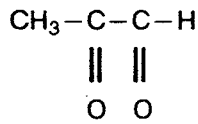
METHYLGLYOXAL

1. Chemical and Physical Data

1.1 Synonyms

Chem. Abstr. Services Reg. No.: 78-98-8
Chem. Abstr. Name: 2-Oxopropanal
Synonyms: Acetylformaldehyde; 2-ketopropionaldehyde; pyruvaldehyde

1.2 Structural and molecular formulae and molecular weight



$C_3H_4O_2$ Mol. wt: 72.06

1.3 Chemical and physical properties of the pure substance

From Windholz (1983)

(*a*) *Description*: Yellow liquid with pungent odour
(*b*) *Boiling-point*: 72°C
(*c*) *Density*: d^{24} 1.0455
(*d*) *Solubility*: Soluble in water, ethanol, diethyl ether and benzene
(*e*) *Reactivity*: Polymerizes very readily; hygroscopic

1.4 Technical products and impurities

No data were available to the Working Group.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(a) *Production*

Methylglyoxal is not produced commercially. It can be obtained by warming isonitrosoacetone with dilute sulfuric acid; by distilling a dilute solution of dihydroxyacetone from calcium carbonate (Windholz, 1983); by the catalytic dehydrogenation of glycerol (Baltes & Leupold, 1981); and by the oxidation of acetone with selenium dioxide (Musashino Chemical Research Institute Ltd, 1981).

(b) *Use*

No commercial use of methylglyoxal has been reported.

2.2 Occurrence

(a) *Natural occurrence*

Methylglyoxal has been identified as a metabolite during glycolysis (Kasai *et al.*, 1982) and as a sugar fragmentation product. It is one of the most highly reactive compounds in a browning reaction (Hodge, 1953). It is also formed by several bacteria of the human intestine (Baskaran *et al.*, 1989).

(b) *Occupational exposure*

No data on exposure levels were available to the Working Group.

(c) *Air*

Methylglyoxal has been reported to be a degradation product of toluene under simulated atmospheric conditions (Dumdei & O'Brien, 1984). It has been found in cigarette smoke at levels ranging from 5 to 60 μg per cigarette (Moree-Testa & Saint-Jalm, 1981).

(d) *Water and sediments*

Methylglyoxal has not been detected in US industrial effluents (Perry *et al.*, 1979) or in drinking-water (National Research Council, 1977).

(e) *Food and beverages*

Methylglyoxal has been detected in a broad range of commercial food products and beverages, including bread (Wiseblatt & Kohn, 1960; Nagao *et al.*, 1986a), toast

(Nagao *et al.*, 1986a), tomatoes (Schormüller & Grosch, 1964), boiled potatoes (Kajita & Senda, 1972), caramelized sucrose (Lukesch, 1956), soya sauce and soya bean paste (Hayashi & Shibamato, 1985; Nagao *et al.*, 1986a), roast turkey (Hrdlicka & Kuca, 1965), alcohol from sugar cane (Matsubara & Tamura, 1970), wine, saké, apple brandy and bourbon whiskey (Nagao *et al.*, 1986a), apple, orange and tomato juices, maple syrup, beer, root-beer and cola, non-fat dry milk (Hayashi & Shibamato, 1985), instant, brewed and decaffeinated coffees (Kasai *et al.*, 1982; Hayashi & Shibamato, 1985; Nagao *et al.*, 1986a; Shane *et al.*, 1988), and cocoa and instant tea (Hayashi & Shibamato, 1985). Table 1 summarizes the amounts of methylglyoxal determined in various foods and beverages (Nagao *et al.*, 1986a) and Table 2 gives the amounts in foods and the calculated intake.

