5-(Hydroxymethyl)-2-furfural

CAS NO. 67-47-0

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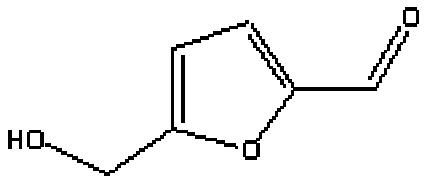
CHEMICAL IDENTIFICATION

CAS Registry Number: 67-47-0

Chemical Abstracts Name: 5-(Hydroxymethyl)-2-furancarboxaldehyde

Synonyms: 5-(Hydroxymethyl)-2-furaldehyde; HMF; 5-(hydroxymethyl)-2-furancarbonal; 5-(hydroxymethyl)-2-furfural; 5-hydroxymethyl-2- formylfuran; 5-oxymethylfurfurole; hydroxymethyl furfuraldehyde

Structure, Molecular Formula and Molecular Weight:



$C_6H_6O_3$ Mol. Wt.:126.11

Chemical and Physical Properties:

Description: Crystalline solid (needles) (Lewis, 1993); dark-yellow liquid or powder (Aldrich Chemical Co., 1994); odor of chamomile flowers (Budavari, 1989)

Boiling Point: 110°C at 0.02 mm Hg (Budavari, 1989); 114-116°C at 1 mm Hg (Aldrich Chemical Co., 1994)

Melting Point: 31.5°C (Budavari, 1989); 32-35°C (Aldrich Chemical Co., 1994)

Solubility: Freely soluble in water, methanol, ethanol, acetone, ethyl acetate, dimethylformamide; soluble in ether, benzene, chloroform; less soluble in carbon tetrachloride; sparingly soluble in petroleum ether (Budavari, 1989)

Density: 1.2062 (Budavari, 1989)

Refractive Index: 1.5627 at 18°C (Budavari, 1989)

Flash Point: 79°C (Aldrich Chemical Co., 1994)

Reactivity: Very slightly volatile with steam (Budavari, 1989). Keep protected from light and air (Budavari, 1989). When heated to decomposition it emits acrid smoke and irritating fumes (Sax & Lewis, 1989). Incompatible with strong bases, strong oxidizing agents, strong reducing agents. Releases toxic fumes of carbon monoxide and carbon dioxide upon combustion or decomposition (Aldrich Chemical Co., 1994).

UV Absorption Maximum: 283 nm

Technical Products and Impurities: HMF is commercially available with a purity range of 95-99% (Aldrich Chemical Co., 1994; TCI America, 1994).

EXPOSURE INFORMATION

Commercial Availability

Production and Producers: HMF is prepared from the fructose portion of the sugar molecule in 57% yield (Budavari, 1989). HMF can also be obtained by acid-catalyzed dehydration of hexoses (Considine, 1974).

HMF is listed on EPA's TSCA Inventory (Sax & Lewis, 1989).

HMF is produced and supplied domestically in both bulk and smaller specialty quantities by many manufacturers and distributors. Eight domestic suppliers are listed in the Fine Chemicals Database and other catalog sources (Dialog Information Services, 1994).

Distributors/Suppliers

Sigma Chemical Co.

Aldrich Chemical Co.

TCI America

Atomergic Chemetals Corp.

Pfaltz & Bauer, Inc.

Lancaster Synthesis Ltd.

Janssen Chimica

luka Chemical Corp.

Davos Chemical Corp.

ICN Biochemicals

Use Pattern: HMF is used in the synthesis of dialdehydes, glycols, ethers, amino alcohols and acetals. As an aqueous acid it catalyzes ring opening (Budavari, 1989). Generation of HMF (from starch) in the presence of phenol is used to produce phenol/furfural novolak-type resins (Brode, 1982). In this application, HMF is produced from glucose in the presence of phenol, with which it is polymerized using a basic catalyst. Acid-catalyzed dehydration of HMF yields OMBF (5,5'-oxydimethylenebis(2-furfural)), an intermediate in the synthesis of several crown ethers (Larousse *et al.*, 1992). Due to its various functionalities, it has been proposed that HMF could be utilized to produce a wide range of products such as polymers, surfactants, solvents, pharmaceuticals, and plant protection agents (Kunz, 1993).

