Mean (range)		5544 (267.9–15701)
Shih *et al.* (2007)	Taiwan, China, NR	3 factories, MOCA	54	Range	1.7–1663	2.5–2267
		3 factories, acetyl-MOCA	54	Range	0.08–93.1	0.16–102.8

[a] several months after production ceased
NR, not reported

measures (ventilation, protective clothing, dry-cleaning scheme for overalls) (Thomas & Wilson, 1984). Concentrations dropped from an average of 50 nmol/mmol creatinine to less than 5 nmol/mmol creatinine.

More than 340 analyses were performed on urine samples from 150 workers from 19 polyurethane factories in France (Ducos *et al.*, 1985). In 17 factories where MOCA was used as solid pellets or in solutions for manufacturing a variety of products by coating, moulding or foaming urethane resins, mean concentrations of excreted MOCA in the urine of exposed workers varied from undetectable to 660 μg/L, with a maximum of 1600 μg/L (1540 μg/g creatinine). Process improvements resulted in a significant reduction in urinary MOCA concentrations, to averages of 20–62 μg/L.

The Michigan Department of Public Health tested urine samples of nine manufacturing workers, and reported MOCA concentrations ranging from 13 to 458 μg/L (mean, 145 μg/L) (Keeslar, 1986).

In a NIOSH study of mixers and moulders in a polyurethane elastomer factory, MOCA urinary concentrations were increased during the week and dropped over the weekend (NIOSH 1986).

Between 1980 and 1983, 3323 urinary samples from 54 companies in the USA were analysed: MOCA concentrations exceeded 50 μg/L in 16.9% of the samples and exceeded 100 μg/L in 9.2% (Ward *et al.*, 1987). In 1985, the urinary concentration exceeded 50 μg/L in 12% of all samples tested. In 1990, 8% of the samples showed concentrations that were still above that level (Lowry & Clapp, 1992).

Urinary MOCA concentrations were measured on a regular basis in seven factories in Australia that used MOCA from 1984 onwards (Wan *et al.*, 1989). The measurements were done in five factories before and after a training programme promoting the safe use of MOCA; concentrations decreased from 29.6 μg/L to 10.4 μg/L.

In a study from Japan, MOCA urinary concentrations were measured in five workers over one week (Ichikawa *et al.*, 1990). The concentrations at the beginning and the end of the workshift were 3.1–81.5 and 2.4–96.6 μg/g creatinine, respectively. The highest concentrations were measured for workers pouring the MOCA mix.

Following the accidental spill of MOCA onto the face of a polyurethane worker, urinary MOCA concentrations reached 1700 ppb [μg/L] 4 hours after the accident (Osorio *et al.*, 1990).

In 1986 a study was conducted at a company in the USA that used large amounts of MOCA in manufacturing polyurethane products (Clapp *et al.*, 1991). Sixty-six percent of the urine samples had detectable levels of MOCA, and the highest concentration measured was 159 μg/L.

Urine samples were obtained from five workers involved in the production of polyurethane elastomers in Australia (Vaughan & Kenyon, 1996). The urine contained MOCA at 4.5–2390 nmol/L. Blood samples contained MOCA at 0.13–17.37 nmol/L.

In another study from Australia (Murray & Edwards, 1999), 12 workers in the manufacture of polyurethane showed a median MOCA urinary concentration of

6.5 μmol/mol creatinine (range, 0.4–48.6 μmol/mol creatinine). MOCA was not detected in the urine of control workers.

In a study from France (Robert *et al.*, 1999), postshift urinary MOCA concentrations were determined in 40 workers from four factories producing polyurethane resin. Workers exposed directly to crystallized MOCA on a daily basis had the highest concentration of MOCA in their urine, with a median value of 84 μg/L (49 μg/g creatinine). Concentrations by job category and trends between 1982 and 1996 were also reported (see Table 1.4).

In a small company manufacturing pliable polyurethane, urinary MOCA concentrations were determined (Fairfax & Porter, 2006). None of the 13 employees had detectable amounts of MOCA except the one who performed the urethane casting (15 μg/L total MOCA in urine). Personal air samples collected from the location where the urethane caster worked contained no detectable amounts of MOCA.

A survey of occupational exposure to MOCA in the polyurethane-elastomer industry in Great Britain conducted in 2005–2006 (HSE, 2007a) included 20 polyurethane elastomer manufacturers and two suppliers of MOCA. Urinary concentrations ranged between 1.3–25.0 μmol/mol creatinine.

(d) Occupational surface contamination

Studies from the United Kingdom and USA reported work-surface contamination in the polyurethane industry by determining MOCA concentrations in surface-wipe samples (Table 1.5).

Data from occupational health and safety inspections from 41 polyurethane-production facilities in the USA were assembled (PEDCo Environmental, 1984) and reported surface contamination at facilities using solid and liquid MOCA.

In 1986 a study was conducted at a company in the USA that manufactured polyurethane products and was a large user of MOCA (Clapp *et al.*, 1991). Wipe sampling indicated moderate contamination of the workplace by MOCA dust, with averages up to 19 μg/100 cm^2. The average MOCA concentration found on skin pads worn on workers' hands was generally less than 10 μg/set, with a high of 25 μg/set.

In a small company manufacturing pliable polyurethane (Fairfax & Porter, 2006), the presence of MOCA on work surfaces was reported. Nine locations had non-detectable amounts of MOCA, including a desktop 15 feet from the urethane-casting area; the door handles of the mould oven, respirator locker, gloves locker and restroom; the top of an oven; the handle of the water dispenser in the breakroom; and the handle of a coffee mug. The top of a metal scale table had the highest amount, at 209.7 μg/m^2.

In an occupational exposure survey conducted in 2005–2006 in the polyurethane-elastomer industry in Great Britain, contamination of various surfaces with MOCA was reported (HSE, 2007a). The amounts detected were similar for most surfaces. Contamination around the hopper was generally above that at the other sites, which was thought to be due to excess spillage of MOCA during hopper filling and failure to clean it up immediately.

Table 1.4. Urinary levels of MOCA in polyurethane production workers

Reference	Country, year of study	Task	Number of samples	Measurement	Concentration of MOCA	
					µg/L	µg/g creatinine[1]
Hosein & Van Roosmalen, 1978	Canada, 1976	5 hours after accidental spill (case report)	1			1400
		23 hours after the spill	1			30
Will *et al.*, 1981	Germany	Plastics manufacturing and processing plant	49	range	< 15–100 (LOD = 15)	
PEDCo Environmental, 1984	USA	Without gloves	14	mean	278	
		With gloves	14	mean	80	
		With gloves, respirators and suits	3	mean	3.8	
Thomas & Wilson, 1984	United Kingdom, 1978	Process workers using pelletized MOCA before improvements	~12–15	mean		50 µmol/mol
	United Kingdom, 1982	After improvements	~12–15	mean		< 5 µmol/mol
Ducos *et al.*, 1985	France, 1982	Blending solid MOCA with polyol before improvements	4	range	75–940	31–510
	France, 1983	Blending solid MOCA with polyol after improvements	3	range	ND–9	ND–11
		17 other factories	NR	max	1600	1400
Keeslar, 1986	USA	Manufacturing workers	9	range	13–458	

Table 1.4 (contd)

Reference	Country, year of study	Task	Number of samples	Measurement	Concentration of MOCA	
					µg/L	µg/g creatinine[1]
NIOSH, 1986	USA	Mixers, week	NR	mean	30	
		Mixer, weekend	NR	mean	8.9	
Ward *et al.*, 1987	USA, 1980–83	54 companies	3323	–	16.9% > 50	
				–	9.2% > 100	
Wan *et al.*, 1989	Australia, 1984–87	Workers from 5 factories before training programme		GM (max day)	29.6 (327.2)	
	Australia, 1987	After training programme		GM (max day)	10.4 (21.3)	
Ichikawa *et al.*, 1990	Japan, NR	Production workers (no respirators or gloves)	4	range	[4–120]	2.4–64.0
		Poured mix (no respirators or gloves)	1	–	[200]	96.6
Osorio *et al.*, 1990	USA	Accidental spill of molten MOCA, 4 hours after	1	–	1700 ppb [µg/L]	
Clapp *et al.*, 1991	USA	1 plant	77	–	6.5% > 50	
		Mixers	10	mean (max)	61.9 (158.9)	
		Moulders	35	mean (max)	14.8 (40.0)	
Lowry & Clapp, 1992	USA, 1985	33 companies	1228	–	12% > 50	
	USA, 1990	38 companies	1441	–	8% > 50	