Table 1. Amounts of methylglyoxal found in various beverages and foods[a]

Beverage or food	Methylglyoxal (μg/ml)
Bourbon whiskey	1.5
Apple brandy	0.32
Wine	0.57
Japanese saké	0.26
Instant coffee[b]	1.6
Brewed coffee[c]	7.0
Black tea[d]	0.05
Green tea[e]	Trace
Soft drink	1.4
Bread	0.79 μg/g
Toast	2.5 μg/g
Soya sauce	8.7
Soya bean paste	5.1 μg/g

[a] From Nagao *et al.* (1986a)
[b] Prepared by dissolving 1.5 g coffee powder in 100 ml water
[c] Prepared from 10 g ground coffee beans and 150 ml boiling water
[d] Prepared from 4 g tea leaves and 100 ml boiling water
[e] Prepared from 5 g tea leaves and 20 ml hot water

Table 2. Methylglyoxal in foods and calculated amounts of methylglyoxal intake for each food when consumed[a]

Beverage or food	Amount of item per serving	Methylglyoxal (μg/g)	Methylglyoxal intake per serving (μg)
Brewed coffee	3 g/180 ml	25	75.6
Decaffeinated brewed coffee	3 g/180 ml	47	140.4
Instant coffee	1 g/180 ml	23	22.7
Cocoa	4 g/180 ml	1.2	4.9
Instant tea	0.3 g/180 ml	2.4	0.7
Nonfat dry milk	22.7 g/240 ml	1.4	31.2
Soya sauce A	Not calculated	3-7.6	-
Soya bean paste (Miso)	Not calculated	0.7	-
Cola	354 ml/can	0.23	81.4
Root beer	354 ml/can	0.76	269.0
Beer	355 ml/can	0.08	29.7
Wine (white)	100 ml/glass	0.11	11.0
Apple juice	300 ml/glass	0.26	78.0
Orange juice	354 ml/can	0.04	14.2
Tomato juice	177 ml/can	0.06	11.3
Maple syrup	Not calculated	2.5	-

[a]From Hayashi & Shibamato (1985)

Among the various beverages, coffee contains the largest amount of methylglyoxal (Hayashi & Shibamato, 1985; Nagao *et al.*, 1986a), with a daily intake resulting from the consumption of two to three cups of coffee per day calculated as 1 mg. The content of methylglyoxal in soya sauce (8.7 μg/ml) was comparable to that of brewed coffee (7.0 μg/ml), but the average daily per-caput intake of soya sauce in Japan is 30 ml (Nagao *et al.*, 1986a). In an examination of nine brands of coffee, the concentration of methylglyoxal was highest in roasted instant coffees compared to filtered and to decaffeinated instant and filtered coffees. The mean concentration of methylglyoxal in filtered coffees was 319 μg/g, whereas that in instant coffees was 731 μg/g (Shane *et al.*, 1988). These results are at variance with those of earlier studies in which one cup of instant coffee (1 g/100 ml) contained 100-150 μg methylglyoxal, whereas one cup of coffee prepared from ground coffee beans (8 g/100 ml) contained 470-730 μg methylglyoxal (Kasai *et al.*, 1982). Aeschbacher *et al.* (1989) also determined the amounts of methylglyoxal in brewed coffee and instant coffees (Table 3).

Table 3. Contents of methylglyoxal in coffee

Reference	Methylglyoxal	
	In roasted coffee	In brewed coffee
Kasai *et al.* (1982)	[58-75 μg/g]	470-730 μg/cup[a]
Hayashi & Shibamato (1985)	25 μg/g	76 μg/cup[b]
Nagao *et al.* (1986a)	NA	7 μg/ml[c]
Shane *et al.* (1988)	NA	273-341 μg/g (filtered)[d]
Aeschbacher *et al.* (1989)	[21-39 μg/g][e]	106-197 μg/g[f]

[a] In a brew using 8 g roasted coffee per 100 ml water
[b] In a brew using 3 g roasted coffee per 180 ml water
[c] In a brew using 10 g roasted coffee per 150 ml water
[d] In a brew containing 25 g roasted coffee per 250 ml water
[e] Calculated assuming extraction yield of 20% of dry soluble solids in the brew
[f] μg/g dried product (brew, 1 g roasted coffee per 10 ml water)
NA, not available

Methylgyoxal has been determined in bread at 0.5 ppm (mg/kg) (Borovikova & Reuter, 1971) and in beer at 0.03-11 ppm (mg/l) (Palamand *et al.*, 1970; Wheeler *et al.*, 1971).

