SUMMARY OF DATA FOR CHEMICAL SELECTION

2(5H)-Furanone, 3-chloro-4-(dichloromethyl)-5-hydroxy-(9 CI)

Prepared for NTP by Technical Resources International, Inc. Prepared on 11/94
Under Contract No. N01-CP-56019

Table of Contents

SUMMARY OF DATA FOR CHEMICAL SELECTION

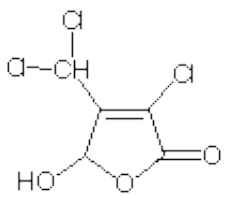
CHEMICAL IDENTIFICATION

CAS Registry No.: 77439-76-0

Chemical Abstracts Service Name: 2(5H)-Furanone, 3-chloro-4-(dichloromethyl)-5-hydroxy-(9 CI)

Synonyms: MX; MX (bacterial mutagen); 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone

Structure: Molecular Formula and Molecular Weight:



$C_5H_3Cl_3O_3$ Mol. wt.: 217.44

Chemical and Physical Properties:

Description: Pale-yellow liquid (Padmapriya, 1985)

Solubility: Solubility in Water (Vartiainen *et al*., 1991)

pH	Solubility (mg/ml)	$LogP_{ow}$
2	50.8	1.13
5	44.7	0.84
7	43.7	-0.44
9	51.0	-1.02

Absorption Spectra:

UVl_{max}: 224.5 nm (e=8129); IR (neat) n $_{max}(cm^{-1})$: 3400, 3000, 1780, 1660, 750, 690. ^{1}H NMR ($CDCl_3$) d: 5.27 (^{1}H, bs, OH), 6.40 (^{1}H, s), 6.60 (^{1}H, s); ^{13}C NMR ($CDCl_3$) : 60.51 (d, $^{1}J_{C6-H6}$= 181Hz, $CHCl_2$), 96.55 (dt, $^{1}J_{C5-H5}$ =178.8 Hz, $^{2}J_{C5-OH}$=3.85 Hz, CHOH), 125.19 (d, d, 2J=0.83 Hz, ^{2}J=3.02 Hz, C=C-$CHCl_2$), 150.45 (bs, C3,=C-Cl), 164.51 (d, $^{3}J_{C2-H5}$=2.72 Hz, CO) (Padmapriya, 1985)

Stability:

Stability in aqueous solution is pH and temperature dependent. Stable in ethyl acetate and acidic water solutions. Order of stability at 23°C (based on mutagenic activity): pH 2>pH 4>pH 8>pH 6. Half-life at pH 8 and 23°C is 4.6 days. (Meier *et al*., 1987a). Stable for up to 5 h in water at pH 2 even at 60°C but is degraded under less acidic conditions

Temperature (°C)	pH	Half-life (hours)
23	8	110.4
60	6	3
40	12	0.167
25	12	0.05

Reactivity:
The water solubility of MX increases rapidly above pH6 because it undergoes ring opening and dissociation (Holmbom *et al*., 1984). At pH>4, MX can tautomerize so that in equilibrium it co-exists with an open (and ionized) form, Z-2-chloro-3-(dichloromethyl)-4-oxoburenoic acid. At 5<pH<7, the open form can isomerize at the double bond to form the geometric isomer E-2-chloro-3-(dichloromethyl)-4-oxobutenoic acid (E-MX). E-MX can be reconverted to MX at lower pH values. Under alkaline conditions, MX can be oxidized to 2-chloro-3-(dichloromethyl)-butenedioic acid (OX-MX) (Bull & Kopfler, 1991.)

Technical Products and Impurities: MX is not commercially available from chemical suppliers.

