METHYL METHANESULFONATE

Data were last reviewed in IARC (1974) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

1.1 Chemical and physical data

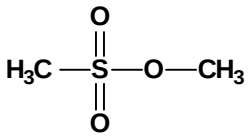
1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 66-27-3

Chem. Abstr. Name: Methanesulfonic acid, methyl ester

Synonym: MMS

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_2H_6O_3S$ Relative molecular mass: 110.13

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid (Budavari, 1996)

(*b*) *Boiling-point*: 202.5°C (Lide, 1997)

(*c*) *Melting-point*: 20°C (Lide, 1997)

(*d*) *Solubility*: Soluble in water (1 part in 5 at 25°C), dimethylformamide and propylene glycol; slightly soluble in non-polar solvents (Budavari, 1996)

(*e*) *Conversion factor*: $mg/m^3 = 4.5 \times ppm$

1.2 Production and use

No indication was found that methyl methanesulfonate is produced commercially, although it has been produced for research purposes. Information available in 1995 indicated that it was produced in one country (India) (Chemical Information Services, 1995).

It is believed to be used currently only for research purposes.

1.3 Occurrence

No data were available to the Working Group.

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has not proposed any occupational exposure limit for methyl methanesulfonate in work-place air.

No international guideline for methyl methanesulfonate in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Methyl methanesulfonate was tested for carcinogenicity in rats by subcutaneous and intraperitoneal injection, producing local tumours and tumours of the nervous system. Following oral administration to mice, it increased the incidence of lung tumours and of lymphomas. In rats, it produced neurogenic tumours after administration of a single dose as well as following prenatal exposure (IARC, 1974).

3.1 Inhalation exposure

Rat: Male Sprague-Dawley rats, 9–10 weeks of age, were exposed by whole-body inhalation to 50 ppm [225 mg/m^3] methyl methanesulfonate (purity, > 95%) for 6 h per day on five days per week for six weeks and were observed for life. Nasal tumours were found in 47/80 animals versus 0/98 air controls. The median life spans for the control and exposed groups were 613 days and 495 days, respectively. The median time of nasal tumour appearance was 513 days (range, 256–775) (Snyder *et al*., 1986; Sellakumar *et al*., 1987).

3.2 Oral or intraperitoneal administration

Mouse: Groups of 16 male and 16 female A/J strain mice, six to eight weeks of age, were dosed either orally or intraperitoneally with methyl methanesulfonate in tricaprylin three times per week for eight weeks and were then observed for an additional 16 weeks. The total cumulative doses were: gavage, 0 and 300 mg/kg bw; intraperitoneal injection, 0, 60, 150 and 300 mg/kg bw. Survival was similar in all groups. At the end of the experiment, in all groups, the numbers of animals with superficial lung adenomas and numbers of lung adenomas per animal were within the range of those observed in a variety of control groups (Stoner *et al*., 1986).

3.3 Multistage models

3.3.1 *Mouse*

Groups of 20 female NMRI mice, seven weeks of age, received a single skin application of either (*a*) 100 nmol 7,12-dimethylbenz[*a*]anthracene (DMBA), (*b*) 100 μmol methyl methanesulfonate or (*c*) 400 μmol methyl methanesulfonate (highest tolerated dose). One week later, all were treated with 10 nmol 12-*O*-tetradecanoylphorbol 13-acetate twice weekly for 24 weeks. While 90% of the DMBA group had skin tumours after 15 weeks, no methyl methanesulfonate-initiated mice had skin tumours after 24 weeks (Fürstenberger *et al*., 1989).