2.3 Analysis

Trace quantities of methylglyoxal have been determined by derivatization with cysteamine to yield 2-acetylthiazolidine in a food or beverage sample at pH 6, then extraction with dichloromethane and analysis by gas chromatography (Hayashi & Shibamato, 1985). Methylglyoxal has been determined in coffee (Kasai *et al.*, 1982; Shane *et al.*, 1988) and in cigarette smoke (Moree-Testa & Saint-Jalm, 1981) as the 2-methylquinoxaline derivative by gas chromatography (Kasai *et al.*, 1982), gas chromatography-mass spectrometry (Shane *et al.*, 1988) or high-performance liquid chromatography (Moree-Testa & Saint-Jalm, 1981) following its initial reaction with *ortho*-phenylenediamine. Methylglyoxal was determined in biological tissues with the fluorescent 2-(2-benzimidiazolyl)-3-methylquinoxaline following separation by high-performance liquid chromatography; the detection limit was 48.4 pmol per 30-μl sample (Matsuura *et al.*, 1985).

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(a) *Oral administration*

Rat: In a study reported as an abstract, 40 male Fischer 344 rats were administered 0.5% methylglyoxal in deionized water as drinking-water for life (854 days; average daily intake, 7.7 mg per rat); 40 controls received deionized water alone. The average body weight of the treated rats was 15% lower than that of the controls. No tumour was found that could be ascribed to administration of methylglyoxal (Fujita *et al.*, 1986).

(b) *Subcutaneous administration*

Rat: Groups of ten male and ten female Fischer 344 rats, eight weeks of age, received subcutaneous injections of 0 or 1.3 mg methylglyoxal solution neutralized with sodium hydroxide (purity, 65.6%; the impurity 'might have been' pyruvic acid) in 0.2 ml saline twice a week for ten weeks. A group of 20 controls received saline solution for ten weeks. After 70 weeks, subcutaneous tumours [type unspecified] were found in two treated animals, but none were seen in controls (Takayama *et al.*, 1984). [The Working Group noted the impurity of the test solution and the limited reporting of the experiment.]

Groups of eight male and ten female Fischer 344 rats [age unspecified] received subcutaneous injections of 2 mg methylglyoxal (unpurified) in 0.2 ml saline twice a week for ten weeks. A control group of 21 males and 19 females received saline only. After 20 months, four of the treated rats (three males and one female) developed malignant tumours (fibrosarcomas) at the injection site, whereas no tumour was seen in controls (Nagao *et al.*, 1986a,b). [The Working Group noted the impurity of the test solution].

(c) *Modifying effects on the activity of known carcinogens*

N-*Methyl*-N′-*nitro*-N-*nitrosoguanidine*: Groups of 30 male Wistar *rats*, seven weeks of age, were administered 100 mg/l *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine in the drinking-water and were simultaneously fed a diet supplemented with 10% sodium chloride for eight weeks; they were then returned to basal diet and maintained on drinking-water containing no additive (controls) or 0.25%

methylglyoxal [purity unspecified] for 32 weeks. Animals were killed at week 40. Methylglyoxal caused a significant increase in the incidence of hyperplasia induced by the nitrosamine but did not enhance the incidence of gastric adenocarcinomas (Takahashi *et al.*, 1989).

3.2 Other relevant data

(*a*) *Experimental systems*

(i) *Absorption, distribution, excretion and metabolism*

No experiment on the metabolism or tissue distribution of methylglyoxal after oral ingestion in animals or man has been reported (Arnaud, 1988). The biosynthesis and degradation of methylglyoxal in animals has been reviewed (Ohmori *et al.*, 1989). There is still uncertainty about the biochemistry of methylglyoxal in animals, owing to the difficulty of determining it in biological tissues, which is due to the active glyoxalase system (Brandt & Siegel, 1979).