Human Exposure: Humans are potentially exposed to HMF through pharmaceutical preparations, cigarette smoke, and the consumption of a number of commonly available beverages and foods.

Occurrence: HMF is naturally occurring. It has been identified in honey, apple juice, citrus juices, beer, brandy, milk, breakfast cereal, baked foods, tomato products and home cooking of sugar and carbohydrates. A sequence of nonenzymatic browning reactions (the so-called Maillard reaction) are initiated during heat treatment of foods containing reducing sugars and amino acids. HMF is a common intermediate product in the Maillard reaction (Blanco Gomis *et al.,* 1991; Li *et al.,* 1988; Jeuring & Kuppers, 1980; Garcia-Villanova *et al.*, 1993; Porretta & Sandei, 1991; Surh & Tannenbaum, 1994).

In apple juice stored for one year, HMF concentration correlated positively with storage temperature (about 1 mg/l at 3°C for apples of different ripeness, 1.6 mg/l at 20°C for juice from unripe apples and 6.7 mg/l at 20°C for juice from ripe apples); also, stored apple juice made from ripe apples had much higher concentrations of HMF than juice from unripe apples stored for the same length of time (Poll, 1985).

HMF is present in sherries and grape concentrate. Medium sherries were found to have HMF concentrations in the range 20-340 ppm; HMF concentration in sweet sherries ranged from 130 to 680 ppm (Meidell & Filipello, 1969). HMF concentration increases with sugar content and baking time.

HMF has been determined in solutions meant for parenteral use. Ulbricht *et al*. (1984) reported the results of several studies that have determined the concentrations of HMF in parenteral solutions. In sterile glucose solutions, HMF concentrations of about 1 to 90 mg of HMF per liter of solution have been reported. Invertose or glucose containing parenteral solutions have been reported to have HMF concentrations ranging from 3 to 56 and 1 to 4 mg/liter, respectively. HMF concentration correlated positively with high acidity (pH<4), high sterilization temperature (>110°C) and a long sterilization time (30 min.).

HMF has been determined in fructose-containing solutions for intravenous injection. It appears to be formed during sterilization whenever a fructose-containing solution with pH lower than 3.5 to 4.0 is heated to 110 to 130°C (Jellum *et al*., 1973). Murty *et al.* (1977) reported that a 50% dextrose injection had a HMF concentration of 0.72 mg/liter, 24 hours after manufacture. After 4 years of storage at 70°F, the level rose to 5.8 mg/liter.

HMF has been determined in cigarette smoke (Crump & Gardner, 1989). The pyrolytic breakdown of cellulose, which occurs in all tobacco varieties, is believed to generate HMF, among other furans (Schlotzhauer & Chortyk, 1987).

HMF has been detected in caramel, which is a widely used coloring agent in food and pharmaceutical syrups (Hewala *et al*., 1993). While the reported levels of HMF in pharmaceutical syrups is very low, the possibility of interaction between HMF and active drugs containing an amino group appears to be of some concern.

HMF is formed in milk when it is treated at temperatures higher than 120°C This is

attributed to the presence of a reducing sugar, which in the case of milk is lactose (Morales *et al*., 1992).

Regulatory Status: No occupational exposure limits for HMF have been set or recommended by OSHA, NIOSH, or ACGIH.

EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

Human Data: No Positive or negative epidemiological studies or case reports associating HMF with a cancer risk in humanswere identified in the available literature.

HMF is considered an irritant and is irritating to eyes, upper respiratory tract, skin and mucous membranes. Human exposures can occur by inhalation, ingestion or skin absorption (Aldrich Chemical Co., 1994).

Animal Data: No 2-year bioassay studies associating HMF either positively or negatively with a carcinogenic effect in animals were found in the available literature. Initiation/promotion studies indicate that HMF may act as an initiator and promoter of colon cancer in rats. HMF does not appear to act as an initiator of skin tumors in mice.

