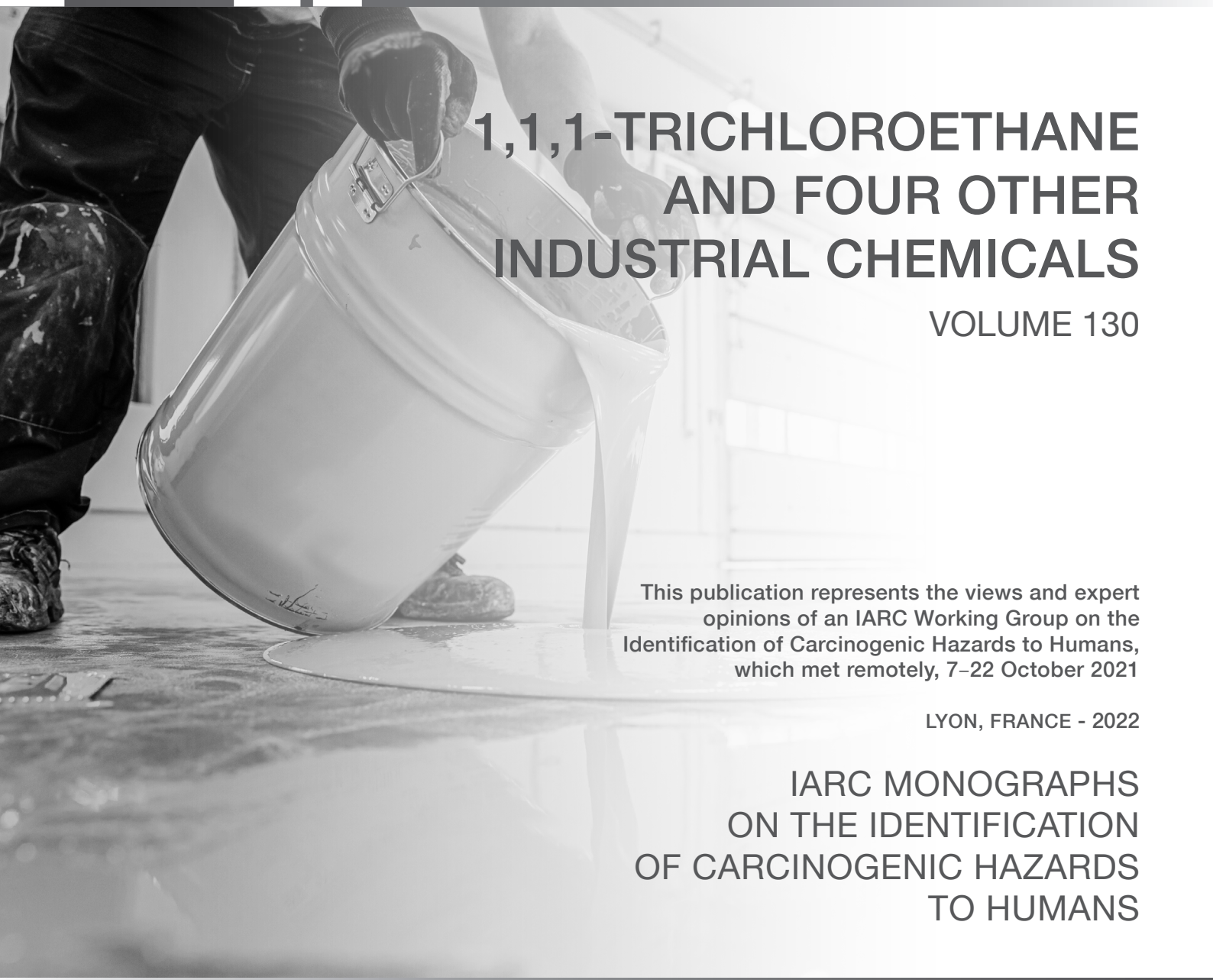
IARC MONOGRAPHS



1,1,1-TRICHLOROETHANE AND FOUR OTHER INDUSTRIAL CHEMICALS

VOLUME 130

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IARC MONOGRAPHS ON THE IDENTIFICATION OF CARCINOGENIC HAZARDS TO HUMANS

International Agency for Research on Cancer

World Health Organization

N-METHYLOLACRYLAMIDE

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 924-42-5

Chem. Abstr. Serv. name: *N*-(hydroxymethyl) acrylamide

EC/List No.: 213-103-2

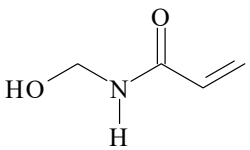
IUPAC systematic name: *N*-(hydroxymethyl) prop-2-enamide

Synonyms: *N*-methylolacrylamide, *N*-methanol-acrylamide, monomethylolacrylamide, *N*-(hydroxymethyl)-2-propenamide, NMA, N-MAM, and other depositor-supplied synonyms and acronyms (NCBI, 2021).

1.1.2 Structural and molecular information

Relative molecular mass: 101.10 (NCBI, 2021)

Chemical structure:



Molecular formula: $C_4H_7NO_2$

1.1.3 Chemical and physical properties

Description: white crystals as a solid, colourless or slightly yellow in aqueous solutions, with a formaldehyde-like odour (ECHA, 2017; NCBI, 2021)

Boiling point: 277 °C (NCBI, 2021)

Melting point: 74.5 °C (NCBI, 2021)

Density: 1.07 g/cm^3 at 25 °C (IFA, 2021)

Vapour pressure: 26.7–40.0 hPa at 25 °C (ECHA, 2021)

Solubility: soluble in water (IFA, 2021); soluble in polar solvents (alcohols) and not soluble in nonpolar solvents (hydrocarbon, chloroform) (ECHA, 2017)

Flash point: > 93 °C (ECHA, 2017)

Stability: sensitive to light, polymerization is possible (IFA, 2021)

Reactivity: tends to polymerize spontaneously and exothermically above 50 °C in the absence of stabilizers (ECHA, 2017; IFA, 2021).

Octanol/water partition coefficient (P): log K_{ow}, –1.81 (NCBI, 2021), log K_{ow}, –1.81 at 20 °C and pH 7 for a 48% aqueous solution (ECHA, 2021).

1.1.4 Impurities

Relevant impurities of toxicological significance are acrylamide (≤ 10%) and formaldehyde (≤ 2%), which are both residues from the production process (ECHA, 2017). Additional impurities can be polymers of *N*-methylolacrylamide (1–2%). The substance is essentially marketed as an aqueous solution only, to which additives such as mequinol (4-methoxyphenol; ≤ 30 ppm), oxygen, or cupric ions are added that function as stabilizers to prevent polymerization. The concentration of the solutions ranges between 40% and 85% (w/w).

1.2 Production and use

1.2.1 Production process

N-Methylolacrylamide is produced in an alkaline environment by hydroxymethylation of acrylamide with formaldehyde in the presence of copper(I) chloride, which acts as a polymerization inhibitor (Feuer & Lynch, 1953; Ashford, 1994; NCBI, 2021).

1.2.2 Production volume

N-Methylolacrylamide is listed as a High Production Volume chemical by the Organization for Economic Co-operation and Development (OECD) (OECD, 2004, 2009). In 2016, the United States Environmental Protection Agency (US EPA) estimated an aggregated production volume of 1 000 000–10 000 000 lb [~450–4500 tonnes] in the USA (NCBI, 2021). The substance is registered under Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation, and 1000–10 000 tonnes per annum are manufactured in and/or imported to the European Economic Area (ECHA, 2021). [The Working Group noted that information on production volumes outside of the abovementioned areas was not available.]

1.2.3 Uses

N-Methylolacrylamide is used as an intermediate for the production of *N*,*N*′-methylenebisacrylamide (Lundberg, 1946; Feuer & Lynch, 1953) and, together with *N*,*N*′-methylenebisacrylamide, for the manufacture of a variety of polymers with acrylic and vinylic monomers, such as acrylonitrile, acrylamide, and substituted acrylamides (NCBI, 2021). Free-radical copolymerization using *N*-methylolacrylamide specifically provides stability to the polymer network via cross-linking (Kamogawa, 1967). Polymers based on *N*-methylolacrylamide are ultimately used in a multitude of industries as adhesives, inks and paints, antistatic compounds, thermoplastic and chromatographic resins, coatings, rubbers, plastics, paper, and textile finishes (NCBI, 2021). Specific examples are food contact plastics (US FDA, 2021), certain laminar flooring sealants and wood glues (DeLima Associates, 2021), viscosity adjustors of paints and colourants, adhesives and binders for papermaking, and finishing and dispersing agents for crease-resistant and antiwrinkle fabrics (ECHA, 2021). *N*-Methylolacrylamide-based polymers are also used as coatings for controlled delivery systems of drugs (Siemoneit et al., 2006; Singh et al., 2006; Yang et al., 2011) and fertilizers (Xie et al., 2012; Louzri & Bennour, 2018), energy storage electrolytes (Silvaraj et al., 2021), as grouting agents to reduce water leakages (Weideborg et al., 2001) and in the production of activity-specific clothing with high moisture absorption and release capabilities (Chaudhuri & Wu, 2020). Specific niche applications involve the use of *N*-methylolacrylamide-based polymers in test kits for medical diagnostics (Albers et al., 2010; Reddy et al., 2012; Sullivan et al., 2021), bioimaging (Mahapatra et al., 2020), food analyses (Hakkoymaz & Mazi, 2020; Zhang et al., 2020), and in experimental dental primers (Fukushima et al., 2001).