Facultative, strictly anaerobic bacteria present in the human gut were shown to produce (and may be one of the most important sources of) methylglyoxal. Several groups of bacteria from human faeces produced methylglyoxal *in vitro* (Baskaran *et al.*, 1989).

Methylglyoxal can be formed from acetoacetate and carbohydrates in glycolysing tissues and from triose phosphates by nonenzymatic processes (Ohmori *et al.*, 1989). An enzyme fraction that specifically catalyses the formation of methylglyoxal from dihydroxyacetone phosphate has been isolated from goat liver (Ray & Ray, 1981). Amine oxidase from goat plasma was shown to catalyse the oxidation of aminoacetone to methylglyoxal (Ray & Ray, 1983). Methylglyoxal has been measured at levels of micrograms per gram in the liver and skeletal muscle of normal and diabetic rats (Ohmori *et al.*, 1989). Methylglyoxal was shown to be present in liver noncovalently bound to protein (Fodor *et al.*, 1978). The biosynthetic routes of methylglyoxal are shown in Figure 1 (Ohmori *et al.*, 1989).

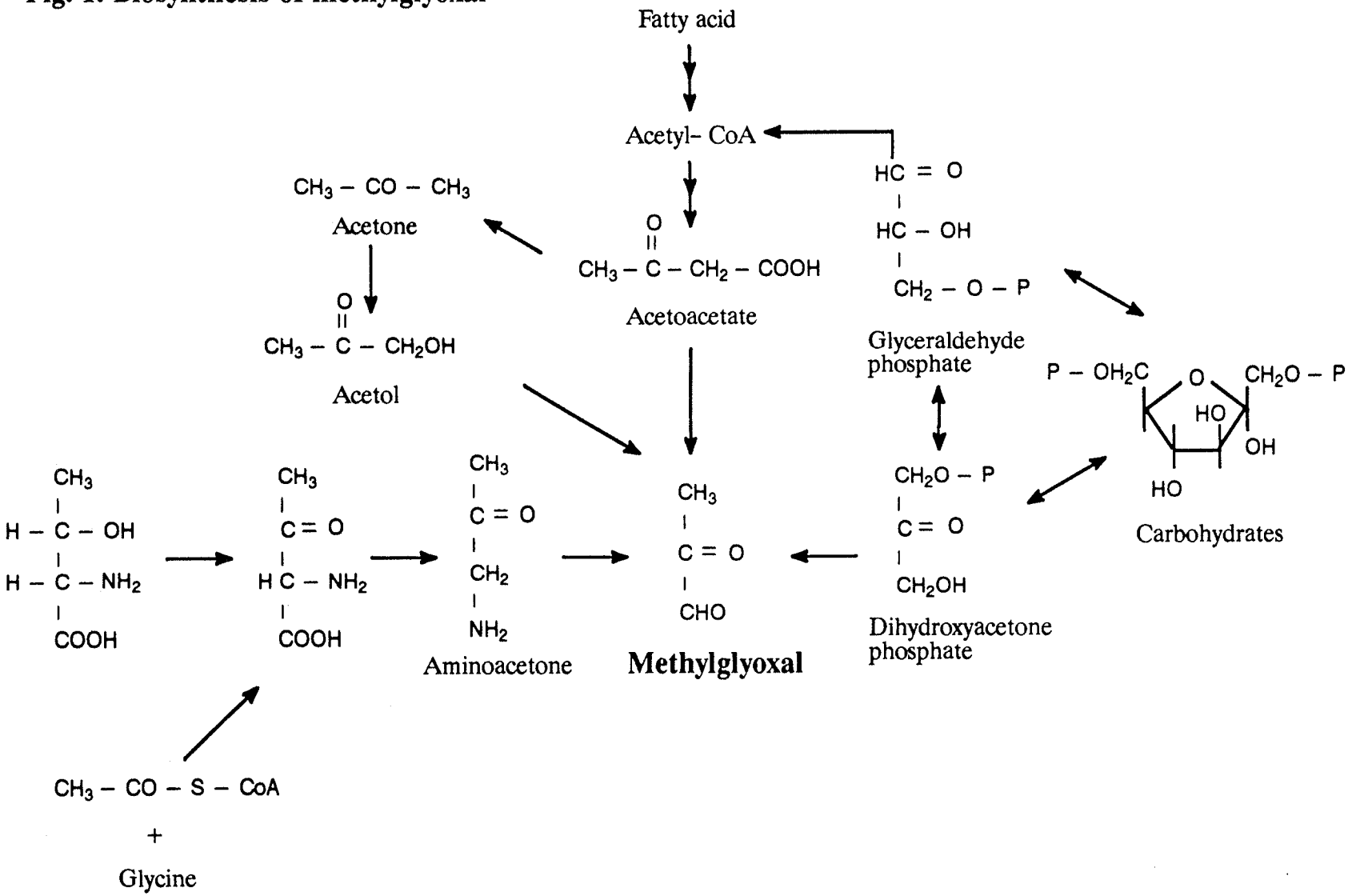
Methylglyoxal can be detoxified by the glyoxalase system present in mammalian intestinal mucosa (Baskaran & Balasubramanian, 1987), and it is converted into D-lactic acid (Neuberg, 1913). Hepatocytes convert methylglyoxal to pyruvate (Ray & Ray, 1982) and to glucose and L-lactate (Sáez *et al.*, 1985).