Several studies suggest that HMF may act as both an initiator and a promoter in the induction of colonic aberrant cryptic foci (ACF) in rats. In the first study, CF1 mice and F344 rats were initiated with the colon carcinogen, azoxymethane. One week after initiation, the animals were fed one of eight diets for 100 days. In each case, 40% of the diet was the same and the remaining 60% was composed of 20% sucrose, 20% casein and 20% beef tallow. The control diet contained uncooked ingredients, while experimental animals were fed diets in which one, two or three of the components were thermolyzed. There were significant increases in the number of ACF and microadenomas in animals fed diets with thermolyzed sucrose and casein. HPLC analysis of the thermolyzed sucrose showed that it contained 1% HMF (Archer *et al*., 1992a; Bruce *et al.*, 1993).

To determine whether the HMF was responsible for the promotion of ACF by thermolyzed sucrose, a subsequent study was conducted in which F344 female rats were initiated with azoxymethane and a week later were fed diets containing untreated sucrose, 20% thermolyzed sucrose, 20% butanol extracted thermolyzed sucrose (HMF free), or 1% HMF for 100 days. There were significant increases in the average size of the foci and in the number of ACF in animals receiving the thermolyzed sucrose and 1% HMF diets, which is consistent with the hypothesis that HMF is the active component in the thermolyzed sucrose. The increase in the average size of the foci suggests that HMF does act as a promoter. However, the increase in the number of ACF suggests that HMF may also act as an initiator (Archer *et al.*, 1992b; Bruce *et al*., 1993; Zhang *et al.*, 1993).

To determine whether HMF is an initiator, Zhang *et al*. (1993) gave female F344 rats a single dose of 0, 100, 200, 250, or 300 mg/kg HMF by gavage; treatment was repeated one week later and the animals were sacrificed 30 days later. There was a significant dose-related increase in ACF in the treated animals. These data suggest that HMF acts as an initiator, although much weaker than azoxymethane.

Miyakawa *et al*. (1991) examined the potential of HMF to act as an initiator of skin

tumors in mice. As the initiation treatment, 50 umol HMF was applied to the shaven backs of 20 female CD-1 mice twice weekly for 5 weeks; as a positive control, 10 ug 7,12- dimethylbenz[a]anthracene (DMBA) was used and dimethylsulfoxide (DMSO) was the vehicle control. The promotion treatment began one week later and consisted of applications of 2.5 ug TPA twice weekly for 47 weeks. Tumors were counted and recorded weekly; surviving animals were autopsied at the end of the experiment. Initiation with HMF resulted in skin tumors in 20% of the animals compared to 5% in the control group; the difference was not statistically significant.

In rats, the acute oral LD_{50} has been reported at 2.5 g/kg for males and between 2.5 and 5.0 g/kg for females (US EPA, 1992). The lowest published subcutaneous toxic dose for rats is 200 mg/kg (Sax & Lewis, 1989).

Short-Term Test: The available mutagenicity studies indicate mutagenic activity at high doses of HMF (greater than 1 mg per test system).

HMF was not mutagenic, with or without activation by S9, to *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 at a dose level of 3 umol/plate, the only dose tested (Florin *et al*., 1980). Kasai *et al*. (1982) found no mutagenic activity of HMF, with and without activation by S9, towards *S. typhimurium* TA100; details on dose levels were not provided. Aeschbacher *et al*. (1981) found no mutagenic activity of HMF, with and without activation by S9, towards *S. typhimurium* TA98 and TA100 at doses of 1-50 ul/plate. In contrast to these studies, Shinohara *et al*. (1986) found that HMF was mutagenic to strain TA98, with and without activation, at a dose of 0.165 umol/plate. However, the significance of this is unclear since mutagenic activity was not noted at doses of 0.330, 0.495, and 0.660 umol/plate. In addition, Shinohara *et al*. (1986) found that HMF with metabolic activation was mutagenic to strain TA100 at dose levels of 0.165 and 0.330 umol/plate. Again, the meaning is unclear since no mutagenic activity was noted at higher doses. Shinohara *et al*. (1986) also found HMF to be positive in the Rec-assay with *B. subtilis* H 17 Rec+ and M 45 Rec-, both with and without activation, at dose levels greater than 2.5 mg/disc.