Groups of 20 female NMRI mice were treated with DMBA as above and subsequently treated with (*a*) acetone (the vehicle used for all substances in the experiment) 6 h before 10 nmol 12-*O*-retinoylphorbol 13-acetate, (*b*) 100 μmol methyl methanesulfonate 6 h before acetone, (*c*) 100 μmol methyl methanesulfonate 6 h before 10 nmol 12-*O*-retinoylphorbol 13-acetate, (*d*) 10 μmol methyl methanesulfonate 6 h before 10 nmol 12-*O*-retinoylphorbol 13-acetate or (*e*) 10 nmol 12-*O*-tetradecanoylphorbol 13-acetate 6 h before acetone. Two weeks after DMBA treatment, all groups received 10 nmol 12-*O*-retinoylphorbol 13-acetate once a week for 23 weeks. At the end of the experiment, > 90% of the mice were alive. The numbers of papillomas per survivor at 24 weeks [figures read from a graph] were (*a*) 0.4, (*b*) 1.6, (*c*) 1.7, (*d*) 2.9 and (*e*) 3.0 (Fürstenberger *et al*., 1989).

Following a single intraperitoneal injection of 120 mg/kg bw to an unspecified number of four-week-old AKR mice, all of the methyl methanesulfonate-treated mice had developed thymomas after 50 weeks versus 50% tumour incidence in the controls (Warren *et al*., 1990). [The Working Group noted the inadequate reporting.]

3.3.2 *Rat*

Groups of female Fischer 344 rats, six to eight weeks old, were either left untreated (group A) or received a single intravesicular instillation of 0.3 mg *N*-methyl-*N*-nitrosourea (group B), six intravesicular instillations of 2.5 mg methyl methanesulfonate at 14-day intervals (group C) or sequential treaments with 0.3 mg *N*-methyl-*N*-nitrosourea followed by six intravesicular instillations of 2.5 mg methyl methanesulfonate at 14-day intervals (group D). The numbers of rats with bladder tumours were (A) 0/25, (B) 7/29 (24%), (C) 2/27 (7%) and (D) 19/33 (58%) (Tudor *et al*., 1984).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Methyl methanesulfonate is rapidly distributed throughout the body of mice and rats, including the central nervous system, and rapidly crosses the placenta. After intravenous injection of 100 mg/kg bw methyl methanesulfonate to rats, none was detected in serum after 2 h (IARC, 1974).

In rats injected with [*methyl*-^{14}C]methyl methanesulfonate, about 30% of the label was exhaled as CO_2 within 30 h and 20% was found in the urine. The corresponding values for mice given an intraperitoneal dose were 27% and 34%, respectively (IARC, 1974).

Urinary metabolites recovered within the first 16 h and representing 80% of the excretion products resulted from an initial methylation of cysteine residues by methyl methanesulfonate. These were methylmercapturic acid sulfoxide, 2-hydroxy-3-methylsulfinylpropionic acid, methylsulfinylacetic acid, methylmercapturic acid and *N*-(methylthioacetyl)glycine. Glutathione conjugation has been shown to occur in rat liver (IARC, 1974).

4.2 Toxic effects

4.2.1 *Humans*

Therapeutic application to cancer patients of total doses ranging from 2.8 to 800 mg/kg bw over a period of up to 350 days led to significant gastrointestinal and hepatic toxic effects (IARC, 1974).

4.2.2 *Experimental systems*

Methyl methanesulfonate (250 μM) induced neurite formation in 71% of mouse neuroblastoma N-18 cells, when cell growth was inhibited by 83% (Yoda *et al*., 1982).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Groups of adult female (C3H/R1 × 101/R1)F_1 mice received a single intraperitoneal injection of 75 mg/kg bw methyl methanesulfonate within four days before mating or at 1, 6, 9 or 25 h after mating with untreated males. Control groups were treated with vehicle only (1 mL water) four days before mating or 6 or 25 h after mating. Control and treated females were killed and their uterine contents examined 17–18 days after mating. Resorptions were significantly increased ($p < 0.01$) following treatment at 1, 6, 9 or 25 h after mating (21.2%, 25.2%, 28.2% and 22.7%, respectively) in comparison with before mating and 6 h and 25 h after mating control group frequencies of 4.8%, 3.6% and 4.6%, respectively. Treatment before mating had no effect. Mid-gestational deaths were unaffected at any time, while late deaths were significantly increased only at 1 h (3.4%) in comparison with the 6 h control frequency of 0.7%. The incidences of live fetuses with malformations were (numbers of fetuses examined in parentheses): before-mating