1.3 Detection and quantification

1.3.1. Environmental samples

No analytical methods were available that specifically described the quantitation of *N*-methylolacrylamide in air, water, and soil samples, or in consumer products, with the exception of the detection of *N*-methylolacrylamide in drainage water by high-performance liquid chromatography-ultraviolet (HPLC-UV), with a limit of detection of 5 µg/L [no additional analytical details were provided] (Weideborg et al., 2001). An analytical method based on gas chromatography (GC) and tandem mass spectrometry (MS/MS) has been reported for the analysis of the sum of acrylamide and *N*-methylolacrylamide in aqueous samples including drinking-water, brewed coffee, and water extracts of snuff (Pérez & Osterman-Golkar, 2003). L-Valine has been used as a nucleophilic trapping agent during sample preparation for both acrylamide and *N*-methylolacrylamide. The two reaction products are then converted to a single pentafluorophenylthiohydantoin derivative using pentafluorophenyl isothiocyanate during further sample preparation. The limit of detection for the sum of acrylamide and *N*-methylolacrylamide was estimated to be ~0.003 µg/L.

1.3.2. Biological specimens

No specific biomarkers for *N*-methylolacrylamide could be traced in the literature; however, there were multiple methods for the determination of haemoglobin adducts of acrylamide based on GC or HPLC and MS/MS analyses (Bergmark et al., 1993; Vesper et al., 2006; Schettgen et al., 2010; von Stedingk et al., 2010; Yang et al., 2018). These methods can also be used to analyse haemoglobin adducts of *N*-methylolacrylamide (Hagmar et al., 2001). All methods are based on a modified Edman degradation using pentafluorophenyl isothiocyanate which, similarly to the analyses of aqueous samples described above (see Section 1.3.1), converts the haemoglobin adducts of acrylamide and/or *N*-methylolacrylamide at the N-terminal valine of haemoglobin to a single pentafluorophenylthiohydantoin derivative. The methods have been used for several decades and there are only minor variations in the limits of detection, which are in a narrow range around 0.01 µg/L blood. As previously mentioned, the methods per se cannot distinguish between exposures to acrylamide or *N*-methylolacrylamide unless the exact source of exposure is known, e.g. grouting agents containing monomers of *N*-methylolacrylamide (Hagmar et al., 2001).

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

N-Methylolacrylamide is not known to occur naturally. It may enter the environment via its use in polyacrylamide polymers and other applications described in Section 1.2.3. The US EPA Toxic Release Inventory reported that, between 2009 and 2019, 2.9–146 tonnes of *N*-methylolacrylamide (3–13 tonnes for most years except for 2016 to 2018, when it was 129–146 tonnes) were released annually on-site (air emission, 5%; surface water discharge, < 0.1%; and land release, 95%) by 26–33 facilities (US EPA, 2021). Facilities emitting or sending *N*-methylolacrylamide off-site included hazardous-waste treatment and disposal (2009–2019, 411 tonnes), manufacture of all plastic material and resin (56 tonnes), manufacture of other basic organic chemicals (0.6 tonnes), manufacture of all other miscellaneous chemical products and preparations (4 tonnes), manufacture of synthetic rubber (0.4 tonnes), and manufacture of paint and coatings (0.2 tonnes).

On the basis of similar chemical characteristics to those of acrylamide, *N*-methylolacrylamide would be expected to have a low bioconcentration potential and be readily biodegradable

(Weideborg et al., 2001). Transient environmental contamination has been reported after use in grouts in tunnels in Sweden and Norway, where *N*-methylolacrylamide-based products were used as a replacement for the more hazardous acrylamide-based grouts (Hagmar et al., 2001; Weideborg et al., 2001). A grouting agent containing about 37% *N*-methylolacrylamide and about 1–5% acrylamide in a quantity of 1500 tonnes was used to prevent water leaks in a tunnel in Sweden over a period of 2 months in 1997 (Hagmar et al., 2001). Incomplete polymerization caused leakage of the grouting agents into a brook, leading to contamination of groundwater and wells in the area, with highest concentrations in the brook water (at the end of the 2-month period) of 92 mg/L for acrylamide and 342 mg/L for *N*-methylolacrylamide (Godin et al., 2002). Weideborg et al. (2001) described a similar situation in a tunnel in Norway, where the same grouting agent was used between 1995 and 1997. Both acrylamide and *N*-methylolacrylamide were monitored in drainage water during the entire period. The highest measured concentrations were 9.7 mg/L and 16.6 mg/L, respectively, and decreased to non-detectable when the grouting activities ceased (Weideborg et al., 2001). [The Working Group noted that the water contamination with acrylamide observed by the authors was much higher than expected from the grout formula (water levels for acrylamide were similar to those for *N*-methylolacrylamide, despite the latter being ~10 times more concentrated than acrylamide in the original product). They attributed this observation to the chemical instability of *N*-methylolacrylamide, which tends to rapidly degrade to acrylamide.]

1.4.2 Occupational exposure

Several reports indicated that occupational exposure to *N*-methylolacrylamide occurred in construction workers in Sweden and Norway, where *N*-methylolacrylamide-based grouting agents were used in waterproofing tunnels. In Sweden, Hagmar et al. (2001) described airborne exposure conditions of workers who pumped the grouting agent under pressure into drill holes. The study reported two measurements of 0.27 and 0.34 mg/m^3, which were the sum of acrylamide and *N*-methylolacrylamide concentrations, taken by the construction company's health service (Hagmar et al., 2001). The authors further indicated that *N*-methylolacrylamide represented 50% of the sum based on two additional samples [levels not reported] taken on the day operations were stopped after the leakage was discovered, and that none of the workers wore adequate protective equipment. [No information was provided about sampling and analysis of these air samples.] The study also reported quantification of haemoglobin adducts in 228 workers (210 exposed, and 18 unexposed) who provided blood samples approximately 1 month after the end of the 2-month grouting operation. Although it was impossible to differentiate between adducts originating from acrylamide or *N*-methylolacrylamide, adduct levels were higher in exposed workers than in unexposed referents. In a similar occupational setting in Norway, Kjuus et al. (2004) measured haemoglobin adducts in 23 exposed workers and 8 unexposed referents. Distinct from the previous study, workers had been engaged in grouting work for an average of 19 months (rather than 2 months), and blood samples were taken 2–5 months after the cessation of work (instead of 1 month). Mean adduct levels were also higher in the exposed workers than in the unexposed referents (Kjuus et al., 2004). In both studies, the higher level of adducts in exposed workers was mainly attributed to dermal exposure (Hagmar et al., 2001; Kjuus et al., 2004). [The Working Group noted that, despite the inability to differentiate adducts from *N*-methylolacrylamide and those from acrylamide, the combined evidence of the studies in Sweden and Norway demonstrated possible internal exposure to *N*-methylolacrylamide

from both the dermal, and, probably to a lesser extent, airborne routes.]

Some information was available to the Working Group regarding the extent of the use of *N*-methylolacrylamide-based grouts, for Europe from a commissioned United Kingdom risk assessment document prepared in 2000 after the incidents in Sweden and Norway (Risk & Policy Analysts Limited, 2000), and for the USA from the United States Federal Register about the proposal for a ban on these products in 1991 by the US EPA (Office of the Federal Register, 1991), followed in 2002 by the withdrawal of the proposed ban (Office of the Federal Register, 2002). According to the United Kingdom document, acrylamide and *N*-methylolacrylamide-based grouts were not produced in Europe as of 2000, and their use was rare. They were, however, in much wider use before the incidents in Sweden and Norway. According to the US EPA proposal for a ban, acrylamide-based grouts represented approximately half of the total chemical grout usage in the USA in 1989, with ~300 tonnes consumed (of which ~10% was *N*-methylolacrylamide-based), mostly used for sewer operations. The US EPA withdrew the proposal for a ban in 2002, proposing that affordable and appropriate protective equipment had become available such that a ban of the products was no longer warranted. In a case report of toxicity in two acrylamide-grout workers, it was reported in 2017 that acrylamide grouts were widely used in the Republic of Korea (Kim et al., 2017). [To the Working Group, this would suggest that occupational exposure via *N*-methylolacrylamide-based grouts may also have occurred recently in this country.]

Exposure to *N*-methylolacrylamide in sealant was also described for four workers working in a window-manufacturing company that used an interlayer product similar to the grouting agent described in the Swedish tunnel studies mentioned above (Paulsson et al., 2006).

No direct information on exposure to *N*-methylolacrylamide for other uses or types of workplaces was available to the Working Group. The National Institute for Occupational Safety and Health (NIOSH) National Occupational Exposure Survey estimated that 20 665 workers (13 852 of whom were women) were exposed to *N*-methylolacrylamide in 1981–1983 (NIOSH, 1983, 1988a, b, 1990). These included mainly workers in the textile-mill product industry (major occupation as "mixing and blending machine operators") and in the apparel and other textile-product industries (major occupation as "textile sewing machine operators"). In 1982, 101 510 000 workers were employed in the USA (Silvestri et al., 1983); thus 0.02% of the working population in the USA in 1982 was potentially occupationally exposed to *N*-methylolacrylamide. [The Working Group noted that it is unclear how representative these estimates are of current exposure prevalence.] During 2003–2018, 98% of *N*-methylolacrylamide used in the Nordic countries (i.e. Denmark, Finland, Norway, and Sweden) was used in the manufacture of chemicals and chemical products (SPIN, 2021). [The Working Group noted that these numbers for Europe are consistent with values for toxic releases for 2009–2019 reported by the US EPA (see Section 1.4.1), primarily in plastics material and resin manufacturing (after excluding landfills).]