The desmutagenic, or antimutagenic, activity of HMF has been studied in conjunction with numerous mutagens in the Ames test (Kim *et al*., 1987; Kong *et al*., 1989). The antimutagenic activity is presumed to be a result of the presence of carbonyl groups and their reaction with amino groups as might occur during cooking or processing of foods. In the first study, HMF was mixed with each of five different mutagenic heterocyclic amines (Trp-P-1, Trp-P-2, Glu-P- 1, Glu-P-2, and IQ[1], autoclaved (to simulate cooking/processing of foods), and tested for mutagenicity in the Ames strain TA98 with metabolic activation and strain TA100 without metabolic activation (Kim *et al*. 1987). In addition to regular controls, the heterocyclic amines were tested separately without HMF. The results showed a decrease in mutagenicity (in terms of revertants per plate) in the presence of HMF. Only one HMF dose was tested and so a dose- response analysis is not possible.

Kong *et al*. (1989) studied the desmutagenic effect of combining HMF (four doses ranging from 0.8 to 3.2 ug/plate) with three mutagens [Trp-P-1 (1 ug/plate), benzo[a]pyrene (BaP) (1 ug/plate), or 2-aminofluorene (2-AF) (2 ug/plate)] in Ames

assays with strains TA98 and TA100, both with metabolic activation. Unlike the Kim *et al*. (1987) study, the mixtures were not autoclaved. Results indicated that the mutagenicity of each mutagen decreased with increasing HMF dose. At the highest HMF dose level, mutagenicity decreased to approximately 20% of what was observed without HMF present. The authors noted that HMF, like other furan compounds tested, appeared to show a bifunctionality by exhibiting a weak mutagenic effect (doses on the order of 1 or more mg/plate) and a desmutagenic effect (doses in the 1 or more ug/plate range). However, the results of the Ames test conducted by Shinohara *et al*. (1986) are not consistent with this hypothesis.

Surh and Tannenbaum (1994) found no mutagenic activity or cytotoxicity of HMF in TK6 human lymphoblast cells at doses as high as 75 ug/ml.

HMF was shown to significantly induce chromosomal aberrations in Chinese hamster V79 cells at a dose of 2 mg/ml, the highest dose tested. In the same study and experimental system, HMF significantly reduced mitotic activity at all doses tested (1.0, 1.5, and 2.0 mg/ml) (Nishi *et al*., 1989).

[1] Trp-P-1 (3-amino-1,4-dimethyl-*5H*-pyrido[4,3-*b*]indole, Trp-P-2 (3-amino-1-methyl-*5H*-pyrido[4,3-*b*]indole), Glu-P-1 (2-amino-6-methyldipyrido [1,2-*a* :3', 2'-*d*]imidazole), Glu-P-2 (2-aminodipyrido [1,2-*a* :3', 2'-*d*]imidazole), and IQ (2-amino-3-methylimidazo[4,5-*f*]quinoline.

Metabolism: Jellum *et al*. (1973) found HMFA and furan-2,5-dicarboxylic acid (FDCA) in the urine of two infants receiving parenteral solutions which contained HMF. Both infants were involved in surgical procedures during which quantitative urine samples were obtained before, during and after administration of the HMF-containing parenteral nutrition solutions. Thus, the authors were able to quantify the amount of HMF going into the infant intravenously and calculated that approximately 50% (38% in one patient and 74% in the other) of the administered HMF was excreted via the urine as HMFA and FDCA. Neither unchanged HMF nor the glycine conjugates of HMFA and FDCA were found in the urine samples. The authors postulated that the remaining 50% of the HMF was probably retained in the body and was bound to proteins [however, see Germond *et al.* (1987) below].

Based on a radiolabelled study in rats, it appears that HMF is rapidly absorbed from the gastrointestinal tract and almost totally excreted via the urine as 5-hydroxymethyl-2-furoic acid (HMFA) and N-(5-hydroxymethyl-2-furoyl)-glycine (HMFG), the latter being the glycine conjugate of HMFA (Germond *et al*., 1987).