control, 1.0% (298); treated, 0.0% (292); 1 h treated, 4.4% (411); 6 h after mating control, 0.8% (392); 6 h treated, 3.8% (448); 9 h treated, 3.6% (249); 25 h after mating control, 0.6% (350); 25 h treated, 2.6% (546). By comparison with other alkylating agents with similar DNA-binding properties but different effects upon exposed zygotes, there appeared to be no site-specific alkylation product identifiable as the critical target (Generoso *et al*., 1991).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Methyl methanesulfonate is a direct-acting alkylating agent that was active in all of the standard short-term tests for genetic and related effects *in vivo* and *in vitro*. It induced SOS response in the *umu* test using *Salmonella typhimurium* strain TA1535/pSK1002 and it induced mutations in strain TA100. In *Drosophila melanogaster*, somatic and sex-linked recessive lethal mutations were induced following exposure of adults or larvae to methyl methanesulfonate in their feed.

DNA damage was induced in rabbit alveolar macrophages *in vitro* and Clara cell cultures incubated with methyl methanesulfonate. DNA single-strand breaks and unscheduled DNA synthesis were induced in rat primary hepatocytes. Unscheduled DNA synthesis was also induced in rat tracheal epithelium, in Syrian hamster and mouse primary hepatocytes and in mouse epidermal keratinocytes *in vitro*. Methyl methanesulfonate induced gene mutations at the *hprt* locus in Chinese hamster ovary cells and lung V79 fibroblasts. One study reported gene mutation in V79 cells transfected with a retroviral vector carrying the *tag* gene of *Escherichia coli***.** This gene encodes for 3-methyladenine DNA glycosylase I, which excises 3-alkyl-adenine. The results showed that the majority of the mutations induced by methyl methanesulfonate were GC→AT transitions. Gene mutations were induced at the *tk* locus in mouse lymphoma L5178Y cells, and ouabain-resistant mutants were induced in mouse C3H 10T½ and L5178Y cells *in vitro*. Methyl methanesulfonate increased the frequency of sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary and mouse lymphoma cells. It also induced micronuclei in mouse lymphoma cells in a single study. It induced morphological cell transformation in virally enhanced Syrian hamster ovary cell cultures but not in the same cell line without viral enhancement.

Methyl methanesulfonate induced DNA single-strand breaks and alkali-labile sites in human lymphocytes *in vitro*. It induced unscheduled DNA synthesis in human epidermal keratinocytes and in oral epithelial and fibroblast cell cultures. Methyl methanesulfonate induced gene mutations in human lymphoblasts at the *hprt* locus and sister chromatid exchanges and micronuclei in HepG2 human liver cells *in vitro*.

Methyl methanesulfonate induced DNA strand breaks in mouse kidney and spermatozoa and DNA fragmentation in rat brain cells following in-vivo treatment.