[In terms of co-exposures, at least in the grouting sector, the Working Group noted that acrylamide is omnipresent alongside *N*-methylolacrylamide, both as a component of grouting agents as well as a product of *N*-methylolacrylamide degradation. No other specific co-exposures were noted, but it is expected that the various uses described in Section 1.2.3 also imply potential exposure to multiple chemicals (e.g. in paints or adhesives).]

1.4.3 Exposure of the general population

No empirical measurement data were available to the Working Group regarding exposure of the general population. In a recent report on polymers containing *N*-methylolacrylamide in Australia, the National Industrial Chemicals Notification and Assessment Scheme indicated that products manufactured using these polymers can contain low levels of *N*-methylolacrylamide as an impurity, and that consumers might be exposed dermally or through inhalation when in contact with coated surfaces, although it was concluded that this type of exposure would be too low to pose a health risk (National Industrial Chemicals Notification and Assessment Scheme, 2019).

1.5 Regulations and guidelines

There are no reported occupational standard or guidelines for *N*-methylolacrylamide. According to the harmonized classification and labelling framework implemented in the European Union, Classification, Labelling and Packaging (CLP) Regulation (1272/2008/EC), *N*-methylolacrylamide has the following classification: mutagen 1B; carcinogen 1B; specific target organ toxicity-repeated exposure category 1. Employers are obligated under the CLP Regulation to minimize worker exposure to *N*-methylolacrylamide and must arrange for medical surveillance of exposed workers (Council Directive 98/24/EC; European Council, 1998). *N*-Methylolacrylamide is also regulated under the United States Food and Drug Administration, listed as a minor monomer in the production of acrylic plastics in contact with food. Its use is restricted in plastic items for repeated food contact and the main polymer material cannot include more than 5% in weight of polymer units derived by copolymerization with *N*-methylolacrylamide (US FDA, 2020). Acrylamide residues in polyacrylamide polymers are also regulated under the European regulation on cosmetic products. The final cosmetic product maximum residual acrylamide content (including *N*-methylolacrylamide) is 0.1 mg/kg and 0.5 mg/kg for body-leave-on and other products, respectively (European Council, 2009). *N*-Methylolacrylamide is also regulated under the European regulation on plastic materials and articles intended to come into contact with food, with permitted use as a monomer. *N*-Methylolacrylamide should not be released to foods in quantities exceeding 0.01 mg/kg of food (European Commission, 2011). Pregnant workers and workers who have recently given birth or are breastfeeding may not be exposed; young persons (age < 18 years) may not be exposed at the workplace (European Council, 1992, 1994). In 2021, the European Commission developed a Regulatory Management Option Analysis (RMOA) aiming to assess the regulatory needs for *N*-methylolacrylamide. This assessment, performed by the Swedish Chemicals Agency, concluded that *N*-methylolacrylamide fulfils the criteria for inclusion in the Candidate List of Substances of Very High Concern (SVHC) according to Article 57 (a) and (b) of REACH (ECHA, 2021). The RMOA report also states that this inclusion will raise awareness of the substance, represents an incentive for substitution, and may furthermore prevent regrettable substitution of acrylamide with *N*-methylolacrylamide, as acrylamide is already included in the Candidate list of SVHC.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1.

Table 3.1 Studies of carcinogenicity in experimental animals exposed to *N*-methylolacrylamide

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 8 wk 105 wk NTP (1989)	Oral administration (gavage) *N*-Methylolacrylamide, ~98% Deionized water 0, 25, 50 mg/kg bw 5 days/wk for 103 wk 50, 50, 50 30, 20, 21	*Harderian gland* Adenoma 1/48, 14/49*, 29/50*	$P < 0.001$, Cochran–Armitage trend test, life-table trend test, logistic-regression trend test *$P < 0.001$, Fisher exact test, life-table test, logistic regression test	Principal strengths: well-conducted study that complied with GLP; covered most of the life span; used multiple doses; used males and females; adequate number of mice per group Historical controls: hepatocellular carcinoma, gavage studies, 56/347 (16.1 ± 8.03%), range, 4–28%; hepatocellular carcinoma, all routes, 379/2032 (18.7 ± 6.50%), range, 8–30%; hepatocellular adenoma or carcinoma (combined), gavage studies, 106/347 (30.5 ± 5.83%), range, 20–36%; hepatocellular adenoma or carcinoma (combined), all routes, 609/2032 (30.0 ± 7.59%), range, 16–58% No historical control data for Harderian gland carcinoma
		Carcinoma 1/48, 0/49, 2/50	NS	
		Adenoma or carcinoma (combined) 2/48, 14/49*, 30/50*	$P < 0.001$, Cochran–Armitage trend test, life-table trend test, logistic-regression trend test *$P < 0.001$, Fisher exact test, life-table test, logistic regression test	
		Liver Hepatocellular adenoma 8/50, 4/50, 19/50*	$P = 0.005$, Cochran–Armitage trend test; $P < 0.001$, life-table trend test; $P = 0.002$, logistic-regression trend test *$P = 0.012$, Fisher exact test; $P = 0.004$, logistic regression test	
		Hepatocellular carcinoma 6/50 (12%), 13/50 (26%)*, 12/50 (24%)**	$P = 0.027$, life-table trend test *$P = 0.023$, logistic regression test; $P = 0.012$, life-table test **$P = 0.031$, life-table test	
		Hepatocellular adenoma or carcinoma (combined) 12/50, 17/50, 26/50*	$P = 0.003$, Cochran–Armitage trend test; $P < 0.001$, life-table trend test, logistic regression trend test *$P < 0.001$, life-table test; $P = 0.001$, logistic regression test; $P = 0.004$, Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 8 wk 105 wk NTP (1989) (cont.)		*Lung* Bronchioloalveolar adenoma 3/49, 6/50, 11/50*	*P* = 0.005, life-table trend test; *P* = 0.010, logistic-regression trend test **P* = 0.006, life-table test; *P* = 0.015, logistic regression test; *P* = 0.022, Fisher exact test	
		Bronchioloalveolar carcinoma 2/49, 4/50, 10/50*	*P* = 0.003, life-table trend test; *P* = 0.005, logistic-regression trend test **P* = 0.006, life-table test; *P* = 0.011, logistic regression test; *P* = 0.015, Fisher exact test	
		Bronchioloalveolar adenoma or carcinoma (combined) 5/49, 10/50, 18/50*	*P* = 0.001, Cochran–Armitage trend test; *P* < 0.001, life-table trend test, logistic-regression trend test **P* < 0.001, life-table test; *P* = 0.001, logistic regression test; *P* = 0.002, Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 8 wk 105 wk NTP (1989)	Oral administration (gavage) *N*-Methylolacrylamide, ~98% Deionized water 0, 25, 50 mg/kg bw 5 days/wk for 103 wk 50, 50, 50 41, 35, 33	*Harderian gland* Adenoma 5/47, 8/45, 20/48*	$P < 0.001$, Cochran–Armitage trend test, life-table trend test, logistic-regression trend test *$P < 0.001$, life-table test, logistic regression test, Fisher exact test	Principal strengths: well-conducted study that complied with GLP; covered most of the life span; used multiple doses; used males and females; adequate number of mice per group Historical controls: Harderian gland carcinoma, gavage studies, 3/350 (0.9 ± 1.57%), range, 0–4%; Harderian gland carcinoma, all routes, 7/2040 (0.3 ± 0.88%), range, 0–4%; bronchioloalveolar carcinoma, gavage studies, 8/349 (2.3 ± 1.80%), range, 0–6%; bronchioloalveolar carcinoma, all routes, 45/2026 (2.2 ± 1.78%), range, 0–6%; bronchioloalveolar adenoma or carcinoma (combined), gavage studies, 33/349 (9.5 ± 3.66%), range, 4–14.3%; bronchioloalveolar adenoma or carcinoma (combined): all routes, 145/2026 (7.2 ± 4.21%), range, 0–16%
		Carcinoma 0/47, 3/45 (6.7%), 2/48 (4.2%)	NS	
		Adenoma or carcinoma (combined) 5/47, 11/45*, 22/48**	$P < 0.001$, Cochran–Armitage trend test, life-table trend test, logistic-regression trend test *$P = 0.031$, logistic regression test **$P < 0.001$, life-table test, logistic regression test, Fisher exact test	
		Liver Hepatocellular adenoma 3/50, 4/50, 17/49*	$P < 0.001$, Cochran–Armitage trend test, life-table trend test, logistic-regression trend test *$P < 0.001$, life-table test, logistic regression test, Fisher exact test	
		Hepatocellular carcinoma 3/50, 3/50, 2/49	NS	
		Hepatocellular adenoma or carcinoma (combined) 6/50, 7/50, 17/49*	$P = 0.004$, Cochran–Armitage trend test; $P = 0.001$, life-table trend test; $P = 0.002$, logistic-regression trend test *$P = 0.002$, life-table test; $P = 0.003$, logistic regression test; $P = 0.007$, Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 8 wk 105 wk NTP (1989) (cont.)		*Lung* Bronchioloalveolar adenoma 4/50, 4/50, 7/49 Bronchioloalveolar carcinoma 2/50, 5/50, 7/49*	 NS *P* = 0.034, life-table trend test **P* = 0.045, life-table test	
		Bronchioloalveolar adenoma or carcinoma (combined) 6/50, 8/50, 13/49*	*P* = 0.019, life-table trend test; *P* = 0.042, logistic-regression trend test; *P* = 0.041, Cochran–Armitage trend test **P* = 0.025, life-table test	
		Ovary: benign granulosa cell tumours 0/50, 5/45*, 5/47**	*P* = 0.017, life-table trend test; *P* = 0.017, logistic-regression trend test; *P* = 0.031 Cochran–Armitage trend test ** *P* = 0.015, life-table test; *P* = 0.015, logistic regression test; *P* = 0.021, Fisher exact test ** *P* = 0.016, life-table test; *P* = 0.016, logistic regression test; *P* = 0.024, Fisher exact test	
Full carcinogenicity Mouse, C57BL/6 (M) 15–18 wk 30 wk Tennant et al. (1995)	Oral administration (gavage) *N*-Methylolacrylamide, assumed to be ~98% purity Corn oil 0, 50 mg/kg bw 5×/wk for 24 wk 5, 5 5, 5	*Liver*: tumours 0/5, 0/5	 NA	Principal limitations: small number of mice per group; an unspecified number of mice were killed at interim; inadequate duration