(ii) *Toxic effects*

The average acute oral LD_{50}s of methylglyoxal in rats were 531 mg/kg bw in newborn, between 1165 and 1623 mg/kg bw in females depending on age and/or stage of pregnancy and 1990 mg/kg bw in adult males (Peters *et al.*, 1978).

The effects of pre- (initiation) and post-treatment (promotion) with methylglyoxal (0.05 or 0.2% in drinking-water) on the induction of γ-glutamyl-

Fig. 1. Biosynthesis of methylglyoxal



transpeptidase-positive foci in the livers were studied in Fischer 344 rats (weighing 150-200 g; five to six animals per group). Foci were induced in dose-related amounts both in the absence and presence of initiation with 0.02% 2-acetylaminofluorene in the diet (Martelli *et al.*, 1988). [The Working Group noted the limited number of animals used.]

Intraperitoneal treatment of mice with methylglyoxal at 600 mg/kg bw enhanced aminopyrine *N*-demethylase and *para*-nitroanisole-*O*-demethylase activities, while ethoxycoumarin -*O*-deethylase activity and total cytochrome P450 content was only weakly increased (Bronzetti *et al.*, 1987). Administration of 300-600 mg/kg bw by gastric intubation to male Fisher 344 rats induced a 100-fold increase in ornithine decarboxylase activity within 7 h, a 26-fold increase in DNA synthesis within 16 h and a 16-fold increase in the labelling index of S-phase cells within 16 h in the glandular stomach mucosa (Furihata *et al.*, 1985).

(iii) *Effects on reproduction and prenatal toxicity*

While no data on reproductive or developmental toxicity were available to the Working Group, available information on acute toxicity suggests that neonates are more sensitive to methylglyoxal than adult male rats (Peters *et al.*, 1978).

(iv) *Genetic and related effects*

The results described below are listed in Table 4 on p. 453, with the evaluation of the Working Group, as positive, negative or inconclusive, as defined in the footnotes. The results are tabulated separately for the presence and absence of an exogenous metabolic system. The lowest effective dose (LED), in the case of positive results, or the highest ineffective dose (HID), in the case of negative results, are shown, together with the appropriate reference. The studies are summarized briefly below.

Methylglyoxal induced mutations in *Salmonella typhimurium* strains containing the pKM101 plasmid and in *Escherichia coli* WP2 *uvrA* and WP2 *uvrA* (pKM101). Mutagenicity in *S. typhimurium* was partially dependent upon the pKM101 plasmid and *uvrB* deletion, as shown by comparing the responses between TA104 and TA2659 and between TA104 and TA2638, respectively (Marnett *et al.*, 1985). Methylglyoxal was also active in the forward mutation ara^R test in *S. typhimurium*. Mutagenicity in *S. typhimurium* reversion tests was suppressed by sulfite, glutathione, dithiothreitol (Nagao *et al.*, 1984, 1986b) and cysteine, but not by catalase (Fujita *et al.*, 1985). There is no evidence for the formation of a stable conjugate of methylglyoxal with cysteine, the most active of the thiol inhibitors of mutagenic activity (Nagao *et al.*, 1986b).