Table 1. Genetic and related effects of methyl methanesulfonate

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, Prophage, *umu* induction/SOS response/strand-breaks or cross-links	+	NT	27	Nakamura *et al*. (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	280	McCann *et al*. (1975)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	500	Bruce & Heddle (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	5	De Flora (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	15	Eder *et al*. (1989)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	100	Koch *et al*. (1994)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	NT	280	McCann *et al*. (1975)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	5	De Flora (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	330	Eder *et al*. (1989)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	150	De Flora (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	NT	280	McCann *et al*. (1975)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	150	De Flora (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	NT	280	McCann *et al*. (1975)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	150	De Flora (1981)
DMM, *Drosophila melanogaster*, somatic mutation	+		10 ppm feed	Mitchell *et al*. (1981)
DMM, *Drosophila melanogaster*, somatic mutation	+		275 µg/mL sol	Vogel & Zijlstra (1987)
DMM, *Drosophila melanogaster*, somatic mutation	+		275 µg/mL sol	Vogel (1989)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	?[c]		550ppm feed	Vogel & Zijlstra (1987)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		22 µg/mL sol	Vogel & Nivard (1997)
DIA, DNA single-strand breaks, rat hepatocytes *in vitro*	+	NT	33	Sina *et al*. (1983)
DIA, DNA damage, rabbit (macrophage, Clara and type II) lung cells *in vitro*	+	NT	5	Becher *et al*. (1993)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	NT	11	Kornbrust & Barfknecht (1984)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
UIA, Unscheduled DNA synthesis, male B6C3F$_1$ mouse hepatocytes *in vitro*	+	NT	80	McQueen *et al*. (1983)
UIA, Unscheduled DNA synthesis, male Syrian hamster hepatocytes *in vitro*	+	NT	80	McQueen *et al*. (1983)
UIA, Unscheduled DNA synthesis, Syrian hamster hepatocytes *in vitro*	+	NT	11	Kornbrust & Barfknecht (1984)
UIA, Unscheduled DNA synthesis, rat tracheal epithelium cells *in vitro*	+	NT	11	Doolittle & Butterworth (1984)
UIA, Unscheduled DNA synthesis, mouse epidermal keratinocytes *in vitro*	+	NT	0.1	Sawyer *et al*. (1988)
GCO, Gene mutation, Chinese hamster ovary cells, *hprt* locus *in vitro*	+	NT	5	Couch *et al*. (1978)
GCO, Gene mutation, Chinese hamster ovary cells, *hprt* locus *in vitro*	+	NT	11	Lee *et al*. (1986)
GCO, Gene mutation, Chinese hamster ovary cells, *hprt* locus *in vitro*	+	NT	6	Moore *et al*. (1989)
GCO, Gene mutation, Chinese hamster ovary cells, *hprt* locus *in vitro*	+	NT	5	Oberly *et al*. (1990)
GCO, Gene mutation, Chinese hamster ovary cells, *hprt* locus *in vitro*	+	NT	5	Moore *et al*. (1991)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	(+)	NT	50	Nishi *et al*. (1984)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	+	NT	175	Slamenova *et al*. (1990)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	+	7.5	Clive *et al*. (1979)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	5	Oberly *et al*. (1984)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	4	Moore *et al*. (1989)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	13.2	Cole *et al*. (1990)
G51, Gene mutation, mouse lymphoma L5178Y cells, *hprt* locus *in vitro*	NT	+	7.5	Clive *et al*. (1979)
G51, Gene mutation, mouse lymphoma L5178Y cells, ouabain resistance *in vitro*	+	NT	13.2	Cole *et al*. (1990)
GIA, Gene mutation, mouse lymphoma LC98.16 cells, *tk* locus *in vitro*	+	NT	7.5	Blazak *et al*. (1986)