EXPOSURE INFORMATION

Commercial Availability

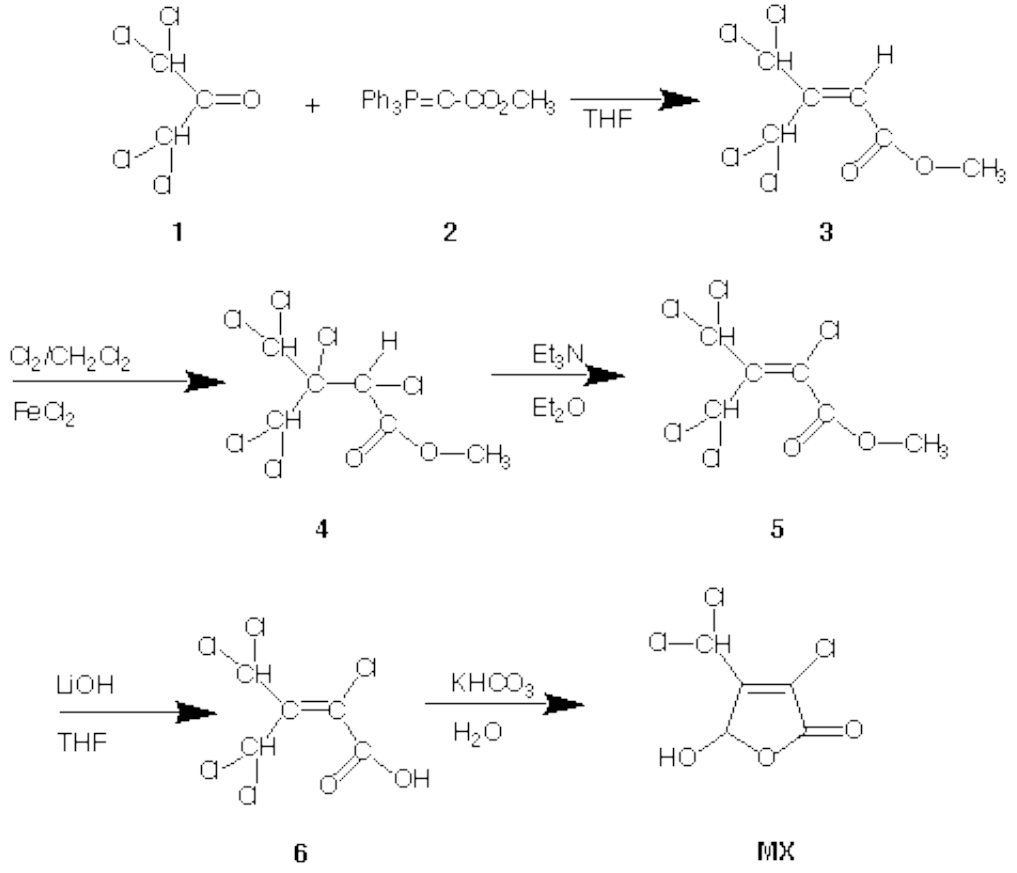
Production and Producers:
MX is one of the by-products formed from the chlorine disinfection of drinking water and it also has been found as a contaminant of chlorinated wood pulp mill effluents (bleaching liquors). MX is not commercially produced in U.S. Neither producers nor annual production volumes for MX can be found in the available literature.

Synthesis:
Synthesis of MX was originally reported by Padmapriya and Just (1985). The step-wise synthesis is shown in Scheme 1. 1,1,3,3-Tetrachloroacetone **[1]** was condensed with methyl (triphenylphosphoranylidene) acetate **[2]** to form methyl 3-(dichloromethyl)-4,4-dichlorocrotonate **[3]**. The resulting compound **[3]** was then saturated with chlorine in ferric chloride and methylene chloride solution to give the hexachloro compound **[4]**, which is then further reacted with triethylamine in ether to give the pentachloro-olefin ester **[5]**. Treatment of the resulting olefin ester **[5]** with LiOH in THF solution yielded the corresponding acid **[6]**. Subsequent treatment of the acid **[6]** with aqueous potassium bicarbonate solution results in the formation of **MX**.

Use Pattern:
MX is a by-product found both in chlorination liquors from wood pulp bleaching and in chlorinated drinking waters and, as such, does not have a pattern of application and use. No other uses of MX have been found in the available literature as a laboratory research chemical except for mutagenicity related studies.

Human Exposure:
There is potential for wide spread, very low level human exposures to MX *via* the oral route, especially through ingestion of chlorinated sources of drinking water. Dermal exposures may also occur through contact with contaminated bodies of water.

Environmental Occurrence:
MX is a major mutagen found in chlorination liquors from wood pulp bleaching and in chlorinated drinking waters. The reaction of chlorine and humic materials is believed to be responsible for the formation of MX and its geometric isomer E-MX. Under acidic conditions E-MX converts to MX. Humic materials are complex polymers of lignins, carbohydrates, proteins and fatty acids which are leached out of soils and drain into surface water systems. In the US, concentrations from 0.002 to 0.033 mg/L in chlorinated drinking waters have been reported (Meier *et al.*, 1987a, 1987b). However, the recovery of MX may not have been complete; therefore, these concentrations are very likely to be underestimates. In Finnish chlorinated drinking water supplies, MX concentrations in the range 0.004 to 0.067mg/L (median=0.022, mean=0.027, 20 samples) have been reported (Kronberg & Vartiainen, 1988). MX concentrations in chlorinated drinking water samples in Great Britain in the range 0.002 to 0.023 mg/L (median=0.005, mean=0.007, 8 samples)have been reported (Horth *et al.*, 1989).