Table 1.4 (contd)

Reference	Country, year of study	Task	Number of samples	Measurement	Concentration of MOCA	
					µg/L	µg/g creatinine[1]
Vaughan & Kenyon, 1996	Australia	5 manufacturers of polyurethane elastomers		range	4.5–2390 nmol/L	
Murray & Edwards, 1999	Australia, 1998	Polyurethane manufacture workers	12	range		0.4–48.6 µmol/mol
Robert *et al.*, 1999	France	Mixer (n = 6)	17	range	0.5–375	0.5–149
		Moulder (n = 10)	26	range	0.5–58	0.5–22
		Maintenance (n = 6)	17	range	5–570	0.5–456
		Others (n = 13)	38	range	0.5–35	0.5–28
Fairfax & Porter, 2006	USA	Urethane casting	1	–	15	
		Other tasks	NR		ND	
HSE, 2007a	United Kingdom, 2005–06	Casting	3	range		3.3–17.0 µmol/mol
		Moulding	12	range		2.2–25.0 µmol/mol
		All exposed	40	range		1.3–25.0 µmol/mol

ND, not detected; NR, not reported; GM, geometric mean
[1] concentration in µmol/mol creatinine when indicated

Table 1.5. MOCA levels in workplace surface wipe samples

Reference	Country, year	Task	Number of samples	Amount of MOCA (μg/100 cm^2)
				Median
PEDCo Environmental, 1984	USA	Solid MOCA: Storage and manual transfer of solid MOCA to melting operations	37	847
		Solid MOCA: Melting	38	11
		Solid MOCA: Transfer of molten MOCA to mixing operations and mixing	9	1650
		Solid MOCA: Transfer of mixture to moulds and pouring of mould	19	5
		Solid MOCA: Storage and manual transfer to melting operations, after controls	19	25
		Solid MOCA: Melting, after controls	2	369
		Solid MOCA: Transfer of molten MOCA to mixing operations and mixing, after controls	12	8
		Solid MOCA: Transfer of mixture to moulds and pouring of mould, after controls	8	8
		Liquid MOCA: Storage and transfer to mixing, uncontrolled	25	30
		Liquid MOCA: Mixing, uncontrolled	4	50 000
		Liquid MOCA: Transfer to moulding and moulding, uncontrolled	6	4.3
		Liquid MOCA: Storage and transfer to mixing, controlled	19	9
		Liquid MOCA: Mixing, controlled	6	4.4
		Liquid MOCA: Transfer to moulding and moulding, controlled	1	0.25

Table 1.5 (contd)

Reference	Country, year	Task	Number of samples	Amount of MOCA (μg/100 cm^2)
				Mean
Clapp *et al.*, 1991	USA, 1986	MOCA room near melting pot	3	19.1
		Top of standing cabinet (moulding dept)	2	4.7
		MOCA room dispensing counter	3	1.4
		Trimmer work table	1	0.1
		Moulder work table	14	0.5
				Mean
Fairfax & Porter, 2006	USA	Top of transformer adjacent to electric oven (where MOCA is heated)	1[a]	0.533
		Top of metal scale table	1	2.097
		Seal of urethane caster respirator	1	0.015
		Top of scale table	1	0.051
				GM
HSE, 2007a	United Kingdom	Fume cupboard	27	3800
		Storage	34	2700
		Weighing/pouring	21	2660
		Mixing	9	1910
		Oven	21	1910
		Hopper	4	96 000
		Casting	6	790
		Other	34	2340

[a]Only those samples with detectable levels are presented (LOD, 0.2 μg per sample)
GM, geometric mean.

1.3.3 *Exposure to the general population*

The general population can be exposed to MOCA in an area that has been contaminated with MOCA or upon consumption of certain types of plants (e.g. root crops) grown in MOCA-contaminated soil. Also, immediate family members of workers exposed to MOCA can be affected. Concentrations of MOCA in urine of up to 15 μg/L have been reported (Keeslar, 1986).

1.3.4 *Accidental release of MOCA in the environment*

Extensive environmental contamination with MOCA on several hundred hectares of land surrounding a MOCA plant occurred in 1979 in Adrian, MI, USA (Keeslar, 1986). Levels up to several milligrams per kilogram were found in gardens and community recreation areas. Of 12 selected children, aged 2 to 16 years, half were found to have detectable concentrations of MOCA ranging from 0.3 to 1.0 ppb [μg/L] in the urine. These children were all under the age of six years. Contact with contaminated soil during playing and going barefoot was considered the most likely route of exposure. The general adult population living within the MOCA-contaminated area had no detectable levels of MOCA in the urine samples tested.

The concentrations in sediment samples collected from the lagoon used by the MOCA plant mentioned above ranged from 1600 to 3800 ppm [mg/kg dry weight]. Effluent water from the lagoon had a concentration of 250 ppb [μg/L], deep-well water from under the plant had a concentration of 1.5 ppb [μg/L], and surface runoff water contained 1 ppb [μg/L]. Activated sludge from the sewage-treatment plant contained an estimated 18 ppm [mg/kg]. MOCA was not detected in sewage treatment-plant influent or effluent water (detection limit 0.5 μg/L) or in the water of a river located near the plant (detection limit 0.1 μg/L) (Parris *et al.*, 1980).

1.4 Regulations and guidelines

1.4.1 *Europe*

(a) Directive 97/56/EC

According to Directive 97/56/EC on the restrictions on the marketing and use of certain dangerous substances and preparations, the packaging of 4,4′-methylenebis(2-chloroaniline) and preparations containing this compound must be marked legibly and indelibly as follows: “Restricted to professional users” (European Commission, 1997).

(b) Directive 2002/61/EC

Directive 2002/61/EC restricts the marketing and use of azocolourants (European Commission, 2002). In this Directive, Annex I to Directive 76/769/EEC is amended. Azodyes which, by reductive cleavage of one or more azo groups, may release 4,4′-

methylenebis(2-chloroaniline) (MOCA) in detectable concentrations, i.e. above 30 ppm in the finished articles or in the dyed parts thereof, according to the testing method established in accordance with Article 2a of this Directive, may not be used in textile and leather articles that may come into direct and prolonged contact with the human skin or oral cavity.

(c) Directive 2004/37/EC

4,4′-Methylenebis(2-chloroaniline) (MOCA) is regulated by the Directive 2004/37/EC (European Commission, 2004), which applies to activities in which workers are exposed to carcinogens or mutagens of Category 1 and 2. Rules are fixed regarding the employers' obligations of reduction and replacement, prevention and reduction of exposure, unforeseen exposure, foreseeable exposure, access to risk areas, hygiene and individual protection, information for the competent authority, information and training of workers, consultation and participation of workers, health surveillance, record keeping and limit values.

1.4.2 *Japan*

The Japan Society for Occupational Health (JSOH; 2007) has reported an occupational exposure limit (OEL) value of 0.005 mg/m^3 with a skin notation for 3,3′-dichloro-4,4′-diaminodiphenylmethane [MOCA]. An Occupational Exposure Limit based on Biological Monitoring (OEL-B) was mentioned of 50 µg/g-creatinine (total MOCA) in urine sampled at the end of the shift at the end of the work week. The JSOH follows the classification by IARC of 3,3′-dichloro-4,4’-diaminodiphenylmethane in Group 2A.

1.4.3 *Germany*

4,4′-Methylenebis(2-chloroaniline) (MOCA) is classified as a Category-2 carcinogen by the MAK Commission. The MAK Commission listed 4,4′-methylenebis(2-chloroaniline) as a substance where percutaneous absorption may significantly contribute to systemic exposure. A MAK value was not set (MAK, 2007).

1.4.4 *USA*

(a) ACGIH

4,4′-Methylenebis(2-chloroaniline) (MOCA) has been assigned an A2 notation, *suspected human carcinogen* (ACGIH, 2001). A TLV-TWA (threshold limit value–time-weighted average) of 0.01 ppm (0.11 mg/m^3) is recommended. A skin notation is assigned (*potentially significant contribution to the overall exposure by the cutaneous route*) in recognition of the consensus that skin absorption from direct contact is the major source of occupational exposure. Implementation of a urine-monitoring programme to ensure the effectiveness of dermal exposure control is encouraged.