GIA, Gene mutation, C3H10T½ mouse cells, ouabain resistance *in vitro*	(+)	NT	120	Smith *et al*. (1988)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
GIA, Gene mutation, Chinese hamster AS52/hprt cells, *hprt* locus *in vitro*	+	NT	20	Oberly *et al*. (1993)
GIA, Gene mutation, Chinese hamster fibroblasts, *hprt* locus *in vitro*	+[d]	NT	180	Klungland *et al*. (1995)
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	NT	7.4	Natarajan *et al*. (1983)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	+	NT	5	Nishi *et al*. (1984)
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	NT	5.5	Lee *et al*. (1986)
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	NT	17.6	Darroudi *et al*. (1989)
SIM, Sister chromatid exchange, mouse fetal liver erythroblasts *in vitro*	+	NT	5	Cole *et al*. (1983)
SIT, Sister chromatid exchange, transformed cells (CHO 43-3B) *in vitro*	+	NT	17.6	Darroudi *et al*. (1989)
MIA, Micronucleus test, mouse lymphoma L5178Y cells *in vitro*	+	NT	13.2	Cole *et al*. (1990)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	+	NT	22	Natarajan *et al*. (1983)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	+	NT	11	Lee *et al*. (1986)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	+	NT	44	Darroudi *et al*. (1989)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	+	NT	33	Lin *et al*. (1989)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	+	NT	6	Moore *et al*. (1989)
CIM, Chromosomal aberrations, mouse lymphoma L5178Y cells *in vitro*	+	NT	7.5	Blazak *et al*. (1986)
CIM, Chromosomal aberrations, mouse lymphoma L5178Y cells *in vitro*	+	NT	4	Moore *et al*. (1989)
CIT, Chromosomal aberrations, transformed cells (CHO 43-3B) *in vitro*	+	NT	17.6	Darroudi *et al*. (1989)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay	–	NT	330	Dusinska & Slamenova (1994)
T7S, Cell transformation, SA7/Syrian hamster embryo cells *in vitro*	+	NT	50	Casto *et al*. (1979)
DIH, DNA single-strand breaks/alkaline-labile sites, human lymphocytes *in vitro*	+	NT	5.5	Munzer *et al*. (1988)
UIH, Unscheduled DNA synthesis, human oral epithelium and fibroblasts *in vitro*	+	NT	11	Ide *et al*. (1982)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
UIH, Unscheduled DNA synthesis, human epidermal keratinocytes *in vitro*	+	NT	1.1	Lawrence & Benford (1993)
GIH, Gene mutation, GM6804 human lymphoblasts, *hprt* locus *in vitro*	+	NT	5	Aubrecht *et al*. (1995)
SIH, Sister chromatid exchange, human HepG2 liver cells *in vitro*	+	NT	44	Natarajan & Darroudi (1991)
MIH, Micronucleus test, human HepG2 liver cells *in vitro*	+	NT	88	Natarajan & Darroudi (1991)
HMM, Host-mediated assay, *Escherichia coli* K-12 in NMRI mice *in vivo*	+		83 po × 1	Hellmer & Bolcsfoldi (1992)
DVA, DNA single-strand breaks, NMRI mouse kidney *in vivo*	+		33 ip × 1	Solveig Walles & Erixon (1984)
DVA, DNA strand breaks, mouse spermatozoa *in vivo*	+		10 ip × 1	Sega *et al*. (1986)
DVA, DNA fragmentation, Sprague-Dawley rat brain *in vivo*	+		27.5 iv × 1	Robbiano & Brambilla (1987)
UVM, Unscheduled DNA synthesis, ICR mouse skin epithelial cells *in vivo*	+		6 sc × 1	Ishikawa *et al*. (1982)
UVR, Unscheduled DNA synthesis, Fischer 344 rat kidney cells *in vivo*	+[e]		100 ip × 1	Tyson & Mirsalis (1985)
UVR, Unscheduled DNA synthesis, Fischer 344 rat spermatocytes *in vivo*	+		10 ip × 1	Bentley & Working (1988)
GVA, Gene mutation, Fischer 344 rat fibroblasts, *hprt* locus *in vivo*	–		100 ip × 1	Khan & Heddle (1991)
GVA, Gene mutation (*lac*I), male Big Blue™ mouse liver cells, *in vivo*	–		20 ip × 21	Mirsalis *et al*. (1993)
GVA, Gene mutation (*lac*I or *Dbl*-1), male transgenic C57BL/6 mouse intestinal epithelium *in vivo*	+[f]		100 ip/wk × 10	Tao *et al*. (1993)
GVA, Gene mutation (*lacz*), Muta™ Mouse germ cells *in vivo*	–		40 ip × 1	Brooks & Dean (1997)
GVA, Gene mutation (*lac*I), Big Blue™ mouse germ cells *in vivo*	–		40 ip × 1	Gorelick *et al*. (1997)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