Ulbricht *et al*. (1984) provide a review of the earlier pharmacokinetic studies of HMF. No *in vivo* radiolabelling studies were reported. Species tested included rabbits, dogs, chickens, rats and mice. In rabbits, dogs and chickens, HMFA was found in the urine at levels of 44 to 67% of the HMF administered (dose, route, species differences were not reported) (Karashima 1927; as cited in Ulbricht *et al*., 1984). Lang *et al*. (1970; as cited in Ulbricht *et al*., 1984) found 12 mg of HMF in the urine of rabbits administered 1 gram of HMF in feed; no other metabolites were found. Rats and mice given 100 mg HMF/kg, either orally or intravenously, showed only trace amounts in urine, blood plasma, and bile samples (Czok, 1970; as cited in Ulbricht *et al*., 1984). No information was provided

pertaining to dosing regimen (single or repeated), time between dosing and sampling, or metabolite(s) found.

Male Sprague-Dawley rats were given single doses of radiolabelled HMF (0 to 330 mg/kg) either by gavage or intravenous (tail vein) administration. Expired air was trapped and analyzed for $^{14}CO_2$, urine and feces were collected, and all rats (2 per dose) were sacrificed 24 hours after dosing for radioactivity analyses. Results of the gavage experiment showed less than 1% of the radioactivity was recovered in expired air, and less than 1% was found in the feces. Almost all of the radioactivity was recovered in the urine (85% of administered dose 8 hours after dosing) and consisted of three major metabolites: HMFA, N-(5-hydryoxymethyl-2-furoyl)glycine (HMFG), and a third, unidentified polar product. The authors noted that earlier investigators (specifically mentioning Jellum *et al*., 1973) did not find HMFG (the glycine conjugate of HMFA) in urine because they performed ether extracts on acidified urine and HMFG is not soluble in ether. In addition, the HMFA/HMFG ratio changed with HMF dose: as the HMF dose increased (from 13 to 330 mg/kg), the ratio increased from 2 to 15-20 (Germond *et al*., 1987).

Whole body radiography one hour after oral administration showed most of the radioactivity to be in the kidney and bladder, with some in the liver, stomach and small intestine. Autoradiography 24 hours after HMF administration showed no radioactivity anywhere in the body. Similar results were observed with rats given HMF intravenously, except some radioactivity was observed in the brain, and, as expected, was not observed in the stomach.

Miller (1994) proposed that sulfonation of HMF may lead to mutagenicity and carcinogenicity. This is based on the fact that mouse and rat liver cytosols contain sulfotransferases and so are able to convert HMF to its reactive sulfuric acid ester, which is mutagenic and carcinogenic (according to reference 31 in Miller 1994). However, none of the metabolism studies reviewed commented on the presence or absence of sulfate conjugates following HMF administration.

Other Biological Effects:

Ulbricht *et al*. (1984) reviewed the early toxicity studies of HMF. The oral LD_{50} was to be 1910 mg/kg and 3100 mg/kg for the mouse and rat, respectively. Oral administration of 310 mg/kg/day to rats for 2 months resulted in some disruption of liver function (a change in the ratio of serum-protein fractions), and hepatic tributyrinase and intestinal (mucosal) phosphomonoesterase 1 and enterokinase activities were elevated. Oral administration of doses of 0, 40, 80, or 160 mg/kg/day to rats for 11 months resulted in a temporary rise in the serum gamma globulin level, an increase in the relative weight of the spleen, and a tendency toward increased hepatic tributyrinase activity at 160 mg/kg/day; no effects were observed at 40 and 80 mg/kg/day. In another study, no effects were noted in rats administered dietary levels of 250 mg/kg/day HMF for 40 weeks.

Rasmussen *et al*. (1982) gave rabbits, 9 per group, two daily subcutaneous injections of 400 mg HMF for 6 days; the rabbits were sacrificed on day 7. There were no significant effects of HMF treatment on mean body weight, hemoglobin, leucocytes, platelets,

serum-protein, serum- alanine-aminotransferase, or alkaline phosphatase. Histologic examination of the liver revealed no treatment-related effects. In a second study, Rasmussen *et al*., (1982) examined the vein damaging properties of HMF. Twelve rabbits were given an isotonic NaCl solution containing 200 mg HMF/l in one ear vein for 5 hours; a pure isotonic NaCl solution was given in the corresponding ear vein of the other ear. The rabbits were sacrificed 24 hours later and the infusion area was examined histologically for inflammatory changes. There was no evidence of a treatment-related vein irritating effect.