(b) NIOSH

4,4'-Methylenebis(2-chloroaniline) is listed with a TWA of 0.02 ppm (0.22 mg/m^3) [skin] as a recommended exposure limit (REL) not in effect (NIOSH, 1989).

(c) NTP

4,4′-Methylenebis(2-chloroaniline) is listed in the NTP *Report on Carcinogens* as *reasonably anticipated to be a human carcinogen* (NTP, 2005).

1.4.5 *United Kingdom*

The Health and Safety Commission (HSE, 2007b) has set an 8-hour time-weighted average Workplace Exposure Limit (WEL) of 0.005 mg/m^3 for 2,2'-dichloro-4,4'-methylenedianiline (MOCA), with a skin notation. HSE also lists MOCA as capable of causing cancer and/or heritable genetic damage, and has defined a Biological Monitoring Guidance Value (BMGV) of 15 μmol total MOCA/mol creatinine in urine sampled after the work-shift.

1.4.6 *Other*

(a) GESTIS

Table 1.6 presents some international limit values for MOCA (GESTIS, 2007).

Table 1.6. International limit values (2007) for MOCA

Country	Limit value – Eight hours		Limit value – Short-term		Comments
	ppm	mg/m^3	ppm	mg/m^3	
Austria		0.02		0.08	TRK value (based on technical feasibility)
Canada, Québec	0.02	0.22			
Denmark	0.01	0.11	0.02	0.22	
France	0.02	0.22			
The Netherlands		0.02			
Spain	0.01 (skin)	0.1 (skin)			
Switzerland		0.02			
United Kingdom		0.005			

TRK, technical guiding concentration

2. Studies of Cancer in Humans

2.1 Screening studies

Ward *et al.* (1988; 1990) reported three cases with noninvasive papillary tumours of the bladder identified in a screening study of 385 workers who had been exposed to MOCA in a chemical plant in Michigan, USA, from 1968 to 1979. Later on, Hogan (1993) assessed detailed occupational exposures for the three cases by reviewing the chemical plant's product catalogues, material safety-data sheets, and written documentation about chemicals used to produce specific products, and by examining job histories, the time frames of exposure, and job classifications. The study found that the three cases had been exposed to many other chemicals in addition to MOCA during their time at the chemical plant and throughout their work history. Some of these chemicals are known or suspected bladder carcinogens (*ortho*-toluidine, 4-chloro-*ortho*-toluidine).

In 1989, Fox Chase Cancer Center worked with DuPont Chamber Works in New Jersey, USA to develop a bladder-cancer screening programme for the high-risk population of workers who had been exposed to MOCA, β-naphthylamine, benzidine, or *ortho*-toluidine at this plant (Mason and Vogler 1990; Mason *et al.* 1992). During the first seven quarterly periods of screening, two new cases and one recurrent case of transitional cell carcinoma of the bladder were detected, but they had been exposed to occupational bladder carcinogens other than MOCA.

Chen *et al.* (2005) screened for bladder cancer at four MOCA-manufacturing factories in Taiwan, China that employed 70 workers who were directly involved in MOCA-manufacturing processes, including the reaction, neutralization, washing, purification, and packing, or who were indirectly involved in the research and development laboratory. Ninety-two workers were not involved in the MOCA manufacturing or packing, nor were they working in the same building as the employees involved in these activities. The prevalence of atypical urinary cells and the nuclear matrix protein 22 tumour marker was not significantly different between the two groups of workers. However, the prevalence of positive occult blood was borderline-significantly ($P = 0.055$) greater in male exposed workers (18%) than in male non-exposed workers (7%). Among the 70 workers who had exposure to MOCA, there was one person with suspected malignant cells on urine cytology, one person with atypical cytology combined with gross haematuria, and one simply with atypical cytology. One worker was absent from the screening programme because he was admitted to the hospital, where he was diagnosed with bladder cancer. Liu *et al.* (2005) presented detailed information for the confirmed bladder-cancer case in his occupational history and environmental monitoring data. The confirmed bladder-cancer case had worked in the purification process area for 14 years (1987–2001), where the concentration of MOCA in the air was the highest (0.23–0.41 mg/m^3) and exceeded permissible OSHA exposure levels (0.22mg/m^3), without wearing any personal protective equipment at work. Furthermore, the worker was a non-smoker without a history of exposure to any other bladder carcinogen, except for

occasional pesticides application during agricultural work before he began working at this factory.

3. Studies of Cancer in Experimental Animals

Animal bioassays conducted with 4,4′-methylenebis(chloroaniline) (MOCA) were reviewed in IARC Monograph Volume 57 (IARC, 1993).

3.1 Oral administration

3.1.1 *Mouse*

Groups of 25 male and 25 female HaM/ICR mice, 6–8 weeks of age, were fed diets containing 0, 1000 or 2000 ppm MOCA as the hydrochloride (purity 97%) for 18 months. The doses were chosen on the basis of preliminary tests, the highest dose being the maximum tolerated dose. The effective numbers of animals at the end of the study were 18, 13, and 20 males, and 20, 21, and 14 females in the control, low-dose and high-dose groups, respectively. Haemangiomas or haemangiosarcomas (mainly subcutaneous) combined occurred in 0/18, 3/13 (23%), and 8/20 (40%) in the control, low-dose, and high-dose groups of the male mice, and in 1/20 (5%), 0/21, and 6/14 (43%) of the female mice. 'Hepatomas' occurred in 0/20, 9/21 (43%), 7/14 (50%) in the control, low-dose, and high-dose groups of female mice ($P < 0.01$, Fisher exact test) and in 3/18 (17%), 3/13 (23%), 4/20 (20%) male mice. 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 groups was comparable with that in historical controls of the same strain [and probably not treatment-related] (Russfield *et al.*, 1975).

3.1.2 *Rat*

Groups of 25 male and 25 female Wistar rats, 100 days of age, were fed 0 or 1000 ppm MOCA (purity unspecified) in a protein-deficient diet [not otherwise specified] for 500 days (total dose, 27 g/kg bw), followed by an observation period on protein-deficient diet. Animals were killed when moribund; mean survival of treated males and females was 565 days and 535 days, respectively, and mean survival of male and female controls on the protein-deficient diet was 730 days. Of the 25 treated males, 23 died with tumours; 'hepatomas' occurred in 22/25 (88%) ($P < 0.001$, Fisher exact test), and lung tumours (mainly carcinomas) in 8/25 (32%) ($P = 0.002$, Fisher exact test). Among the treated females, 20 rats died with tumours; 'hepatomas' occurred in 18/25 (72%) ($P < 0.001$ Fisher exact test), and lung tumours were observed in 5/25 (20%) ($P = 0.025$, Fisher exact test). No 'hepatoma' or lung tumour was observed among 50 control animals (Grundmann & Steinhoff, 1970).

Groups of 25 male Charles River CD-1 rats, 6–8 weeks of age, were given diets containing 0, 500 or 1000 ppm MOCA as the hydrochloride (purity 97%) for 18 months. The doses were chosen on the basis of preliminary tests, the highest dose being the maximum tolerated dose. 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 (5%) low-dose and 4/19 (21%) high-dose rats ($P < 0.05$, Cochran-Armitage trend test) (Russfield *et al.*, 1975).

Groups of 50 male and 50 female Charles River CD rats, 38 days of age, were given 0 (control) or 1000 ppm MOCA (purity approximately 95%) in a standard diet (23% protein) for two years. The average duration of the experiment was 560 days for treated males, 548 days for treated females, 564 days for male controls and 628 days for female controls. Six animals from each group were sacrificed after one year for interim evaluation. Lung adenocarcinomas occurred in 21/44 (48%) ($P < 0.05$, χ^2-test) treated males and 27/44 (61%) ($P < 0.05$, χ^2-test) treated females. 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 (32%) treated males and 11/44 (25%) treated females, and in 1/44 (2%) males and 1/44 (2%) females in the controls ($P < 0.05$). Pleural mesotheliomas occurred in 4/44 (9%) treated males and 2/44 (5%) treated females; no such tumour was observed among controls. Hepatocellular adenomas and hepatocellular carcinomas occurred in 3/44 (7%) and 3/44 (7%) treated males and in 2/44 (5%) and 3/44 (7%) 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 (2%) *vs* 12/44 (27%)) (Stula *et al.*, 1975).