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, C57BL/6 (F) 15–18 wk 30 wk Tennant et al. (1995)	Oral administration (gavage) *N*-Methylolacrylamide, assumed to be ~98% purity Corn oil 0, 50 mg/kg bw 5×/wk for 24 wk 5, 5 5, 4	*Liver*: tumours 0/5, 0/5	NA	Principal limitations: small number of mice per group; an unspecified number of mice were killed at interim; inadequate duration
Full carcinogenicity Mouse, C57BL/6 *p53*$^{+/-}$ (M) 15–18 wk 30 wk Tennant et al. (1995)	Oral administration (gavage) *N*-Methylolacrylamide, assumed to be ~98% purity Corn oil 0, 25, 50 mg/kg bw 5×/wk for 24 wk 7, 7, 10 7, 6, 8	*Liver*: tumours 0/7, 0/7, 0/10	NA	Principal limitations: small number of mice per group; an unspecified number of mice were killed at interim
Full carcinogenicity Mouse, C57BL/6 *p53*$^{+/-}$ (F) 15–18 wk 30 wk Tennant et al. (1995)	Oral administration (gavage) *N*-Methylolacrylamide, assumed to be ~98% purity Corn oil 0, 25, 50 mg/kg bw 5×/wk for 24 wk 7, 7, 10 7, 6, 8	*Liver*: tumours 0/7, 0/7, 0/10	NA	Principal limitations: small number of mice per group; an unspecified number of mice were killed at interim

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, $CB6F_1$ (M) 7 wk 26 wk Tsuji et al. (2015)	Oral administration (drinking-water) *N*-Methylolacrylamide, NR Water 0, 135 mg/kg bw, ad libitum 5, 5 5, 5	*Lung* Adenoma 0/5, 0/5 Adenocarcinoma 0/5, 0/5	 NA NA	Principal limitations: only one sex used; only one dose used; small number of mice per group; inadequate duration of experiment
Full carcinogenicity Mouse (transgenic, Tg), $CB6F_1$ *rasH2* (M) 7 wk 26 wk Tsuji et al. (2015)	Oral administration (drinking-water) *N*-Methylolacrylamide, NR Water 0, 135 mg/kg bw, ad libitum 5, 5 5, 4	*Lung* Adenoma 0/5, 5/5* Adenocarcinoma 0/5, 3/5	 *[$P = 0.0040$, one-tailed Fisher exact test] [NS]	Principal limitations: only one sex used; only one dose used; small number of mice per group; lack of historical control data
Full carcinogenicity Rat, F344/N (M) 7 wk 105 wk NTP (1989)	Oral administration (gavage) *N*-Methylolacrylamide, ~98% Deionized water 0, 6, 12 mg/kg bw 5 days/wk for 103 wk 50, 50, 50 28, 22, 27	No significant increase in tumour incidence in treated animals		Principal strengths: well-conducted study that complied with GLP; covers most of the life span; used multiple doses; used males and females; adequate number of rats per group

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/N (F) 7 wk 105 wk NTP (1989)	Oral administration (gavage) *N*-Methylolacrylamide, ~98% Deionized water 0, 6, 12 mg/kg bw 5 days/wk for 103 wk 50, 50, 50 35, 22, 33	No significant increase in tumour incidence in treated animals		Principal strengths: well-conducted study that complied with GLP; covers most of the life span; used multiple doses; used males and females; adequate number of rats per group

bw, body weight; F, female; GLP, Good Laboratory Practice; M, male; NA, not applicable; NR, not reported; NS, not significant; wk, week.

3.1 Mouse

3.1.1 Oral administration (gavage)

In a well-conducted study that complied with Good Laboratory Practice (GLP), groups of 50 male and 50 female B6C3F$_1$ mice (age, 8 weeks) were given *N*-methylolacrylamide (purity, approximately 98%) at a dose of 0, 25, or 50 mg/kg body weight (bw) for the control group and the groups at the lower and higher dose, respectively, in deionized water, by gavage, 5 days per week for 105 weeks (NTP, 1989). Surviving animals were killed at age 113 weeks. At study termination, survival was 30/50, 20/50, and 21/50 in males and 41/50, 35/50, and 33/50 in females, for the control group and the groups at the lower and higher dose, respectively. The mean body weights of all groups of treated mice were significantly increased, being up to 13% (males) and 25% (females) higher than those of controls. *N*-Methylolacrylamide treatment had no significant effect on survival. All mice underwent complete necropsy. Histopathological evaluation was performed on main tissues and organs.

In male mice, there was a significant positive trend in the incidence of Harderian gland adenoma ($P < 0.001$; Cochran–Armitage trend test, logistic-regression trend test and life-table trend test). The incidence of Harderian gland adenoma was significantly increased ($P < 0.001$; Fisher exact test, logistic regression test and life-table test) in all exposed groups. No significant changes were reported for the incidence of Harderian gland carcinoma [and no data on historical controls were reported]. [The Working Group noted that the reported increased incidence of Harderian gland adenoma or carcinoma (combined) in males may not have been related to treatment, in view of the lack of a significant positive trend in the incidence of Harderian gland carcinoma, lack of a significant increase in the incidence of Harderian gland carcinoma at the lower and higher dose, and lack of data on Harderian gland carcinoma in historical controls, making the contribution of the numerical increase at the higher dose negligible.] There was a significant positive trend in the incidence of hepatocellular adenoma ($P = 0.005$, Cochran–Armitage trend test; $P < 0.001$, life-table trend test; $P = 0.002$, logistic-regression trend test) with the incidence being significantly increased in males at the higher dose ($P = 0.004$, logistic regression test; $P = 0.012$, Fisher exact test). There was a significant positive trend in the incidence of hepatocellular carcinoma ($P = 0.027$, life-table trend test). The incidence of hepatocellular carcinoma in males – controls, 6/50 (12%); lower dose, 13/50 (26%); and higher dose, 12/50 (24%) – was significantly increased at the lower dose ($P = 0.023$, logistic regression test; $P = 0.012$, life-table test) and higher dose ($P = 0.031$, life-table test), but did not exceed the upper bound of the range observed in historical controls in this laboratory – gavage, 56/347 (mean ± standard deviation, 16.1 ± 8.03%); range, 4–28% – and all routes, 379/2032 (18.7 ± 6.50%); range, 8–30%). There was a significant positive trend in the incidence of hepatocellular adenoma or carcinoma (combined) – control, 12/50 (24%); lower dose, 17/50 (34%); and higher dose, 26/50 (52%); $P = 0.003$, Cochran–Armitage trend test; $P < 0.001$, life-table trend test and logistic-regression trend test – and a significant increase in the incidence at the highest dose ($P = 0.001$, logistic regression test; $P = 0.004$, Fisher exact test) that exceeded the upper bound of the range observed in historical controls in this laboratory – gavage, 106/347 (mean ± standard deviation, 30.5 ± 5.83%); range, 20–36% – and all routes, 609/2032 (30.0 ± 7.59%); range, 16–58%. There were significant positive trends in the incidence of bronchioloalveolar adenoma ($P = 0.010$, logistic-regression trend test; $P = 0.005$, life-table trend test) and bronchioloalveolar carcinoma ($P = 0.005$, logistic-regression trend test; $P = 0.003$, life-table trend test). The incidence of both bronchioloalveolar adenoma and of

bronchioloalveolar carcinoma was significantly increased at the higher dose ($P = 0.022$, Fisher exact test; $P = 0.006$, life-table test; $P = 0.015$, logistic regression test; and $P = 0.015$, Fisher exact test; $P = 0.006$, life-table test; $P = 0.011$, logistic regression test, respectively) compared with controls. There was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined) ($P = 0.001$, Cochran–Armitage trend test; $P < 0.001$, logistic-regression trend test and life-table trend test), with the incidence being significantly increased at the higher dose ($P = 0.002$, Fisher exact test; $P < 0.001$, life-table test; $P = 0.001$, logistic regression test).