Shinohara *et al*. (1990) examined the effects of HMF on the viability and the activity of some enzymes of a human histiocytic lymphoma cell line, U-937. The cells were incubated with 3.9- 117 nmol HMF for 12 hours. Incubation of U-937 with 117 nmol HMF resulted in a 20% reduction in viability; lower concentrations of HMF had no effect. There was a dose-related increase in the activity of NADPH-cytochrome c reductase at all concentrations of HMF, whereas HMF had no effect on the activity of glutamic oxaloacetic transaminase.

The effect of HMF on the metabolism of human blood cells was investigated by incubating human blood cells (granulocytes, erythrocytes and thrombocytes) with concentrations of 0.735, 7.35 and 73.5 mM HMF followed by determination of heat production using a microcalorimetric technique (Nassberger, 1990). There was a 60% increase in heat output in erythrocytes and a significant reduction of heat output (17%) in granulocytes at the highest concentration of HMF. No effects on thrombocytes were noted.

Structure Activity Relationships: The available literature was screened for relevant structural analogs of HMF. Ten structural analogs of HMF were then searched to identify relevant data associating the compounds structurally similar to HMF with mutagenic or carcinogenic activity. Chemical names, CAS registry numbers and structures of these compounds are shown in Appendix A. Information was found on eight of these compounds as follows.

Furfuryl alcohol was negative in an Ames assay in *Salmonella typhimurium* strains TA98, TA100, and TA102 (Aeschbacher *et al*., 1989; Shinohara *et al*., 1986). Furfuryl alcohol was evaluated for mutagenic activity in *D. melanogaster* by means of the sex-linked recessive lethal test (SLRLT) and the sex-chromosome loss test (SCLT); no evidence of a mutagenic effect was found after adult injection and larval feeding (Rodriguez-Arnaiz *et al*., 1989). Jansson *et al*. (1986) stated that furfuryl alcohol did not induce sister-chromatid exchanges (SCEs) in human lymphocytes. In addition, furfuryl alcohol was positive in the Rec assay (Kong *et al*., 1988).

Furfural: In the NTP 2-year gavage studies (NTP, 1990), furfural showed *some evidence* of carcinogenic activity for male F344/N rats, based on the occurrence of uncommon cholangiocarcinomas in two animals and bile duct dysplasia with fibrosis in two other animals. There was *no evidence* of carcinogenic activity for female F344/N rats that received doses of 0, 30, or 60 mg/kg furfural. There was *clear evidence* of carcinogenic activity for male $B6C3F_1$ mice, based on increased incidences of hepatocellular adenomas and hepatocellular carcinomas, and *some evidence* of carcinogenic activity in

female B6C3F$_1$ mice, based on increased incidences of hepatocellular adenomas. Renal cortical adenomas or carcinomas in male mice and squamous cell papillomas of the forestomach in female mice may have been related to exposure to furfural. Miyakawa *et al*., (1991) reported that furfural gave negative results for carcinogenic activity in female CD-1 mice. In gene mutation tests with four strains of *Salmonella* (TA98, TA100, TA1535, and TA1537), no mutagenic activity was observed in the presence or absence of S9 exogenous metabolic activation in one laboratory and an equivocal response was observed in TA100 in the absence of S9 in a second laboratory. However, Zdzienicka *et al*. (1978) reported positive mutagenic activity in TA100 but not in TA98 (with rat liver S9 activation). Exposure to furfural induced trifluorothymidine resistance in mouse L5178Y lymphoma cells in the absence of S9 (no evaluation was made in the presence of S9), sister chromatid exchanges (SCEs) and chromosomal aberrations in CHO cells in the presence or absence of S9, and an increase in sex-linked recessive lethal mutations but no reciprocal translocations in germ cells of *D. melanogaster*; furfural did not induce SCEs or chromosomal aberrations in the bone marrow of B6C3F$_1$ mice (NTP, 1990). Recently, Rodriguez-Arnaiz *et al*. (1992) reported that furfural induced somatic damage as measured in the wing spot test in somatic cells of *D. melanogaster*. In addition, Khan and Hadi (1994) reported that furfural induced single strand breaks in duplex DNA which occur preferentially in AT base pairs, and evidence showed that the strand scissions induced by furfural in DNA account for its biological activity as assayed by inactivation of bacteriophage lambda.