In the same study, another 25 male and 25 female Charles River CD rats, 36 days of age, were given 0 (control) or 1000 ppm MOCA (purity approximately 95%) in a low-protein diet (7%) for 16 months. Six animals from each group were sacrificed after one year for interim evaluation. The average duration of the experiment was 400 days for treated males, 423 days for treated females, 384 days for control males and 466 days for control females. Lung adenocarcinomas occurred in 5/21 treated males ($P < 0.05$, χ^2-test) and 6/21 females ($P < 0.05$, χ^2-test); no such tumour developed in 21 untreated male or female controls. Lung adenomatosis was observed in 8/21 (38%) treated males and 14/21 (67%) treated females and in 1/21 (5%) male controls and 1/21 (5%) female controls ($P < 0.05$, χ^2-test). Hepatocellular adenomas occurred in 5/21 (24%) treated males ($P < 0.05$, χ^2-test) and 2/21 (10%) treated females; hepatocellular carcinomas were observed in 11/21 (52%) treated males ($P < 0.05$, χ^2-test) and 1/21 (5%) treated females; no hepatocellular tumour was observed among 21 untreated males or females. Fibroadenomas of the mammary gland occurred in 1/21 (5%) treated and 7/21 (33%) control female rats ($P < 0.05$). Mammary gland adenocarcinomas developed in 6/21 (29%) treated and in 0/21 untreated females ($P < 0.05$, χ^2-test) (Stula *et al.*, 1975).

Groups of 100, 100, 75 and 50 male Charles River CD rats, 35 days of age, weighing 90–167 gr, were fed either a "protein-adequate" (27%) diet containing 0, 250, 500 or 1000 mg/kg (ppm) MOCA (industrial grade [purity unspecified]) or a "protein-deficient" (8%) diet containing 0, 125, 250 and 500 ppm MOCA for 18 months, after which they were maintained on their respective diet without MOCA for 6 months, followed by a 32-week observation period. The mean survival time (in weeks) for the protein-adequate diet was: control, 89; low-dose, 87; mid-dose, 80 ($P < 0.01$) (two-sided test, but method not specified); high-dose, 65 ($P < 0.001$); for the protein-deficient diet, these values were: 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 in this group). 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; the incidences of the predominant tumours are shown in Table 3.1. 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 the 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%) (Kommineni *et al.*, 1979).

3.1.3 *Dog*

A group of six pure-bred female beagle dogs, approximately one year of age, were given a daily oral dose of 100 mg MOCA (~90%, ~10% polyamines with a three-ring structure and ~0.9% *ortho*-chloroaniline) in a gelatin capsule on three days per week for six weeks, then on five days per week for up to nine years. A further group of six female dogs 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 4/5 (80%) treated dogs, and a composite tumour (transitional-cell carcinoma/adenocarcinoma) of the urethra developed in the 5th treated dog. No such tumours were observed among the six untreated control dogs ($P < 0.025$, Fisher exact test) (Stula *et al.*, 1978).

3.2 Subcutaneous administration

3.2.1 *Rat*

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

Table 3.1. Percentages of male rats with tumours at specific sites after feeding of MOCA in diets with different protein contents

Dietary protein	MOCA (ppm)	No. of rats autopsied	Lung adeno-carcinomas	All lung tumours	Mammary adeno-carcinomas	Zymbal gland carcinomas	Hepatocellular carcinomas	Haemangio-sarcomas	Pituitary adenomas[a]
Adequate	0	100	0	1	1	1	0	2	42
(27%)	250	100	14***	23***	5	8*	3	4	36
	500	75	27***	37***	11**	7	4	4	25*
	1 000	50	62***	70***	28***	22***	36***	0	4***
Deficient	0	100	0	0	0	0	0	1	23
(8%)	125	100	3	6**	1	0	0	2	16
	250	75	9**	15***	4	5*	0	5	12*
	500	50	16***	26***	6*	12***	18***	8*	20

From Kommineni *et al.* (1979).

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

[a] Includes pituitary adenocarcinomas (0–2 per group).

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 (26%) ($P < 0.0042$, Fisher exact test), and malignant lung tumours (six adenocarcinomas, one carcinoma) were observed in 7/34 (20%) animals ($P < 0.016$, 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 in the control group (Steinhoff & Grundmann, 1971). [The Working Group noted the inadequate reporting of the experiment.]

3.3 Initiation-promotion studies

3.3.1 *Mouse*

Groups of 80 male and female SENCAR mice, 7–9 weeks of age, were given a single dermal application to the dorsal area of 0, 0.1, 1, 10, 100 or 200 mg MOCA (purity not specified), and after a one-week interval 2 µg of 12-*O*-tetradecanobylphorbol-13-acetate (TPA) was applied twice a week for 26 weeks as a promoter. MOCA did not induce significant numbers of mouse skin papillomas (Table 3.2) (Nesnow *et al.*, 1985).

Groups of 20 female hairless albino HRA/Skh mice, about six weeks of age, were given a single dermal application of 12.5, 25, 50 or 100 mg MOCA (purity ≥ 90–100%). After one week, 5 µg of 12-*O*-tetradecanonylphorbol-13-acetate (TPA) was applied twice a week for 21 weeks as a promoter. In this experiment MOCA was tested as an initiator. Mice were observed for one year; the minimum diameter of the papillomas scored was approximately 1 mm. The group treated with 25 mg MOCA had a slightly higher tumour incidence (35%) than the controls (27%) at 25 weeks, and at 52 weeks (20% *vs* 16%). However, comparison of the control group response with any of the MOCA-treated groups did not show any statistically significant difference (Rozinova *et al.*, 1998).

Table 3.2. Mouse skin-tumour initiation by MOCA

Dose µg[a]	Number of mice surviving		Mice bearing papillomas	
	Male	Female	Male	Female
0	40	36	10	11
100	35	38	6	3
1000	39	39	23	15
10 000	38	39	5	5
100 000	38	40	16	15
200 000	37	37	14	5

From Nesnow *et al.*, 1985.

[a] dermal application of MOCA, dermally promoted with 2 µg TPA

In the same study, another group of 20 female hairless albino HRA/Skh mice, about six weeks of age, were given single dermal application of 2.56 µg 7,12-dimethylbenz[*a*]anthracene (DMBA). After one week, dermal applications of 2.5 or 5 mg of MOCA (purity ≥ 90–100%) were started and continued twice a week for twenty weeks. In this experiment MOCA was tested as a promoter. Mice were observed for one year; the minimum diameter of the papillomas scored was approximately 1 mm. The negative control group received only a single application of DMBA. In this group, the first tumour occurrence was observed at week 19; the maximum tumour yield was 0.55 ± 0.21 tumours/mouse. In the 2.5-mg and 5-mg MOCA-promoted groups, the first tumour occurrence was observed at weeks 9 and 11, and the maximum tumour yields were 0.65 ± 0.17 and 0.30 ± 0.14 tumours/mouse, respectively (Rozinova *et al.*, 1998).

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, excretion

4.1.1 *Humans*

Vaughan and Kenyon (1996) measured the concentrations of MOCA in urine, plasma and blood, and the amounts of haemoglobin (Hb) adducts in exposed workers. Samples from five workers involved in the production of polyurethane elastomers were examined. Gas chromatography/negative-ion chemical ionization mass spectrometry (GC-NICI-MS) was used for the determination of pentafluoropropionyl derivatives of MOCA. Both 3, 3′-dichlorobenzidine and [$^2H_6$]-MOCA were used as internal standards. Urinary concentrations of MOCA ranged over 500-fold, from 4.5 nmol/l (worker A) to 2390 nmol/l (worker E). Adducted MOCA was released by alkaline hydrolysis, and adduct levels were similar with or without dialysis of Hb. The Hb adducts ranged from 0.73 pmol/g Hb for worker A, who carried out general duties in the factory, to 43.3 pmol/g Hb for worker E, who was involved in mixing MOCA with a prepolymer. The former value is similar to that reported for 4-aminobiphenyl-Hb adducts in non-smokers (0.19 ± 0.08 pmol/g Hb) and smokers (0.91 ± 0.28 pmol/g Hb) (Skipper & Tannenbaum, 1990). The higher values for MOCA adducts are similar to concentrations of Hb adducts of 4,4'-methylenedianiline (MDA), a structurally similar compound. Concentrations of 10.88 pmol MDA/g Hb and higher values of 25.33 pmol *N*-acetyl-MDA/g Hb were reported for a worker (Bailey *et al.*, 1990), with a ratio of *N*-acetyl-MDA to MDA of 2.3. Although the actual value for pmol *N*-acetyl-MOCA/g Hb was not given, the ratio of *N*-acetyl-MOCA to MOCA was reported to range from 0.016 to 0.05 (Vaughan & Kenyon, 1996). This suggests that *N*-acetylation may not be an important pathway for MOCA metabolism. Blood MOCA concentrations were determined after alkaline hydrolysis. Values for worker A (0.13 nmol/l) and worker E (17.37 nmol/l) represented the lowest and highest levels in blood. Plasma values after alkaline hydrolysis were similar to those

in blood from corresponding workers, with those of workers A and E being 0.05 and 21.95 nmol/l, respectively. When the latter sample was subjected to ultrafiltration, 85% was found to be associated with the high-molecular-weight fraction, representing Hb and possibly other protein adducts. Hb adducts were thought to occur by activation of MOCA in a manner similar to DNA-adduct formation. While the half-life for excretion of MOCA in urine has been estimated to be 23–24 hours, Hb has a life-span of about 120 days, and Hb adducts would reflect exposure over this extended time course (Osorio *et al.*, 1990).