In female mice, there was a significant positive trend in the incidence of Harderian gland adenoma ($P < 0.001$; Cochran–Armitage trend test, logistic-regression trend test, and life-table trend test), with the incidence being significantly increased at the higher dose ($P < 0.001$, Fisher exact test, life-table test, and logistic regression test). Although there was no significant positive trend in the incidence of Harderian gland carcinoma – controls, 0/47; lower dose, 3/45 (6.7%); and higher dose 2/48 (4.2%) – the incidence exceeded the upper bound of the range observed in historical controls in this laboratory – gavage, 3/350 (mean ± standard deviation, 0.9 ± 1.57%); range, 0–4% – and all routes, 7/2040 (0.3 ± 0.88%); range, 0–4%. A significant positive trend in the incidence of Harderian gland adenoma or carcinoma (combined) was observed ($P < 0.001$, Cochran–Armitage trend test, logistic-regression trend test, and life-table trend test), with a significant increase in incidence at both the lower and higher doses ($P = 0.031$, logistic regression test; and $P < 0.001$, Fisher exact test, logistic regression test and life-table test, respectively). There was a significant positive trend in the incidence of hepatocellular adenoma ($P < 0.001$, Cochran–Armitage trend test, life-table trend test, and logistic-regression trend test), with the incidence being significantly increased at the higher dose ($P < 0.001$, Fisher exact test, logistic regression test, and life-table test). The incidence of hepatocellular carcinoma was: controls, 3/50; lower dose, 3/50; and higher dose, 2/49. [A significant positive trend in the incidence of hepatocellular adenoma or carcinoma (combined) and a significant increase in the incidence at the higher dose were reported, but the Working Group concluded that this was attributable to the increased incidence of hepatocellular adenoma alone.] There was a significant positive trend in the incidence of bronchioloalveolar carcinoma ($P = 0.034$, life-table trend test) with the incidence being significantly increased at the higher dose ($P = 0.045$, life-table test). There was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined) – controls, 6/50 (12%); lower dose, 8/50 (16%); and higher dose, 13/49 (26%); $P = 0.019$, life-table trend test; $P = 0.041$, Cochran–Armitage trend test; $P = 0.042$, logistic-regression trend test – and a significant increase in the incidence at the higher dose ($P = 0.025$, life-table test) that exceeded the upper bound of the range observed in historical controls in this laboratory – gavage, 33/349 (mean ± standard deviation, 9.5 ± 3.66%); range, 4–14.3% – and all routes, 145/2026 (7.2 ± 4.21%); range, 0–16.0%. There was a significant positive trend in the incidence of benign granulosa cell tumours of the ovary ($P = 0.031$, Cochran–Armitage trend test; $P = 0.017$, life-table trend test and logistic-regression trend test) with the incidence being significantly increased at both the lower and higher doses ($P = 0.021$, Fisher exact test; $P = 0.015$, logistic regression test and life-table test; and $P = 0.024$, Fisher exact test; $P = 0.016$, logistic regression test and life-table test, respectively).

Regarding non-neoplastic lesions, chronic inflammation and alveolar epithelium hyperplasia were observed at an increased incidence in the lungs of treated male and female mice. These two lesions generally occurred together and appeared to be part of the same lesion (NTP, 1989). [The

Working Group noted that this was a well-conducted GLP study, males and females were used, the duration of exposure and observation was adequate, and an adequate number of animals per group and multiple doses were used.]

A concurrent study (Tennant et al., 1995) in male and female C57BL/6 mice homozygous (wildtype) or hemizygous for *Tp53* (C57BL/6 *p53*$^{+/-}$) was performed using the same chemical doses as the above study by the NTP (1989). Treatment began in a staggered fashion at age 15–18 weeks. Male mice were housed singly, and female mice were housed in groups. The authors stated that the wildtype sibling groups and hemizygous *Tp53* mouse control group contained 10 mice each [15 mice were probably used for the hemizygous *Tp53* mouse control group], and the hemizygous *Tp53* treatment groups contained 15 (lower dose) or 20 (higher dose) mice. *N*-Methylolacrylamide [purity assumed to be approximately 98%] was administered daily, by gavage, five times per week, for 24 weeks, at a dose of 0, 25 mg/kg bw (lower dose, hemizygous *Tp53* treatment groups only), or 50 mg/kg bw (higher dose) in corn oil. Mice were held an additional 6 weeks. An unspecified number of animals were killed at interim. All mice underwent gross necropsy and microscopic examination of gross lesions and of the liver.

There was no significant effect of *N*-methylolacrylamide treatment on survival. *N*-Methylolacrylamide treatment decreased body-weight gain in all groups of treated male mice (highest decrease in wildtype siblings at the higher dose). No liver tumours were observed in any groups of mice (Tennant et al., 1995). [The Working Group noted the small number of animals per group and the inadequate duration of the study. Therefore, the Working Group judged the study in wildtype mice inadequate for the evaluation of the carcinogenicity of *N*-methylolacrylamide in experimental animals.]

3.1.2 Oral administration (drinking-water)

A group of eight $CB6F_1$ *rasH2* transgenic mice (Tg) and a group of eight non-Tg mice (age, 7 weeks) were given drinking-water containing *N*-methylolacrylamide [purity not reported] at a dose of 135 mg/kg bw (1000 ppm) ad libitum for up to 26 weeks. One control group of eight Tg mice and one control group of eight non-Tg mice received drinking-water alone, ad libitum. After 4 weeks of treatment, three mice from each group were killed, and the study was continued until experimental week 26. Full histopathological examination was performed on major tissues and organs (Tsuji et al., 2015). None of the non-Tg mice died during the experimental period; one mouse from the *N*-methylolacrylamide-treated group of Tg mice died on the day of necropsy (at experimental week 26). No significant difference in average body weight was observed in the groups of Tg or non-Tg mice treated with *N*-methylolacrylamide compared with their respective controls.

In the *N*-methylolacrylamide-treated group of Tg mice, there was a significant increase in the incidence of adenoma of the lung [$P = 0.0040$, Fisher exact test]. In both groups of non-Tg mice, no lung tumours were observed. [The Working Group noted that the study had several limitations: the small number of animals per group, the lack of data on historical controls for Tg mice, the administration of a single dose, the use of only one sex and, the short duration of the study for non-Tg mice. Despite the small numbers of animals analysed, the incidence of adenoma in the lung of Tg mice was 0/5 in the untreated group and 5/5 in the *N*-methylolacrylamide-treated group, representing a significant increase in the treated group of $P < 0.005$ by Fisher exact test, a highly significant *P* value. The Working Group considered that this increase was treatment-related.]

3.2 Rat

Oral administration (gavage)

In a well-conducted study that complied with GLP, groups of 50 male and 50 female F344/N rats (age, 7 weeks) were given *N*-methylolacrylamide (purity, approximately 98%) at a dose of 0, 6, or 12 mg/kg bw in deionized water, for the control group and the groups at the lower and higher dose, respectively, by gavage, on 5 days per week for 105 weeks (NTP, 1989). Surviving animals were killed at age 112 weeks. The mean body weights of males at the higher dose were 6–7% lower than those of controls. The mean body weights of females at the higher dose were 5–6% lower than those of controls. *N*-Methylolacrylamide treatment has no significant effect on survival. At study termination, survival was 28/50, 22/50, and 27/50 for males; and 35/50, 22/50 and 33/50 for females, for the control group and the groups at the lower and higher dose, respectively. All rats underwent complete necropsy. Histopathological evaluation was performed on the main tissues and organs.

No increased incidence of any neoplasm was attributable to the administration of *N*-methylolacrylamide in male and female rats (NTP, 1989). [The Working Group noted that this was a well-conducted GLP study, both sexes were used, the duration of exposure and observation was adequate, and an adequate number of animals per group and multiple doses were used.]

3.3 Evidence synthesis for cancer in experimental animals

The carcinogenicity of *N*-methylolacrylamide has been assessed in one well-conducted GLP study in male and female B6C3F_1 mice (NTP, 1989) and one well-conducted study in male and female F344/N rats (NTP, 1989) treated by oral administration (gavage), in a concurrent oral administration (gavage) study in male and female C57BL/6 mice homozygous (wildtype) or hemizygous for *Tp53* (C57BL/6 *p53*$^{+/-}$) (Tennant et al., 1995), and in an oral administration study (drinking-water) in male CB6F_1 *rasH2* transgenic mice (Tg) and non-Tg mice (Tsuji et al., 2015).