2-Furoic acid gave a negative result when tested in an Ames/*Salmonella typhimurium* assay in strains TA98 and TA100 (Ichikawa *et al*., 1986).

2-Methylfuran was negative in an Ames/*Salmonella typhimurium* assay (Aeschbacher *et al*., 1989; Shinohara *et al*., 1986). 2-Methylfuran was found positive in a micronucleus test, but negative in a SOS chromotest and an umu test (Kong *et al*., 1988).

5-Methylfurfural also tested negative in an Ames/*Salmonella typhimurium* assay (Aeschbacher et al., 1989; Shinohara *et al*., 1986). In a micronucleus test, 5-methylfurfural was positive, but it was negative in a SOS chromotest and an umu test (Kong *et al*., 1988).

Methyl 2-furoate was negative in an Ames/*Salmonella* assay (Shinohara *et al*., 1986).

5-(Sulfooxymethyl)-furfural (SMF), an allylic sulfuric acid ester metabolite from HMF, showed intrinsic direct mutagenicity toward *Salmonella typhimurium* TM677 without a metabolic activation system. It induced 8-azaguanine-resistant mutants in a dose-dependent manner (at concentrations from 25 to 250 μM). SMF also exhibited direct mutagenicity at both thymidine kinase and hypoxanthine-guanine phosphoribosyltransferase loci in human lymphoblasts. The mutagenicity and cytotoxicity were enhanced significantly when extra chlorine ion was added to the incubation medium (Surh & Tannenbaum, 1994). Toxicity of SMF was also enhanced with increased mutagenicity.

5-(Chloromethyl)-furfural (CMF) was also mutagenic and cytotoxic in *Salmonella typhimurium* TM677, and it was much more active (too toxic to assess the correct mutagenicity) than SMF (analog 10 in the Appendix A) in the bacterial mutagenicity assay; considerable toxicity was still observed above the concentration of 2.5 μM (Surh & Tannenbaum, 1994).

Mutagenic activities of SMF and CMF, which lack the aldehyde functionality of HMF (both compounds were derivatized from furfuryl alcohol), were also studied by Surh and Tannenbaum (1994). The results showed no significant mutagenicity. In addition, the acetic acid ester of HMF was neither mutagenic nor cytotoxic under the same experimental conditions used for SMF and CMF.

Neither short-term test data nor chronic bioassay data were found addressing the potential mutagenicity or carcinogenicity for the first two analogs shown in Appendix A.

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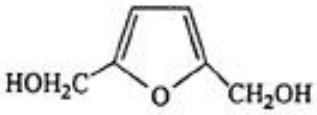
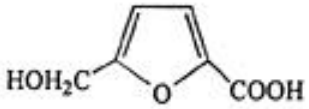
APPENDIX A

Structural Analogs of HMF

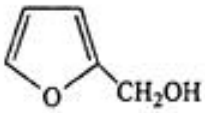
1) 2,5-Furandimethanol
[1883-75-6]



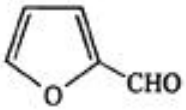
2) 5-(hydroxymethyl)-2-furoic acid
[6338-41-6]



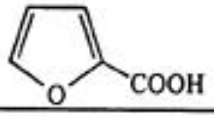
3) Furfuryl alcohol
[98-00-0]



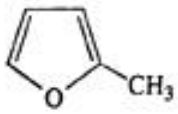
4) 2-Furaldehyde (or Furfural)
[98-01-1]



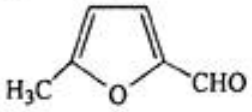
5) 2-Furoic acid
[88-14-2]



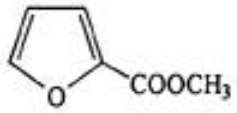
6) 2-Methylfuran
[534-22-5]



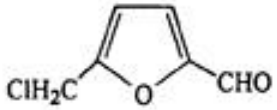
7) 5-Methylfurfural
[620-02-0]



8) Methyl 2-furoate
[611-13-2]



9) 5-(Chloromethyl)-furfural
[1623-88-7]



10) 5-(Sulfooxymethyl)-furfural
[159091-35-7]