While *N*-acetylation has been shown to be an important detoxifying pathway in the human biotransformation of many aromatic amines, this may not be the case for MOCA (see previous study). In workers exposed to benzidine, more than 95% of measured urinary benzidine metabolites were present in *N*-acetylated form (Rothman *et al.*, 1996). Urinary levels of MOCA and its metabolites have been monitored in exposed workers, with only low concentrations of *N*-acetyl-MOCA and *N*,*N*′-diacetyl-MOCA observed (Ducos *et al.*, 1985; Cocker *et al.*, 1988). To evaluate whether *N*-acetylated products might be heat-labile conjugates, urine was heated for 1.5 hours at 80°C (Cocker *et al.*, 1988). This improved the detection of *N*-acetyl-MOCA. However, this did not produce ratios of *N*-acetylated to parent amine of greater than 0.09. More than half of the urine samples in this study had undetectable levels of *N*-acetyl-MOCA and thus were not used in the analysis. For heat-treated urine, ratios for *N*-acetylated MOCA to MOCA ranged from 1 to 24 (Cocker *et al.*, 1988). Human liver homogenates catalysed *N*-acetylation of MOCA with rates for rapid and slow acetylators similar to that observed with benzidine (Glowinski *et al.*, 1978). These results suggest that MOCA is either not *N*-acetylated *in vivo* or that it is acetylated and then rapidly deacetylated. The latter was suggested when human liver slices incubated with [^{3}H]-benzidine doubled their production of *N*-acetylated products with paraoxon (Lakshmi *et al.*, 1995). In urothelial cells of workers exposed to benzidine, the most predominant DNA adduct formed was *N*-acetylated, i.e. *N*′-(deoxyguanosin-8-yl)-*N*-acetylbenzidine (Rothman *et al.*, 1996). In contrast, in urothelial cells of workers exposed to MOCA, the major adduct was shown to be a non-*N*-acetylated monocyclic adduct, *N*-(deoxyadenosin-8-yl)-4-amino-3-chlorobenzyl alcohol (Kaderlik *et al.*, 1993). *N*-Acetyl-MOCA is less mutagenic than MOCA as measured by the Ames test (Hesbert *et al.*, 1985), and no difference has been observed in the induction of DNA repair in hepatocytes from either rapid or slow acetylator rabbits (McQueen *et al.*, 1983). Thus, the metabolism and activation of MOCA seems significantly different from that of benzidine or even 4,4′-methylenedianiline (MDA).

4.1.2 *Experimental animals*

N-Acetylation plays an important role in metabolism of aromatic amines in rats. For example, [^{3}H]-labelled benzidine (180 mCi/mmol) is rapidly metabolized to its mono- and diacetylated products by male Fischer 344 rats. After 30 min, the recirculating perfusate from the isolated perfused rat liver showed a ratio of *N*-acetylated metabolites to benzidine of 14 (Lynn *et al.*, 1983; 1984). In contrast, *N*-acetylated heterocyclic amines

are not observed following administration of the parent compound to male Fischer 344 rats (Armbrecht *et al.*, 2007). A study of female LAC:Porton rats given an intraperitoneal injection of [methylene-^{14}C]-MOCA (8.3 mCi/mmol, radiochemical purity unspecified) reported at least nine metabolites and showed evidence of an *O*-glucuronide, an *O*-sulfate, and amino-chlorophenol. However, no *N*-acetylated metabolites were reported (Farmer *et al.*, 1981). Another extensive study with male CD rats (30 days old; immature) assessed the metabolism of orally dosed [methylene-^{14}C]-MOCA (4 to 7 mCi/mmol; ~93% radiochemical purity). Evidence of an *O*-glucuronide and an *O*-sulfate in urine was found, along with the major metabolite in bile, i.e. the mono-*N*-glucuronide of MOCA. However, no *N*-acetylated products were reported (Morton *et al.*, 1988).

Rat microsomal CYPs catalysed formation of three hydroxylated MOCA metabolites, *N*-OH, 5-OH, and methylene-OH. *N*-OH-MOCA is thought to participate in MOCA binding to DNA. The major MOCA-DNA adduct in rats was shown to be a non-*N*-acetylated monocyclic adduct, *N*-(deoxyadenosin-8-yl)-4-amino-3-chlorobenzyl alcohol (Silk *et al.*, 1989). This adduct was also formed by reaction of *N*-OH-4-amino-3-chlorobenzyl alcohol with DNA.

While MOCA is a substrate for rat *N*-acetyltransferases, *N*-acetylated products of MOCA have not been reported in urine or bile of rats that were given this amine.

Sabbioni and Neumann (1990) developed a quantitative method to determine MOCA-protein adducts. Female Wistar rats received oral doses of 3.82, 14.2 and 16.2 μmol/kg bw [^{14}C]-ring-labelled MOCA or 0.25 and 0.50 mmol/kg bw unlabelled MOCA. MOCA bound in decreasing amounts to macromolecules in the lung, liver and kidney. Fractions of 0.19% and 0.026% of the dose were bound to the blood proteins haemoglobin and albumin, respectively. MOCA was released by hydrolysis from haemoglobin, and determined by HPLC with electrochemical detection or by GC-MS. However, in contrast to many other aromatic amines, only 54% of the haemoglobin-bound [^{14}C]-labelled metabolites are hydrolysable, while none of the radioactivity could be released from albumin. This implies the existence of additional reactive species, presumably originating from metabolic cleavage at the methylene-bridge in MOCA, which yields stable protein adducts.

Macromolecular binding in rats after oral and dermal application of [^{14}C]-labelled MOCA has also been described (Cheever *et al.*, 1990).

Chen *et al.* (1991) studied the capacity of *N*-oxidized metabolites of MOCA to form haemoglobin (Hb) adducts in animals with or without induction of CYP enzymes with phenobarbital or β-naphthoflavone. Intravenous administration of as little as 0.04 μmol/kg *N*-hydroxy-MOCA to rats resulted in measurable formation of MOCA-Hb adducts (0.9 ng/50 mg Hb). Intraperitoneal administration of 0.5–50 mg/kg MOCA to rats, and subcutaneous administration of 5–500 mg/kg MOCA to rats and 4–100 mg/kg to guinea-pigs resulted in dose-related formation of Hb adducts. MOCA-Hb adducts remained elevated in blood for longer than 10 weeks following a single subcutaneous dose in guinea-pigs. Pretreatment of rats with phenobarbital would induce microsomal benzphetamine *N*-demethylase (BND) activity in rats, and result in a small increase in in-

vitro *N*- and *ortho*-hydroxylation of MOCA, but did not increase in-vivo Hb adduct levels. Pretreatment of rats with β-naphthoflavone induced microsomal aryl hydrocarbon hydroxylase as well as ethoxyresorufin-*O*-deethylase and increased the formation of MOCA-Hb adducts when the animals were dosed with MOCA at 100 and 500 mg/kg, but not 20 mg/kg subcutaneously (Chen *et al.* 1991).

Cheever *et al.* (1991) studied the multiple oral administration of MOCA in adult male rats. As many as 28 consecutive daily doses of [^{14}C]MOCA at 28.1 μmol/kg bw (5 μCi/day) were given. Rats were killed at weekly intervals for seven weeks. MOCA adduct formation for globin and serum albumin was evaluated by determination of [^{14}C]MOCA covalent binding. The covalent binding associated with globin showed a linear increase over the 28-day exposure period with 342 fmol/mg globin 24 hours after the final dose. The covalent binding with albumin was 443 fmol/mg albumin. After cessation of dosing, the albumin and globin adduct levels decreased rapidly with a half-life of the respective proteins.