In the well-conducted GLP study in male and female B6C3F_1 mice treated by oral administration (gavage) (NTP, 1989), there was a significant positive trend in the incidence of Harderian gland adenoma in male mice with the incidence being significantly increased in all treated groups. In male mice, there was a significant positive trend in the incidence of hepatocellular adenoma, of hepatocellular carcinoma, and of hepatocellular adenoma or carcinoma (combined) with a significant increase in the incidence of hepatocellular adenoma at the higher dose, of hepatocellular carcinoma at all doses, and of hepatocellular adenoma or carcinoma (combined) at the higher dose. In male mice, there was a significant positive trend in the incidence of bronchioloalveolar adenoma, of bronchioloalveolar carcinoma, and of bronchioloalveolar adenoma or carcinoma (combined) with a significant increase in the incidence at the highest dose. In female mice, there was a significant positive trend in the incidence of Harderian gland adenoma and of Harderian gland adenoma or carcinoma (combined), with the incidence being significantly increased for Harderian gland adenoma in females at the highest dose, and for Harderian gland adenoma or carcinoma (combined) in all treated groups of females. There was a significant positive trend in the incidence of hepatocellular adenoma in female mice, with the incidence being significantly increased at the higher dose. There was a significant positive trend in the incidence of bronchioloalveolar carcinoma and of bronchioloalveolar adenoma or carcinoma (combined) in female mice, with the incidence being significantly increased at the highest dose. The incidence of benign granulosa cell tumours of the ovary was significantly increased in both treated

groups, with a significant positive trend (NTP, 1989).

In the study in male $CB6F_1$ *rasH2* transgenic mice (Tg) and non-Tg mice given drinking-water containing *N*-methylolacrylamide, there was a significant increase in the incidence of adenoma of the lung in the group of treated Tg mice. No lung tumours were observed in treated and control non-Tg mice (Tsuji et al., 2015).

In the study by Tennant et al. (1995) in male and female C57BL/6 mice homozygous (wildtype) or hemizygous for *Tp53* (C57BL/6 $p53^{+/-}$) treated by gavage, no liver tumours were observed in any groups of hemizygous mice; the study in homozygous mice was considered inadequate for the evaluation of the carcinogenicity of *N*-methylolacrylamide in experimental animals.

In the well-conducted GLP study in male and female F344/N rats treated by gavage (NTP, 1989), no increased incidence of any neoplasm was attributable to the administration of *N*-methylolacrylamide in male or female rats.

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

N-Methylolacrylamide was widely distributed in the blood and tissues of male rats given a single intravenous injection of 140 mg/kg bw (Hashimoto & Aldridge, 1970) and of male mice given a single dose by intraperitoneal injection (150 mg/kg bw) or by oral administration (in the drinking-water) (150 or 1.5 mg/kg bw) (Witt et al., 2003). In the study by Witt et al. (2003, there was no difference in tissue distribution between intraperitoneal and oral administration in mice, and for both administration routes the tissue/blood concentration ratios were < 1. In the same study, comparison of the excretion profiles after oral or intraperitoneal administration indicated lower absorption via the oral route as the percent of the administered dose recovered in urine was higher after intraperitoneal injection.

In rats given a single intravenous injection of 140 mg/kg bw, the blood concentration of *N*-methylolacrylamide decreased rapidly, with a half-life of 1.55 hours and a first-order rate of elimination of 0.45 per hour from the blood with distribution in total body water (Edwards, 1975). *N*-Methylolacrylamide was also excreted in the urine and faeces of mice; the percentage of the administered dose recovered in the urine was higher after intraperitoneal injection than after oral administration, whereas the percentage of the administered dose recovered in the faeces was higher after oral administration. For both intraperitoneal injection and oral administration, about 10% of the administered dose was exhaled as radiolabelled carbon dioxide ($^{14}CO_2$) after either intraperitoneal injection or oral administration of *N*-methylolacrylamide [^{14}C]-labelled at the hydroxymethyl group (Witt et al., 2003).

A rapid decrease in liver gluthatione levels was observed after a single intravenous injection of *N*-methylolacrylamide, which was suggestive of conjugation with glutathione as a metabolic pathway (Edwards, 1975). This was further confirmed by the identification of gluthatione conjugates in the bile of exposed rats (Edwards, 1975) and by demonstration of the reaction of *N*-methylolacrylamide with glutathione in vitro (Hashimoto & Aldridge, 1970; Edwards, 1975).

After a single intraperitoneal injection or oral administration of *N*-methylolacrylamide in mice, about 10% of the administered dose was excreted unchanged and 10% as *N*-acetyl-*S*-(3-hydroxymethyl-amino-3-oxopropyl)cysteine (a metabolite derived from reaction with gluta-

thione) in the urine during the first 24 hours of administration (Witt et al., 2003). No evidence was found for the conversion of *N*-methylolacrylamide to acrylamide in vivo (Edwards, 1975). In contrast, *N*-(2-carbamoyl-2-hydroxyethyl)valine (GAVal) adducts, which are derived from glycidamide (the reactive metabolite of acrylamide), were observed in rats given *N*-methylolacrylamide as a single dose at 71 mg/kg bw by gavage (Fennell et al., 2003). [The Working Group noted that this suggests oxidation of *N*-methylolacrylamide, either directly or indirectly after conversion to acrylamide. The Working Group also noted that the purity of the *N*-methylolacryamide used in the study was reported to be 99%. In addition, analytical assessment of purity was performed and there was no indication that either acrylamide or formaldehyde were present. The Working Group considered the identification of glycidamide-specific adducts as an important finding, since glycidamide is genotoxic. However, the Working Group also noted that the possible metabolism of *N*-methylolacrylamide directly or indirectly to glycidamide is not supported by the results of other studies.]

4.2 Evidence relevant to key characteristics of carcinogens

4.2.1 Is electrophilic or can be metabolically activated to an electrophile

(a) Humans

(i) Exposed humans

See Table 4.1.

No studies on DNA adducts were available to the Working Group.

Haemoglobin adducts were reported in the blood of tunnel workers (Hagmar et al., 2001; Kjuus et al., 2004) and glass workers (Paulsson et al., 2006) exposed to grout or sealant containing acrylamide and *N*-methylolacrylamide. [The Working Group noted that it was not possible to attribute these effects only to *N*-methylolacrylamide in these studies.]

(ii) Human cells in vitro

No data were available to the Working Group.

(b) Experimental systems

Haemoglobin adducts were reported in rats given *N*-methylolacrylamide either at 142 mg/kg bw as a single intraperitoneal injection (Paulsson et al., 2002) or at 71 mg/kg bw by gavage (Fennell et al., 2003). In mice, haemoglobin adduct levels were increased after a single intraperitoneal injection of *N*-methylolacrylamide at a dose of 35, 71, and 142 mg/kg bw (Paulsson et al., 2002).

4.2.2 Is genotoxic

(a) Humans

(i) Exposed humans

See Table 4.2.

Chromosomal alterations in blood lymphocytes of tunnel workers exposed to grout containing *N*-methylolacrylamide and acrylamide were assessed in one study. The findings were negative for chromosomal aberrations and breaks, and positive for chromatid gaps (but without an exposure–response relationship) (Kjuus et al., 2005). [The Working Group noted that it was not possible to attribute the effects only to *N*-methylolacrylamide in this study.]

(ii) Human cells in vitro

No data were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.3.

N-Methylolacrylamide induced dominant lethal (germ cell) mutations in male mice after 13 weeks of oral administration via the drinking-water (Chapin et al., 1995; Witt et al., 2003). No induction of dominant lethal mutations in

Table 4.1 Haemoglobin adducts in humans exposed to *N*-methylolacrylamide

End-point	Tissue or cell type	Description of exposed and controls	Results[a]	Comments	Reference
Hb adducts	Blood	210 tunnel workers (3 F/207 M; age, 20–62 years) exposed to grout containing *N*-methylolacrylamide and acrylamide; and 18 controls (7 F/11 M, all non-smokers)	(+)	Increased Hb adduct level was observed for 74 of the exposed workers. Causative effect of *N*-methylolacrylamide alone could not be demonstrated as there was co-exposure to other substances including acrylamide.	Hagmar et al. (2001)
Hb adducts	Blood	23 tunnel workers exposed to grout containing *N*-methylolacrylamide and acrylamide (12 smokers and 11 non-smokers); and 3 controls (2 smokers and 1 non-smoker). The blood samples were collected 60–143 days (mean, 84 days) after the end of working with the grout	(+)	Increased Hb adduct level was observed for two of the exposed workers. Causative effect of *N*-methylolacrylamide alone could not be demonstrated as there was co-exposure to other substances including acrylamide.	Kjuus et al. (2004)
Hb adducts	Blood	A case study of 4 glass workers exposed to sealant containing *N*-methylolacrylamide and acrylamide; and 1 control	(+)	Increased Hb adduct level was observed for one of the exposed workers. Causative effect of *N*-methylolacrylamide alone could not be demonstrated as there was co-exposure to other substances including acrylamide.	Paulsson et al. (2006)

M, male; F, female; Hb, haemoglobin.

[a] (+), positive result in a study of limited quality.

Table 4.2 Genetic and related effects of *N*-methylolacrylamide in exposed humans

End-point	Tissue or cell type	Description of exposed and controls	Results[a]	Comments	Reference
Chromosomal aberrations	Blood lymphocytes	25 tunnel workers exposed to grout containing *N*-methylolacrylamide (26–31%), acrylamide (2.5–5.4%), methylene-bis-acrylamide (0.02–0.03%), methylic diesters (12–17%), formaldehyde (0.9%), and water, were compared with 25 age- and sex-matched tunnel workers who had not been exposed to the grout; both smokers and non-smokers were included; exposure was assessed by questionnaire	(–)	There was co-exposure to other substances including acrylamide.	Kjuus et al. (2005)
Chromosomal gaps and breaks, chromatid breaks	Blood lymphocytes		(–)	There was co-exposure to other substances including acrylamide.	Kjuus et al. (2005)
Chromatid gaps	Blood lymphocytes		(+)	Significant increase; however, there was no exposure–response relationship. Causative effect of *N*-methylolacrylamide alone could not be demonstrated as there was co-exposure to other substances including acrylamide.	Kjuus et al. (2005)

[a] (–), negative result in a study of limited quality; (+), positive result in a study of limited quality.