In *Saccharomyces cerevisiae* (strain D7), methylglyoxal induced gene conversions and reverse mutations. An exogenous metabolic system reduced the effects (Bronzetti *et al.*, 1987).

In cultured mammalian cells, methylglyoxal induced mutations, sister chromatid exchange and (on the basis of alkaline elution and sensitivity to proteinase K) reparable DNA-protein cross-links (Brambilla *et al.*, 1985).

In cultured human lymphocytes, methylglyoxal induced sister chromatid exchange, chromosomal aberrations and micronuclei.

Unscheduled DNA synthesis appeared to be induced in the pyloric mucosa of rats, but only a small proportion of the tritiated thymidine incorporation was not inhibited by hydroxyurea during simultaneous S-phase stimulation by methylglyoxal, making interpretation difficult (Furihata *et al.*, 1985).

A single oral administration of methylgloxal induced neither sister chromatid exchange nor chromosomal aberrations in the ileum of mice.

Combined with hydrogen peroxide in the quantities typically found in a solution of 15 mg instant coffee, methylglyoxal was significantly mutagenic, whereas the individual components (5 μg hydrogen peroxide, 1.5 μg methylglyoxal) had only minor effects (Nagao *et al.*, 1986a). Synergism with hydrogen peroxide was also demonstrated in the ara^R test (Ariza *et al.*, 1988), which the authors suggested was due to inefficient detoxification of methylglyoxal in cells depleted of reduced glutathione (Meister & Anderson, 1983; Alonso-Moraga *et al.*, 1987) by hydrogen peroxide (Smith *et al.*, 1984). This explanation is in agreement with the fact that the addition of glyoxalase I and II together with reduced glutathione abolished the mutagenic activity of methylglyoxal and reduced the mutagenicity of instant coffee (20 mg/plate) by approximately 80% in the Ames test (Friederich *et al.*, 1985). Antimutagenic activity of methylglyoxal was seen against heterocyclic amines, such as Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2 and IQ, in *S. typhimurium* TA98 (Kim *et al.*, 1987).

In *E. coli*, methylglyoxal inhibited protein synthesis and interfered with cell population growth (Fraval & McBrien, 1980). Interaction of methylglyoxal with guanosine triphosphate (Krymkiewicz *et al.*, 1971) and with DNA and RNA has been reported (Krymkiewicz, 1973). An N^2-alkylguanine has been identified from the reaction of methylglyoxal with guanine; glyoxal undergoes a similar reaction (Shapiro *et al.*, 1969).

(*b*) *Humans*

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity to humans

No data were available to the Working Group.