Bailey *et al.* (1993) studied the exposure of rats to [^{14}C]ring-labelled MOCA. At 24 hours after a single intraperitoneal dose (3.74 μmole/kg bw), 0.08% of the administered dose was adducted to haemoglobin (Hb) and alkaline hydrolysis liberated 38% of the bound radioactivity as the parent MOCA. The formation of adducts correlated linearly with the dose of MOCA (3.74–44.94 μmole/kg bw).

The biological availability of the *N*-hydroxylated derivatives of *ortho*-substituted diamines and of known carcinogenic diamines was investigated in female Wistar rats by determining haemoglobin (Hb) adducts. Hb from rats dosed with 0.5 mmol/kg diamine and from untreated control rats were isolated and hydrolysed. The released diamine and monoacetyldiamine were quantified by HPLC with electrochemical detection or GC/MS. MDA, 4,4′-oxydianiline (ODA), 4,4′-ethylenediamine, and 4,4′-thiodianaline (TDA) bound to haemoglobin as diamine and as monoacetyl-diamine. 4,4′-Methylenebis(2,6-dimethylaniline), 4,4′-methylenebis(2,6-diethylaniline), MOCA, and 4,4′-sulfonyl-dianiline (daspone) bound only as diamine to Hb. 4,4′-Methylenebis (2,6-dichloroaniline) did not bind to Hb. Thus, the presence of two substituents in the *ortho* position and the presence of electron-withdrawing groups in the *para* position relative to the amino group drastically reduced the formation of Hb adducts. The extent of haemoglobin binding of the bicyclic diamines (daspone, 3,3′-dichlorobenzidine, MDA, MOCA, TDA, ODA, and benzidine) increases with their carcinogenic potency (Sabbioni & Schütze, 1998).

4.1.3 *In-vitro studies*

Because occupational exposure to MOCA occurs mainly through skin penetration, Hewitt *et al.* (1995) evaluated decontamination procedures *in vitro* using fresh full-thickness human breast skin. Flow-through diffusion cells were used to determine the effect of four washing solutions (100% ethanol, 100% water, 1 and 10% (v/v) aqueous soap). All solutions were equally effective at removing MOCA (ring-[^{14}C]-MOCA, specific activity 59 mCi/mmol; radiochemical purity > 96%; 10–18 μg/cm^2) from the

surface of human skin, with 22–47% of the applied dose removed at 72 hours. The penetration of MOCA into and through human skin at 72 hours was significantly reduced (two- to threefold) by washing the skin surface at 3 or 30 min. Washing at one hour after application significantly reduced total uptake, but this was less effective than at earlier time points. These results suggest that MOCA is rapidly absorbed from the skin surface into the skin. To reduce systemic exposure, the authors suggested that skin should be washed within the first 30 minutes after contamination. The choice of washing solution is less critical than is the time of washing. Similar results were observed for MDA. These results are consistent with previous studies showing that the uptake of MOCA from the skin surface into the skin itself is rapid, reaching a maximum only one hour after application (Hewitt *et al.*, 1993). This suggests the presence of a cutaneous reservoir within the skin.

Wiese *et al.* (2001) showed that recombinant human COX-1 and COX-2, isoforms of prostaglandin H synthase, activate MOCA, given as [4,4'-methylene-^{14}C]MOCA (8.66 mCi/mmol), to bind to DNA. Besides MOCA, several different aromatic and heterocyclic amines were studied. Prostaglandin H synthase is composed of two separate isoenzymes, cyclooxygenase and hydroperoxidase. The latter utilizes these amines as reducing substrates. Binding was found to be arachidonic acid-dependent. The highest DNA binding was observed with MOCA. hCOX-2 activated MOCA nearly twice as effectively as did hCOX-1. In contrast, activation of the other amines by both enzymes was similar. While benzidine-DNA binding was reduced by BSA, this was not observed with MOCA. The peroxidative activity of human lung microsomes was shown to activate MOCA (^{14}C-MOCA, specific activity 46.7 mCi/mmol) to bind DNA (Culp *et al.*, 1997). With specific antisera it was demonstrated that myeloperoxidase was responsible for DNA binding. Binding was dependent on H_2O_2 and was inhibited by azide, but not by indomethacin or eicosatetraynoic acid (Culp *et al.*, 1997). This is consistent with myeloperoxidase-mediated rather than prostaglandin H synthase-mediated binding. The source of myeloperoxidase was thought to be the polymorphonuclear neutrophils. These cells are involved in the inflammatory response, which can contribute to carcinogenesis (Parsonnet, 1999). Results demonstrate that different enzymes mediate peroxidative activation of MOCA to bind to DNA.

While human CYP1A2 *N*-hydroxylates many arylamines, MOCA is an exception, preferring CYP2A6 or CYP3A4 (Butler *et al.*, 1989; Yun *et al.*, 1992).

[^{3}H]*N*-OH-MOCA (20 mCi/mmol) was found to be metabolically activated by 3′-phosphoadenosine-5′-phosphosulfate (PAPS)-dependent human liver sulfotransferases to bind to DNA (Chou *et al.*, 1995). In 12 human hepatic cytosols, binding was significantly correlated with levels of thermostable phenol-sulfotransferase activity, but not with thermolabile phenol- or dehydro-epiandrosterone-sulfotransferase activities. Binding was prevented by 2,6-dichloro-4-nitrophenol, a potent selective inhibitor for human thermostable phenol sulfotransferase. *N*-OH-MOCA was a good substrate for sulfotransferase in human liver cytosols, but not in cytosols from human urinary bladder epithelium. Thermostable phenol-sulfotransferase is polymorphically distributed and

could contribute to individual differences in carcinogen susceptibility. *N*-Hydroxyarylamines are thought to initiate DNA-adduct formation (Chou *et al.*, 1995).

Walraven *et al.* (2006) used recombinant rat *N*-acetyltransferases to assess *N*-acetylation of MOCA by the enzymes Nat1, Nat2, and Nat3 via HPLC analysis of non-radioactive metabolites. MOCA was *N*-acetylated by all three transferases. Nat3 (the recently discovered third rodent *N*-acetyltransferase) metabolized MOCA along with six other arylamines. However, Nat3-mediated metabolism of MOCA was at the limit of detection. Nat1 and Nat2 each metabolized 12 of the 13 arylamines tested. MOCA was only one of three amines found to be selectively *N*-acetylated by recombinant rat Nat1. In contrast, 4,4'-methylenedianiline was one of nine substrates selectively metabolized by rat Nat2. Previous studies have demonstrated *N*-acetylation of MOCA by human and rabbit liver homogenates (Glowinski *et al.*, 1978). *N*-Acetylation of MOCA by human rapid and slow acetylators was similar to that observed with benzidine.

4.2 Genetic and related effects

4.2.1 *Humans*

Exfoliated urothelial cells were recovered from urine samples from a worker accidentally sprayed with molten MOCA. Samples were collected at different times—up to 430 hours—after exposure (Osorio *et al.*, 1990). The occurrence of MOCA-DNA adducts was investogated in these exfoliated urothelial cells by ^{32}P-post-labelling analysis. The major DNA adduct was *N*-(deoxyadenosin-8-yl)-4-aminochlorobenzyl alcohol. This MOCA-DNA adduct was detected in samples obtained between 4 and 98 hours after initial exposure but not in samples collected at later times. The level of DNA adducts 4 hours after exposure was determined to be 516 adducts/10^8 nucleotides. A five-fold decrease in adduct level was observed 14 hours later, followed by a gradual decrease over subsequent days (Kaderlik *et al.* 1993).

An increased frequency of sister chromatid exchange, consistent with their apparent exposure to MOCA, was seen in peripheral lymphocytes from a small number of workers exposed to MOCA during the manufacture of polyurethane (Edwards & Priestly, 1992).