GVA, Gene mutation (*lacz*), Muta™ Mouse germ cells *in vivo*	–		80 ip × 1	Itoh *et al*. (1997)
GVA, Gene mutation (*lacz*), male Muta™ Mouse, germ and bone-marrow cells *in vivo*	–		40 ip × 1	Renault *et al*. (1997)
GVA, Gene mutation (*lacz*), male Muta™ Mouse germ cells *in vivo*	–		40 ip × 1	Suzuki *et al*. (1997)
GVA, Gene mutation (*lacz*), male Muta™ Mouse germ cells *in vivo*	–		100 ip × 1	Tinwell *et al*. (1997)
SLP, Mouse specific locus, postspermatogonia *in vivo*	(+)		7 ip × 1	Ehling (1978)
SLO, Mouse specific locus, other stages *in vivo*	–		5.25 ip × 1	Ehling (1978)
SVA, Sister chromatid exchange, fetal Porton albino mouse liver erythroblasts *in vivo*	+		30 ip × 1	Cole *et al*. (1983)
MVM, Micronucleus test, mouse erythroblasts *in vivo*	+		30 ip × 1	Jenssen & Ramel (1976)
MVM, Micronucleus test, (C57BL/6 × C3H/He)F_1 mice *in vivo*	+		500 ip × 5	Bruce & Heddle (1979)
MVM, Micronucleus test, fetal mouse liver erythroblasts *in vivo*	+		30 ip × 1	Cole *et al*. (1982)
MVM, Micronucleus test, fetal Porton albino mouse liver erythroblasts *in vivo*	+		30 ip × 1	Cole *et al*. (1983)
MVM, Micronucleus test, MS/Ae mouse erythrocytes *in vivo*	+		25 ip × 2	Aeschbacher (1986)
MVM, Micronucleus test, ddY mouse erythrocytes *in vivo*	+		46 inh 21 min × 1	Odagiri *et al*. (1986)
MVM, Micronucleus test, MS/Ae and CD-1 mouse erythrocytes *in vivo*	+		40 ip × 1	Tsuyoshi *et al*. (1989)
MVM, Micronucleus test, MS/Ae and CD-1 mouse erythrocytes *in vivo*	+		40 po × 1	Tsuyoshi *et al*. (1989)
MVM, Micronucleus test, female C57BL/6, DBA2 and BALB/c mouse erythrocytes *in vivo*	+		25 ip × 1	Sato *et al*. (1990)
MVM, Micronucleus test, NMRI mice (during skin carcinogenesis) *in vivo*	+		450 skin × 1	Haesen *et al*. (1993)
MVM, Micronucleus test, Big Blue™ mouse peripheral blood *in vivo*	+		40 ip × 1	Gorelick *et al*. (1997)
MVM, Micronucleus test, Muta™ Mouse reticulocytes *in vivo*	+		40 ip × 1	Suzuki *et al*. (1997)
MVR, Micronucleus test, Wistar rat hepatocytes *in vivo*	–		80 ip × 1	Tates *et al*. (1986)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MVR, Micronucleus test, Wistar rat hepatocytes *in vivo*	+[g]		10 ip × 1	Tates & den Engelse (1989)
MVR, Micronucleus test, Fischer rat lung cells *in vivo*	+		50 ip × 1	Khan & Heddle (1991)
CBA, Chromosomal aberrations, female C57BL mouse bone marrow *in vivo*	+		120 ip × 1	Frei & Venitt (1975)
CVA, Chromosomal aberrations, female NMRI mouse epidermal cells *in vivo*	+		440 skin × 1	Furstenberger *et al*. (1989)
CCC, Chromosomal aberrations, mouse spermatocytes *in vivo*	+		30 ip × 1	Moutschen (1969)
CGC, Chromosomal aberrations, mouse spermatogonia treated *in vivo*, spermatocytes observed	–		50 ip × 1	Leonard & Linden (1972)
COE, Chromosomal aberrations, CD1/CR mouse oocytes or embryos *in vivo*	+		50 iv × 1	Brewen *et al*. (1975)
COE, Chromosomal aberrations, NMRI mouse oocytes or embryos *in vivo*	+		25 ip × 1	Braun *et al*. (1986)
DLM, Dominant lethal test, male mice	+		50 ip × 1	Partington & Bateman (1964)
DLM, Dominant lethal test, male mice	+		50 ip × 1	Ehling *et al*. (1968)
DLM, Dominant lethal test, mice	+		30 ip × 1	Moutschen (1969)
DLM, Dominant lethal test, mice	+		50 ip × 1	Beliles *et al*. (1973)
DLM, Dominant lethal test, male albino mice	+		12.5 ip × 1	Arnold *et al*. (1976)
DLM, Dominant lethal test, male CD1 mice	+		20 ip × 1	Dean & Johnstone (1977)
DLM, Dominant lethal test, male (101 × C3H)F_1 mice	+		10 ip × 1	Ehling (1977)
DLM, Dominant lethal test, male NMRI mice	+		40 ip × 1	Lang & Adler (1977)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MHT, Mouse heritable translocation test	+		40 ip × 1	Lang & Adler (1977)
MHT, Mouse heritable translocation test	+		20 ip × 1	Adler (1980)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day; sol, solution; po, oral; ip, intraperitoneal; sc, subcutaneous; iv, intravenous; wk, week; inh, inhalation