Table 4.3 Genetic and related effects of *N*-methylolacrylamide in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Dominant lethal test (mutation)	Mouse, CD-1 Swiss (M)	Ovary/uterus after mating	+	180 ppm [56.8 mg/kg bw]	Oral via drinking-water, 13 wk	Significant increase in total post-implantation losses and early fetal resorptions; dose calculated using water consumption from the post-mating week; no positive control; purity, 97–99%.	Chapin et al. (1995)
Dominant lethal test (mutation)	Mouse, CD-1 Swiss (M)	Ovary/uterus after mating	+	360 ppm [112.5 mg/kg bw]	Oral via drinking-water, 13 wk	Significant increase in total post-implantation losses, early fetal resorptions, and decrease in live fetuses; dose calculated using water consumption from the post-mating week; no positive control; purity, 97–99%.	Chapin et al. (1995)
Dominant lethal test (mutation)	Mouse, $B6C3F_1$ (M)	Ovary/uterus after mating	–	150 mg/kg bw	Intraperitoneal injection, ×1	No positive control; purity, ~98%.	Witt et al. (2003)
Dominant lethal test (mutation)	Mouse, $B6C3F_1$ (M)	Ovary/uterus after mating	–	50 mg/kg bw	Intraperitoneal injection, ×5	No positive control; purity, ~98%.	Witt et al. (2003)
Dominant lethal test (mutation)	Mouse, $B6C3F_1$ (M)	Ovary/uterus after mating	+	180 ppm (37 mg/kg bw)	Oral via drinking-water, 13 wk	Significant increase in total post-implantation losses and early fetal resorptions; three sets of mating occurred; females were killed and the uterine contents were assessed 2 wk post-mating; dose is estimated using data on water consumption from Chapin et al. (1995); no positive control; purity, ~98%.	Witt et al. (2003)
Micronucleus formation	Mouse, $B6C3F_1$ (M)	Bone marrow PCE	–	150 mg/kg bw	Intraperitoneal injection, ×2	Purity, ~98%.	NTP (1989)
Micronucleus formation	Mouse, CBA (M)	Peripheral blood PCE	+	142 mg/kg bw	Intraperitoneal injection, ×1	Dose-dependent increase; flow cytometric measurements were applied; purity, ~48% in water.	Paulsson et al. (2002)
Micronucleus formation	Rat, Sprague-Dawley (M)	Bone marrow PCE	(–)	142 mg/kg bw	Intraperitoneal injection, ×1	Flow cytometric measurements were applied; purity, ~48% in water.	Paulsson et al. (2002)

Table 4.3 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Micronucleus formation	Mouse, $B6C3F_1$ (M)	Bone marrow PCE	–	150 mg/kg bw	Intraperitoneal injection, ×2 (vehicle, corn oil)	Purity, ~98%.	Witt et al. (2003)
Micronucleus formation	Mouse, $B6C3F_1$ (M)	Bone marrow PCE	–	112 mg/kg bw	Intraperitoneal injection, ×2 (vehicle, PBS)	Purity, ~98%.	Witt et al. (2003)
Micronucleus formation	Mouse, $B6C3F_1$ (M)	Bone marrow PCE	–	150 mg/kg bw	Gavage, ×2 (vehicle, PBS)	Purity, ~98%.	Witt et al. (2003)
Micronucleus formation	Mouse, $B6C3F_1$ (M)	Bone marrow and peripheral blood PCE	–	720 ppm (168 mg/kg bw)	Gavage, daily for 31 days (vehicle, water)	Purity, ~98%.	Witt et al. (2003)
Micronucleus formation	Mouse, $B6C3F_1$ (M)	Bone marrow and peripheral blood PCE	–	720 ppm (~120–125 mg/kg bw)	Oral via drinking-water, 13 wk	Purity, ~98%.	Witt et al. (2003)

bw, body weight; HID, highest ineffective dose; LED, lowest effective dose; M, male; PBS, phosphate-buffered saline; PCE, polychromatic erythrocytes; ppm, parts per million; wk, week.
[a] +, positive; –, negative; (–), negative in a study of limited quality.

male mice was seen after a single intraperitoneal injection or five repeated intraperitoneal injections (Witt et al., 2003). [The Working Group noted that there was no positive control for the dominant lethal test reported in Witt et al. (2003). However, the Working Group considered that the positive result in mice treated by oral administration is sufficient to demonstrate the proficiency of the laboratory in the conduct of the test.]

A significant increase in the frequency of micronucleated erythrocytes was observed in peripheral blood of male mice given a single intraperitoneal injection of *N*-methylolacrylamide (Paulsson et al., 2002). The same route of administration and dose did not induce micronucleus formation in the bone marrow erythrocytes of male rats (Paulsson et al., 2002). Several other studies also reported negative findings regarding the induction of micronucleus formation in bone marrow and peripheral blood cells of male mice exposed to *N*-methylolacrylamide, after either intraperitoneal injection (NTP, 1989; Witt et al., 2003) or oral administration (Witt et al., 2003). [The Working Group noted that several of the studies on micronucleus formation in vivo did not report evidence of bone marrow exposure to *N*-methylolacrylamide; however, the Working Group considered that the toxicokinetic data reported in Witt et al. (2003) showing detection of radiolabelled *N*-methylolacrylamide in blood/plasma and several tissues were sufficient to conclude that this substance is systemically available after both intraperitoneal injection and oral administration. The Working Group also noted that the blood/tissue ratio was < 1, which would indicate that *N*-methylolacrylamide and/or its metabolites are only taken up to some extent by tissues other than blood.]

(ii) Non-human mammalian cells in vitro

See Table 4.4.

An increased frequency of mutation was seen in L5178Y $Tk^{+/-}$ mouse lymphoma cell cultures after incubation with *N*-methylolacrylamide in the presence and absence of metabolic activation (Kirkland & Fowler, 2010).

In one study, *N*-methylolacrylamide with and without metabolic activation induced chromosomal aberrations in Chinese hamster ovary (CHO) cells (NTP, 1989). In another study with CHO cells, induction of chromosomal aberrations was seen only in the absence, but not in the presence, of metabolic activation (Kirkland & Fowler, 2010).

The frequency of sister-chromatid exchanges was increased in CHO cells treated with *N*-methylolacrylamide without metabolic activation. In the presence of metabolic activation, the frequency of sister-chromatid exchange was weakly increased (NTP, 1989).

(iii) Non-mammalian experimental systems

See Table 4.5.

N-Methylolacrylamide did not induce mutation in any of several strains of *Salmonella typhimurium* in the presence or absence of metabolic activation (Hashimoto & Tanii, 1985; Zeiger et al., 1988; NTP, 1989). [The Working Group noted that the mutagenic effect of *N*-methylolacrylamide has not been tested in the strains *Escherichia coli* WP2 (pKM101), *E. coli* WP2 *uvrA* (pKM101), or *S. typhimurium* TA102, which are able to detect cross-linking agents and oxidizing mutagens.]

4.2.3 Other key characteristics of carcinogens

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Regarding oxidative stress, *N*-methylolacrylamide showed reactivity towards glutathione in vitro (Hashimoto & Aldridge, 1970; Edwards, 1975), and a decrease in liver glutathione levels was observed in rats given a single intravenous injection of *N*-methylolacrylamide (Edwards, 1975).

Table 4.4 Genetic and related effects of *N*-methylolacrylamide in non-human mammalian cells in vitro

End-point	Species, cell type	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y *Tk*$^{+/-}$ lymphoma cells	+	+	303.3 µg/mL –S9, 404.4 µg/mL +S9	Exposure, 3 h; S9 from Aroclor-1254-induced rat liver; purity, NR.	Kirkland & Fowler (2010)
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y *Tk*$^{+/-}$ lymphoma cells	+	NT	202.2 µg/mL	Exposure, 24 h; purity, NR.	Kirkland & Fowler (2010)
Chromosomal aberrations	Chinese hamster, ovary (CHO) cells	+	+	250 µg/mL –S9, 2500 µg/mL +S9	No information on whether the increase is statistically significant (no *P* values given); S9 from Aroclor-1254-induced rat liver; purity, ~98%.	NTP (1989)
Chromosomal aberrations	Chinese hamster, ovary (CHO) cells	+	NT	375 µg/mL	No information on whether the increase is statistically significant (no *P* values given); purity, ~98%.	NTP (1989)
Chromosomal aberrations	Chinese hamster, ovary (CHO) cells	+	–	202.2 µg/mL	Exposure, 3 h; S9 from Aroclor-1254-induced rat liver; purity, NR.	Kirkland & Fowler (2010)
Chromosomal aberrations	Chinese hamster, ovary (CHO) cells	+	NT	202.2 µg/mL	Exposure, 20 h; purity, NR.	Kirkland & Fowler (2010)
Sister-chromatid exchange	Chinese hamster, ovary (CHO) cells	+	NT	250 µg/mL	No information on whether the increase is statistically significant (no *P* values given); the highest tested dose is given; purity, ~98%.	NTP (1989)
Sister-chromatid exchange	Chinese hamster, ovary (CHO) cells	NT	+	1700 µg/mL	Weakly positive; no information on whether the increase is statistically significant; S9 from Aroclor-1254-induced rat liver; purity, ~98%.	NTP (1989)

LEC, lowest effective concentration; HIC, highest ineffective concentration; NR, not reported; NT, not tested; Tk, thymidine kinase; S9, 9000 × *g* supernatant.