In a study from Australia, micronucleus induction was measured in peripheral lymphocytes and exfoliated urothelial cells of workers exposed to MOCA. Twelve male workers (age 24–42 years) were recruited for this study from four work locations where exposure was noted. Exfoliated urothelial cells from pre-work urine samples on a midweek work-day were assessed for micronucleus (MN) frequency. Post-work urine samples were analysed for total MOCA. Blood samples were collected on the same day and were cultured for 96 hours. Cytochalasin-B-blocked cells were scored for MN. Eighteen male control subjects (age 23–59 years) provided corresponding urine and blood samples. Average urinary MOCA concentrations were 6.5 µmol/mol creatinine (range 0.4–48.6 µmol/mol creatinine) in post-work samples of MOCA-exposed workers. MOCA was not detected in the urine of control workers. Mean MN frequencies were higher in

urothelial cells and lymphocytes of MOCA-exposed workers (14.27 ± 0.56 and 13.25 ± 0.48 MN/1000 cells) than in control subjects (6.90 ± 0.18 and 9.24 ± 0.9 MN/1000 cells). The mean number of micronucleated cells was also higher in both tissues of exposed workers (9.69 ± 0.32 and 8.54 ± 0.14 MN cells/1000 cells) than in control subjects (5.18 ± 0.11 and 5.93 ± 0.13 MN cells/1000 cells) (Murray & Edwards, 1999).

More recently, the same authors studied cytogenetic endpoints, including the formation of micronuclei (MN) in exfoliated cells and lymphocytes, to estimate the risk for genotoxic events in workers exposed to MOCA and bitumen fumes (which contain skin and lung carcinogens). Twelve men employed in polyurethane manufacture, who had been exposed to MOCA, 12 bitumen road-layers (exposed to bitumen fumes containing PAHs) and 18 hospital-store personnel (controls) were recruited for the study. All provided blood and urine samples on the same day. Blood cultures were prepared by use of a cytochalasin B-block method. Exfoliated urothelial cells were collected from urine and stained for microscopy. The number of MN was higher in MOCA-exposed workers (14.27 ± 0.56 MN/1000 cells; 9.69 ± 0.32 micronucleated cells/1000 cells) than in bitumen-exposed workers (11.99 ± 0.65 MN/1000 cells; 8.66 ± 0.46 micronucleated cells/1000 cells). The MN frequency in control subjects was 6.88 ± 0.18 MN/1000 cells (5.17 ± 0.11 micronucleated cells/1000 cells). Conversely, in lymphocytes, MN frequencies were higher in bitumen-exposed workers (16.24 ± 0.63 MN/1000, 10.65 ± 0.24 MN cells/1000) than in MOCA-exposed workers (13.25 ± 0.48 MN/1000, 8.54 ± 0.14 MN cells/1000) or in control subjects (9.24 ± 0.29 MN/1000 or 5.93 ± 0.13 MN cells/1000). The MN frequency values for the various groups were all significantly different from each other ($P < 0.01$). The results suggest that these genotoxins could cause MN formation to a different extent in different tissues (Murray & Edwards, 2005).

4.2.2 *Experimental systems*

(a) Animals

In an early study, Kugler-Steigmeier *et al.* (1989) showed that MOCA produced DNA adducts in the liver of Sprague-Dawley rats at levels typically found for moderately strong genotoxic carcinogens.

In rats given a single dose of 95 μmol/kg bw [methylene-^{14}C]MOCA by gavage, DNA adducts were found after 24 hours at 7 pmol/mg DNA in liver, 2 pmol/mg in lung and 0.5 pmol/mg in kidney. These adducts, *N*-(deoxyadenosin-8-yl)-4-amino-3-chlorobenzyl alcohol and *N*-(deoxyadenosin-8-yl)-4-amino-3-chlorotoluene were eliminated from rat liver with nonlinear kinetics. The selective reaction of *N*-hydroxy-MOCA with adenine in DNA, and the formation of a single arylamine ring adduct suggest a substitution mechanism involving an intermediate with a strong S_N1 character, aided by the negative inductive effect of the *ortho*-chlorine. Due to tautomer formation, the initial adduct may be inherently unstable and undergo cleavage at the 1′-carbon-methylene bond to yield the observed adducts (Segerbäck & Kadlubar, 1992).

Segerbäck *et al.* (1993) applied ^{32}P-postlabelling analysis to determine DNA adducts of MOCA in target and non-target tissues in dogs. Beagle dogs were treated with single and multiple doses of MOCA, and DNA-adduct levels were determined in liver and bladder epithelium. After a single dose, the level of adducts in the liver was 1.5-fold higher than that in the bladder epithelium. The amounts of adducts in these two organs increased three- to five-fold after 10 doses, and adducts in the liver were then 2.8-fold higher than in the bladder epithelium. The amounts found in these two organs after single exposures were compared, per unit exposure dose, with those reported for other carcinogenic aromatic amines. The comparison showed that MOCA was as effective in DNA-adduct formation as were most other potent urinary bladder carcinogens. These results suggest that MOCA may have high carcinogenic potential in humans.

DeBord *et al.* (1996) studied adduct formation by ^{32}P-postlabelling in rats treated with MOCA and in human uro-epithelial cells treated with *N*-OH-MOCA. In both cases, the major adduct corresponded to *N*-(deoxyadenosin-8-yl)-4-amino-3-chlorobenzyl alcohol.

(b) In-vitro studies

Shivapurkar *et al.* (1987) found that MOCA could bind with DNA of explant cultures of human and dog bladders. In both humans and dogs, there appeared to be a population of cells with high DNA binding and another with low DNA binding. The binding of MOCA to human bladder DNA appeared to be higher than to dog bladder DNA.

MOCA formed adducts with DNA in cultured canine and human bladder cells, and in liver, lung and kidney of rats treated topically, intraperitoneally or orally. One of three HPLC peaks of an enzymatic digest of DNA derived from rats treated *in vivo* was identified tentatively as *N*-(deoxyadenosin-8-yl)-4-amino-3-chlorobenzyl alcohol (Silk *et al.*, 1989).

Kuslikis *et al.*, (1991) found that *N*-Hydroxy-MOCA was mutagenic to *Salmonella typhimurium* TA98 and TA100 in the absence of an exogenous metabolic activation system. Other MOCA metabolites, *ortho*-hydroxy-MOCA, 4-amino-3,3′-dichloro-4′-nitrosodiphenyl-methane (mononitroso derivative) and di-(3-chloro-4-nitrosophenyl) methane (dinitroso derivative), were not mutagenic to *S. typhimurium* TA98 or TA100. The mutagenic activity of the mononitroso derivative towards strain TA100, however, appeared to be masked by its toxicity.

Reaction of *N*-hydroxy[methylene-^{14}C]-MOCA with DNA *in vitro* resulted in the formation of two major adducts, which were identified by mass spectroscopy as *N*-(deoxyadenosin-8-yl)-4-amino-3-chlorobenzyl alcohol and *N*-(deoxyadenosin-8-yl)-4-amino-3-chlorotoluene (Kuslikis *et al.*, 1991).

The SV40-immortalized human uroepithelial cell line SV-HUC.PC was used to study the malignant transformation capacity of *N*-OH-MOCA. SV-HUC.PC cells were exposed *in vitro* to various concentrations of *N*-OH-MOCA. The carcinogen-treated cells were propagated in culture for about six weeks and subsequently injected subcutaneously into athymic nude mice. Two of the 14 different groups of SV-HUC.PC cell cultures treated with different concentrations of N-OH-MOCA formed carcinomas in these mice.

^{32}P-postlabelling analyses of DNA isolated from SV-HUC.PC cells after exposure to *N*-OH-MOCA revealed one major and one minor adduct. The major adduct has been identified as the *N*-(deoxyadenosin-3′,5′-bisphosph-8-yl)-4-amino-3-chlorobenzyl alcohol (pdAp-ACBA) and the minor adduct as *N*-(deoxyadenosin-3′,5′-bisphospho-8-yl)-4-amino-3-chlorotoluene (pdAp-ACT). Furthermore, the SV-HUC.PC cytosols catalysed the binding of *N*-OH-MOCA to DNA in the presence of acetyl-CoA, to yield similar adducts (Swaminathan *et al.*, 1996).

Reid *et al.* (1998) explored the genotoxic potential of MOCA by monitoring the induction of mutations at the *HPRT* locus of AHH-human lymphoblastoid cells. Exposure of AHH-1 cells to *N*-OH-MOCA was found to induce a six-fold increase in mutant frequency and resulted in base-pair substitution primarily at A:T base pairs. The induction of mutations at A:T sites by *N*-OH-MOCA provides a marker of genotoxic damage for an exposed population (Reid *et al.*, 1998).