[c] Significant increase only with treatments of female larvae at the highest dose

[d] Mutation frequency lower in cells transfected with *E. coli tag*-expressing vector (*tag* gene encodes 3-methyladenine DNA glycosylase I activity)

[e] Negative for gavage treatment

[f] *Dbl-1* more sensitive than *lac*I; negative for both loci following acute exposure

[g] Rats received partial hepatectomy 17 h before treatment; bone marrow analysed 24 h after treatment. A weak positive response was also seen in hepatocytes two to three days after treatment.

Unscheduled DNA synthesis was induced in mouse skin epithelium after a single subcutaneous injection of methyl methanesulfonate and in rat kidney cells and spermatocytes after a single intraperitoneal injection of this compound.

Methyl methanesulfonate did not induce mutations at the *hprt* locus in Fischer 344 rat fibroblasts *in vivo*. In a single study, it induced *lac*I and *Dbl*-1 mutations in intestinal epithelium of transgenic mice given 10 weekly injections but did not induce *lac*Z or *lac*I mutations in germ cells of transgenic mice from acute exposure studies. It increased the frequencies of micronuclei in mouse peripheral blood, skin keratinocytes and fetal liver erythrocytes and in rat hepatocytes and lung fibroblasts *in vivo*. It also induced sister chromatid exchanges in fetal mouse liver and chromosomal aberrations in mouse bone marrow and skin epidermal cells after a single intraperitoneal injection. Methyl methanesulfonate was not mutagenic to mouse germ cells: it induced specific locus mutations in only postspermatogonial stages, heritable translocations and chromosomal aberrations in spermatocytes and embryonic cells, and mouse dominant lethal mutations.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Methyl methanesulfonate is a laboratory chemical that has been produced for research purposes. No information was available to the Working Group on potential human exposures.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Methyl methanesulfonate was tested in rats by inhalation exposure and by subcutaneous and intraperitoneal administration, producing nasal tumours, tumours of the nervous system and tumours at the injection site. In rats, it was carcinogenic after administration of a single dose as well as following prenatal exposure. Following instillation into the bladder of rats, it potentiated the effect of *N*-methyl-*N*-nitrosourea. In one study, following oral administration in mice, it increased the incidence of lung tumours and of lymphomas. A subsequent experiment with oral and intraperitoneal administration to mice failed to increase the incidence of lung adenomas in A/J mice. In a multistage mouse skin model, it was not an initiator but was found to be a stage I tumour promoter. It accelerated the occurrence of thymic lymphomas in AKR mice.

5.4 Other relevant data

Methyl methanesulfonate caused an increased frequency of resorptions and congenital malformations after treatment of females 1–25 h after mating.

Methyl methanesulfonate induced mouse germ cell mutations and chromosomal aberrations, and DNA damage, micronuclei, sister chromatid exchanges and chromosomal aberrations in somatic cells of rodents *in vivo*. It increased the frequency of DNA damage, gene mutation, sister chromatid exchanges and micronuclei in human and rodent cell cultures, as well as chromosomal aberrations in rodent cells *in vitro*. Methyl methanesulfonate induced somatic and sex-linked mutations in *Drosophila*. It induced DNA damage in *Escherichia coli* and was mutagenic in bacteria.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of methyl methanesulfonate were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of methyl methanesulfonate.

Overall evaluation

Methyl methanesulfonate is *probably carcinogenic to humans (Group 2A)*.

In making the overall evaluation, the Working Group took into consideration that methyl methanesulfonate is a direct-acting methylating agent which is mutagenic in a wide range of in-vivo and in-vitro test systems.

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