[a] +, positive; –, negative.

Table 4.5 Genetic and related effects of *N*-methylolacrylamide in non-mammalian experimental systems

Species, strain	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium, TA97, TA98, TA100, and TA1535	Reverse mutation	–	–	10 000 µg/plate	Some cytotoxicity was observed; S9 from Aroclor-1254-induced male Syrian hamster liver or male Sprague-Dawley rat liver; purity, ~98%.	NTP (1989) Zeiger et al. (1988)
Salmonella typhimurium, TA98, TA100, TA1535, TA1537, and TA1538	Reverse mutation	–	–	5000 µg/plate	S9 from Aroclor-1254-induced rat liver; purity, > 95%.	Hashimoto & Tanii (1985)

LEC, lowest effective concentration; HIC, highest ineffective concentration; S9, 9000 × *g* supernatant.

[a] –, negative.

Regarding chronic inflammation, in a 13 week study in F344/N rats treated by gavage, *N*-methylolacrylamide caused inflammation and/or haemorrhage of the urinary bladder mucosa at doses of 25 mg/kg bw or greater (NTP, 1989; also reported in Bucher et al., 1990). [The Working Group noted that the mucosal lesions of the urinary bladder were reportedly associated with urinary retention, which was secondary to defects in neural control of the bladder in this 13 week study, and not a direct induction of chronic inflammation by *N*-methylolacrylamide in the bladder.] In $B6C3F_1$ mice, 2-year exposure to *N*-methylolacrylamide administered by gavage caused chronic inflammation of the lung (NTP, 1989). [The Working Group noted that chronic inflammation of the lung was probably attributable to infection with Sendai virus in this 2-year study and was not a direct induction of chronic inflammation by *N*-methylolacrylamide in the lung.] RasH2 transgenic and non-transgenic mice received drinking-water containing *N*-methylolacrylamide at a dose of 135 mg/kg bw per day for 4 weeks. Gene ontology enrichment analysis of non-neoplastic regions of the lungs showed that inflammation-related genes were differentially expressed in both transgenic and non-transgenic mice compared with untreated mice (Tsuji et al., 2015).

Regarding alterations in cell proliferation, cell death, or nutrient supply, hyperplasia and dysplasia of the tracheal mucosa and bronchiolar epithelial hyperplasia of the lung in male and female F344/N rats, and bronchial epithelial hyperplasia of the lung in male and female $B6C3F_1$ mice, were observed after exposure to *N*-methylolacrylamide by gavage for 16 days (NTP, 1989). In $B6C3F_1$ mice, 2-year exposure to *N*-methylolacrylamide by gavage caused alveolar epithelial hyperplasia of the lung (NTP, 1989). [The Working Group noted that alveolar epithelial hyperplasia of the lung observed in this 2-year study was probably attributable to infection with Sendai virus and was not direct induction of cell proliferation by *N*-methylolacrylamide in the lung.]

4.2.4 High-throughput in vitro toxicity screening data evaluation

N-Methylolacrylamide has not been tested in the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA (Thomas et al., 2018).

4.3 Other relevant evidence

Hepatocellular necrosis was observed in rats and mice exposed to *N*-methylolacrylamide by gavage for 16 days and 13 weeks, respectively (NTP, 1989).

N-Methylolacrylamide has been found to be neurotoxic in rats and mice (NTP, 1989; Bucher et al., 1990).

5. Summary of Data Reported

5.1 Exposure characterization

N-Methylolacrylamide is a High Production Volume chemical that is used as an intermediate in the manufacture of some chemicals and acrylamide-based polymers that ultimately appear in a variety of adhesives, sealants, inks, resins, paints, plastics, paper, and textile finishes.

While industrial uses of *N*-methylolacrylamide permit its release into the environment, it is not expected to bioaccumulate and should be readily biodegradable. The only documented environmental contamination involved transient presence in drainage and groundwater after grouting operations in nearby tunnels in Norway and Sweden.

The most substantial human exposures to *N*-methylolacrylamide probably occur in occupational settings, particularly during industrial

processes using *N*-methylolacrylamide (e.g. in textile treatment resins and polymers), although very little empirical information on occupational exposure was available to the Working Group. Documented worker-exposure events involved the use of a grout containing *N*-methylolacrylamide in the construction of one tunnel in Norway and one in Sweden. Airborne exposure was in the milligram per cubic metre range, but most workers also had dermal contact. Additionally, a case study reported the use of *N*-methylolacrylamide as a sealant in the window-manufacturing industry and as hazardous waste generated in the two aforementioned tunnels. Both situations involved co-exposure to acrylamide contained within the *N*-methylolacrylamide products used.

While the general population might be exposed to *N*-methylolacrylamide through uses of products containing its associated polymers, no quantitative information was available to the Working Group.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

Treatment with *N*-methylolacrylamide caused an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in both sexes of a single species in a well-conducted study that complied with Good Laboratory Practice (GLP).

N-Methylolacrylamide was administered by oral administration (gavage) in one well-conducted GLP study in male and female B6C3F$_1$ mice. In males, *N*-methylolacrylamide caused an increase in the incidence of hepatocellular carcinoma, hepatocellular adenoma or carcinoma (combined), bronchioloalveolar carcinoma, and bronchioloalveolar adenoma or carcinoma (combined). In females, *N*-methylolacrylamide caused an increase in the incidence of bronchioloalveolar carcinoma, bronchioloalveolar adenoma or carcinoma (combined), and Harderian gland adenoma or carcinoma (combined).

5.4 Mechanistic evidence

No data on absorption, distribution, metabolism, or excretion in humans were available. One study in mice showed that *N*-methylolacrylamide is widely distributed after oral administration and intraperitoneal injection. There is also evidence that conjugation with glutathione is a metabolic pathway. In mice, parent *N*-methylolacrylamide and/or metabolites are excreted in the urine and faeces and exhaled as carbon dioxide.

Overall, the mechanistic evidence that *N*-methylolacrylamide exhibits key characteristics of carcinogens ("is electrophilic or metabolically activated", "is genotoxic", "induces oxidative stress", "induces chronic inflammation", and "alters cell proliferation, cell death, or nutrient supply") is suggestive but incoherent across experimental systems. There were no studies in humans with exposure specifically attributable to *N*-methylolacrylamide and no studies using human cells in vitro.

There is suggestive evidence that *N*-methylolacrylamide is electrophilic. In two studies, haemoglobin adducts were detected in rodents after intraperitoneal injection. There is suggestive but incoherent evidence that *N*-methylolacrylamide is genotoxic in different experimental systems. In two studies, *N*-methylolacrylamide gave positive results in the dominant lethal test in mice when administered orally, but not by intraperitoneal injection. In rodents, results were positive for micronucleus formation in only one of seven tests. *N*-Methylolacrylamide induced mutations at the *Tk* locus in L5178Y mouse lymphoma cells with

and without metabolic activation in one study. *N*-Methylolacrylamide with and without metabolic activation gave positive results in Chinese hamster ovary (CHO) cells for chromosomal aberrations in two studies and for sister-chromatid exchange in one study. *N*-Methylolacrylamide gave negative results for mutation in bacteria both with and without metabolic activation.

Regarding the key characteristics "induces oxidative stress", "induces chronic inflammation", and "alters cell proliferation, cell death, or nutrient supply", there is suggestive mechanistic evidence. *N*-Methylolacrylamide showed reactivity to glutathione in vitro in two studies and caused decreased liver glutathione levels in rats in another study, indicative of increased oxidative stress. Oral administration of *N*-methylolacrylamide caused chronic inflammation in the lung of mice in one study and increased the expression of inflammation-related genes in the lung of mice 4 weeks after exposure in another. Acute and chronic exposure to *N*-methylolacrylamide by gavage induced epithelial hyperplasia in the respiratory tract of rodents.

N-Methylolacrylamide has not been tested in the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of *N*-methylolacrylamide.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of *N*-methylolacrylamide.

6.3 Mechanistic evidence

There is *limited mechanistic evidence.*

6.4 Overall evaluation

N-Methylolacrylamide is *possibly carcinogenic to humans (Group 2B).*

6.5 Rationale

The Group 2B evaluation for *N*-methylolacrylamide is based on *sufficient evidence* for cancer in experimental animals. This *sufficient evidence* in experimental animals is based on an increased incidence of either malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in both sexes of a single species in a well-conducted study that complied with Good Laboratory Practice. The evidence regarding cancer in humans is *inadequate* because no studies were available. The mechanistic evidence was *limited* as the findings regarding key characteristics of carcinogens across experimental systems were suggestive, but incoherent.

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