Birner *et al.* (1990) have shown that *ortho*-substitution with methyl groups of benzidine to produce 3,3′-5,5′-tetramethylbenzidine results in non-mutagenic derivatives (Chung *et al.*, 2000) that also do not bind to haemoglobin. It is thought that in 4,4′-methylenediamine (MDA) or MOCA, bulky substituents in the *ortho* position eliminate the mutagenicity and carcinogenicity of MDA and MOCA, presumably by inhibiting the formation of the *N*-OH-arylamines, which are the putative genotoxic intermediates.

The major metabolite of MOCA in the urine of dogs is identified as 3,3′-dichloro-4,4′-diaminodiphenylmethane-5-sulfate. Upon hydrolysis of this metabolite with arylsulfatase, time- and enzyme concentration-dependent protein binding and time-dependent DNA binding of this material were observed. However, the metabolites were not mutagenic towards *Salmonella* tester strain TA98 at up to 20 μg/plate, and cytotoxic to the tester strain at 50 μg/plate (Manis & Braselton, 1984).

(c) Summary of short-term genotoxicity tests (IARC, 1993)

MOCA was shown to cause prophage induction in *Escherichia coli* (Thomson, 1981) and differential toxicity in *Bacillus subtilis rec*-deficient strains (Kada, 1981). It was mutagenic to *Salmonella typhimurium* (Bridges *et al.*, 1981), *Escherichia coli* (Matsushima *et al.*, 1981) and at the *Tk* locus in mouse lymphoma L5178Y cells (Mitchell *et al.*, 1988; Myhr & Caspary, 1988), but not to *Saccharomyces cerevisiae* (Mehta & von Borstel, 1981). MOCA caused aneuploidy in *S. cerevisiae* (Parry & Sharp, 1981) but gave equivocal results with regard to gene conversion and did not induce mitotic crossing-over in the same organism (Jagannath *et al.*, 1981). It induced mutation in *Drosophila melanogaster* (Kugler-Steigmeier *et al.*, 1989) and unscheduled DNA synthesis in primary cultures of hepatocytes from mice (McQueen *et al.*, 1981), rats (McQueen *et al.*; 1981; Williams *et al.*; 1982; Mori *et al.*, 1988), and Syrian hamsters (McQueen *et al.*, 1981). Sister chromatid exchange but not chromosomal aberration was induced in Chinese hamster ovary cells (Galloway *et al.*, 1985). MOCA induced cell transformation in mammalian cells (Daniel & Dehnel, 1981; Dunkel *et al.*, 1981; Styles,

1981) and inhibited gap-junctional intercellular communication in cultured rat-liver cells (Kuslikis *et al.*, 1991). MOCA induced sister chromatid exchange in lymphocytes of rats treated *in vivo* (Edwards & Priestly, 1992).

4.3 Mechanistic considerations

The toxicological profile of MOCA is quite similar to that of monocyclic aromatic amines: MOCA causes an increased incidence of bladder tumours in exposed workers, bladder tumours in dogs, lung and liver tumours in rodents, and hemangiosarcoma and hepatoma, among others, in rats and mice. The genotoxic properties of MOCA have been broadly documented previously (IARC, 1993).

MOCA is *N*-oxidized, and the resulting metabolites bind to DNA, RNA and proteins. Interestingly, two different kinds of activation product are formed, and the question of how they contribute to the biological effects has not been answered. A hydrolysable haemoglobin adduct has been found in humans and in experimental animals. MOCA is practically the only cleavage product released from the adduct after oral administration to rats. This indicates that the non-acetylated MOCA and not *N′*-acetyl-MOCA is *N*-oxidized (Sabbioni & Neumann, 1990). MOCA is different from other bifunctional amines, such as benzidine and 3,3′-dimethoxybenzidine, for which predominantly *N′*-acetylated adducts are formed (Neumann, 1988).

The expected 2-chloro-4-methylaniline (2-chloro-*meta*-toluidine) was not detected upon hydrolysis of the haemoglobin adducts found in rats. It should have been formed when it is a major metabolite that is N-oxidized to the respective hydroxylamine and nitroso-derivative. Obviously other reactive forms yield the nonhydrolyzable adducts of haemoglobin and albumin. Structurally, 2-chloro-*meta*-toluidine is related to 4-chloro-*ortho*-toluidine, in which the two substituents are exchanged. In both cases a bulky substituent is in the *ortho*-position to the amine and the *para*-position is blocked. Although experimental data do not seem to exist, similar biological properties would be expected. Overall, MOCA shows many properties typical for aromatic amines and monocyclic amines in particular, which supports the idea of a common mode of action for this class of chemicals.

5. Summary of Data Reported

5.1 Exposure data

4,4′-Methylenebis(2-chloroaniline) (MOCA) is widely used as a curing agent in the polyurethane industry. Occupational exposure to MOCA has been reported in industries that manufacture and use MOCA. Dermal absorption after contact with contaminated surfaces appears to be the most important route of exposure, with inhalation and ingestion representing minor routes. Post-shift urine measurements represent the most appropriate

method to measure workers' exposure. Urinary levels of MOCA have been reported for MOCA production workers and polyurethane production workers in Australia, France, Germany, Japan, Taiwan (China), the United Kingdom, and the USA.

MOCA does not occur in nature. The general population can be exposed to MOCA if they live in an area contaminated with this compound.

5.2 Human carcinogenicity data

No adequate epidemiological studies were available to the Working Group to evaluate an association between exposure to 4,4′-methylenebis(2-chloroaniline) and bladder cancer risk.

5.3 Animal carcinogenicity data

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 and female rats in a further two studies using normal and low-protein diets, and in one study using 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.

5.4 Other relevant data

The toxicological profile of MOCA is typical for biologically active monocyclic aromatic amines. It is metabolically activated by *N*-oxidation to metabolites that react with DNA and proteins. The genotoxicity of MOCA is well documented. The acute toxicity is reflected by methaemoglobin formation, spleen toxicity, fibrosis in spleen and often in liver and kidney of rodents. The profile is comparable with those of *ortho*-toluidine and 4-chloro-*ortho*-toluidine, thus indicating a common mode of action.

MOCA is mutagenic to *Salmonella typhimurium*, *E. coli*, *Drosophila melanogaster*, and human lymphoblastoid cells. It causes prophage induction in *E. coli*, differential toxicity to *Bacillus subtilis* rec-deficient strains, and mutations at the *Tk* locus in mouse lymphoma L5178Y cells. It causes aneuploidy in *S. cerevisiae* and unscheduled DNA synthesis in the primary cultures of hepatocytes from mice, rats, and Syrian hamsters.

MOCA induces sister chromatid exchange in lymphocytes of rats. It forms DNA adducts in cultured canine and human bladder cells, and in the lung, liver, and kidney of rats treated with MOCA. It inhibits intercellular communication in cultured rat liver cells. The MOCA metabolite, *N*-OH MOCA, forms DNA adducts in rat liver, causes malignant transformation in SV40-immortalized human SV.HUC.PC uroepithelial cells, which subsequently produce carcinomas in athymic nude mice. MOCA also binds to protein RNA, and DNA of rats.

MOCA has been shown to interact with DNA to form adducts in urothelial cells, and with haemoglobin to form adducts in the blood of exposed workers. MOCA has been shown to cause the formation of sister chromatid exchange and micronuclei in urothelial cells and lymphocytes of exposed humans. Chromosomal aberrations have been shown to be a good predictor of carcinogenicity.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 4,4′-methylenebis(2-chloroaniline).

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 4,4′-methylenebis(2-chloroaniline).

6.3 Overall evaluation

4,4′-methylenebis(2-chloroaniline) is *carcinogenic to humans (Group 1)*.

6.4 Rationale

In reaching this evaluation the Working Group considered the following:

- MOCA shows many properties typical for monocyclic aromatic amines, *e.g., ortho*-toluidine, which supports the notion of a common mode of action for this class of chemicals.
- MOCA is genotoxic in numerous assays for genotoxicity.
- In rats and dogs, species in which MOCA has shown to produce tumours, this compound is metabolized to *N*-hydroxy-MOCA, which forms DNA adducts.
- One of the two major MOCA-DNA adducts found in the target tissues for carcinogenicity in animals (rat liver and lung; dog urinary bladder) was also

found in urothelial cells from a worker with known occupational exposure to MOCA.

- An increased frequency of sister chromatid exchange and micronuclei was seen in urothelial cells and lymphocytes of workers exposed to MOCA.

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