Table 4. Genetic and related effects of methylglyoxal

Test system	Results		Dose LED/HID	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SAF, Salmonella typhimurium BA13, forward mutation (ara^R)	+	0	140.0000	Ariza et al. (1988)
SA0, Salmonella typhimurium TA100, reverse mutation	+	(+)	5.0000	Kasai et al. (1982)
SA0, Salmonella typhimurium TA100, reverse mutation	+	0	5.0000	Yamaguchi (1982)
SA0, Salmonella typhimurium TA100, reverse mutation	+	0	5.0000	Fujita et al. (1985)
SA0, Salmonella typhimurium TA100, reverse mutation	+	0	40.0000	Kim et al. (1987)
SA2, Salmonella typhimurium TA102, reverse mutation	+	0	0.0000	Nagao et al. (1986b)
SA2, Salmonella typhimurium TA102, reverse mutation	+	+	30.0000	Bronzetti et al. (1987)
SA2, Salmonella typhimurium TA102, reverse mutation	+	+	2.2500	Migliore et al. (1990)
SA4, Salmonella typhimurium TA104, reverse mutation	+	0	5.0000	Marnett et al. (1985)
SA4, Salmonella typhimurium TA104, reverse mutation	+	0	0.0000	Nagao et al. (1986b)
SA4, Salmonella typhimurium TA104, reverse mutation	+	+	1.2500	Migliore et al. (1990)
SA5, Salmonella typhimurium TA1535, reverse mutation	-	0	0.0000	Nagao et al. (1986a)
SA8, Salmonella typhimurium TA1538, reverse mutation	-	0	0.0000	Nagao et al. (1986a)
SA9, Salmonella typhimurium TA98, reverse mutation	(+)	0	0.0000	Nagao et al. (1986b)
SA5, Salmonella typhimurium TA2638, reverse mutation	+	0	25.0000	Marnett et al. (1985)
SA5, Salmonella typhimurium TA2659, reverse mutation	+	0	25.0000	Marnett et al. (1985)
SA5, Salmonella typhimurium TA97, reverse mutation	+	+	115.0000	Bronzetti et al. (1987)
ECW, Escherichia coli WP2 uvrA, reverse mutation	+	0	0.0000	Kasai et al. (1982)
ECR, Escherichia coli WP2 uvrA pKM101, reverse mutation	+	0	0.0000	Kasai et al. (1982)
SCG, Saccharomyces cerevisiae, mitotic gene conversion	+	0	1000.0000	Nagao et al. (1986b)
SCG, Saccharomyces cerevisiae, mitotic gene conversion	+	(+)	1100.0000	Bronzetti et al. (1987)
SCR, Saccharomyces cerevisiae, reverse mutation	+	-	1100.0000	Bronzetti et al. (1987)
GCL, Gene mutation, Chinese hamster lung (CHL) cells, DTr	+	0	30.0000	Nakasato et al. (1984)
G9H, Gene mutation, Chinese hamster V79 cells, 6-thioguanine resistance	+	+	36.0000	Cajelli et al. (1987)
SIC, Sister chromatid exchange, Chinese hamster ovary cells	+	0	7.0000	Faggin et al. (1985)
SHL, Sister chromatid exchange, human lymphocytes in vitro	+	+	110.0000	Migliore et al. (1990)
MIH, Micronucleus test, human lymphocytes in vitro	+	-	110.0000	Migliore et al. (1990)
CHL, Chromosomal aberrations, human lymphocytes in vitro	+	+	110.0000	Migliore et al. (1990)
SVA, Sister chromatid exchange, Swiss mouse ileum	-	0	600.0000 oral	Migliore et al. (1990)
CVA, Chromosomal aberrations, Swiss mouse ileum	-	0	600.0000 oral	Migliore et al. (1990)
DIA, DNA cross-links, Chinese hamster ovary cells	+	-	100.0000	Brambilla et al. (1985)

oral, by gavage

4. Summary of Data Reported and Evaluation

4.1 Exposure data

Methylglyoxal is present in many foods and drinks, including coffee, and is produced during glycolysis and sugar fermentation. It is produced by many strains of bacteria present in the intestinal tract. It is also present in tobacco smoke.

4.2 Experimental carcinogenicity data

No adequate study was available for the evaluation of methylglyoxal.

4.3 Human carcinogenicity data

No data were available to the Working Group.

4.4 Other relevant data

Methylglyoxal induced sister chromatid exchange, chromosomal aberrations and micronuclei in cultured human cells. It induced sister chromatid exchange and gene mutations in cultured mammalian cells. In yeast, it increased the frequencies of reverse mutations and of mitotic gene conversion. In prokaryotes, methylglyoxal was mutagenic in the absence of an exogenous metabolic system. Methylglyoxal forms adducts with guanine bases and nucleic acids.

4.5 Evaluation[1]

There are no data on the carcinogenicity in humans of methylglyoxal.

There is *inadequate evidence* in experimental animals for the carcinogenicity of methylglyoxal.

Overall evaluation

Methylglyoxal is *not classifiable as to its carcinogenicity to humans (Group 3).*

5. References

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[1]For definition of the italicized terms, see Preamble, pp. 27-31.

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