The concentration of MX appears to depend upon whether water treatment is carried out using chlorine (Cl_2) alone, chlorine in combination with chlorine dioxide ($Cl0_2$) or monochloramine (NH_2Cl) (alone, or in combination with ozone pre-treatment). Andrews *et al.* (1988) reported some results of the effects of chlorination method on MX and E-MX concentrations. Treatment by chlorine alone produced the highest concentrations of MX and E-MX. Cl_2-ClO_2 mixtures produced lower concentrations of MX and E-MX; the greater the proportion of ClO_2, the lesser the MX/E-MX concentration. Chlorine dioxide treatment alone produced only 10 percent as much MX/E-MX as Cl_2 treatment alone. Treatment with NH_2Cl produced about 25 percent of the concentration of MX found in chlorinated water. Experiments carried out to determine the effectiveness of ozonation prior to chloramine treatment produced inconclusive results.

No occupational exposure limits for MX have been set or recommended by OSHA, NIOSH or ACGIH.

EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

Human Data:
There are no epidemiological studies associating MX directly with a cancer risk to humans. However, there have been various epidemiological studies which reported an association between drinking chlorinated water and higher incidences of bladder, colon, or rectal cancer, while other such studies found no difference (IARC, 1991; Dayan, 1992; Moller, 1993).

Animal Data:
Only one study on the carcinogenicity of MX was found in the literature. SENCAR mice which were treated topically or orally with MX alone showed no tumors after 24 weeks. Following TPA promotion, the tumor incidence in mice treated topically with MX was similar to that in controls. Mice receiving MX orally and TPA topically had a statistically significant increase in both the percent incidence of tumors and the number of tumors per mouse (Meier *et al.*, 1990).

Toxicity:
In Swiss-Webster mice, the oral LD50 of MX was about 128 mg/kg/day, (based on 2 doses 24 hours apart) while the LD50 for topical application was 160 mg/kg (Ringhand *et al.*, 1990). In male Wistar rats, the oral LD50 of MX was 230 mg/kg in 48 hours. MX is a local irritant for the gastrointestinal tract, may cause tubular damage in the kidneys and causes edema of the lungs (Komulainen *et al.*, 1994).

In mice given MX orally, there were various nuclear anomalies (micronuclei, pyknotic nuclei and karyorrhectic nuclei) in the gastrointestinal tract, with the duodenum being affected most (Daniel *et al.*, 1991). At relatively high doses in rats, MX caused DNA strand scissions in the pyloric mucosa of the stomach, increased replicative DNA synthesis and induced a doubling of ornithine decarboxylase (Furihata *et al.*, 1992). However, when MX was given orally at a dose of 125 mg/kg to rats fasted for two days there was no evidence of induced DNA damage to various organs, including bone marrow, by one hour after dosing as determined by the alkaline elution technique (Bronborg *et al.*, 1990).

In a repeated-dose study of MX at levels of 8, 16, 32, or 64 mg/kg/day by gavage for 14 consecutive days in B6C3F1 mice and F344 rats, Daniel *et al.* (1994) found that mice showed no significant differences in body weight between controls and treated groups. There were some changes in hematological and clinical chemistry parameters and organ weights, but no MX-related gross lesions were noted at necropsy. Histopathological examination revealed treatment-related increases in epithelial hyperplasia of the forestomach as well as extramedullary hematopoiesis in all male and female treated groups of mice.

In the F344 rats, body weight gains and food and water consumption were lower in the animals at the highest dosage levels. There were some changes in hematological and clinical chemical parameters, cholesterol increased significantly in both males and females of all dosage groups. As with the mice, forestomach changes (epithelial hyperplasia, inflammation, hyperkeratosis or ulceration) were found in both males and females of the high dosage groups. There also was a somewhat higher incidence of extramedullary hematopoiesis in treated rats. Liver and kidney weights tended to be higher in treated rats at the higher dosage levels while thymus weights were lower. The lowest adverse effect level (LOAEL) was 8 mg/kg for both the mice and the rats.

Short-Term Test:

MX has been identified as a potent direct-acting mutagen resulting from the chlorine-disinfection of drinking water (Meier *et al.*, 1987; Kronberg *et al.*, 1985) and the chlorine bleaching of softwood pulp (Holmbom *et al.*, 1984). The mutagenicity of MX has been reported in the range of 103-104 revertants/nmol in the Ames *Salmonella* typhimurium TA100 assay. The concentration of MX appears to have a good positive correlation with the degree of mutagenicity (Holmbom *et al.*, 1984; Ishiguro *et al.*, 1988; LaLonde *et al.*, 1991a).

Meier *et al.* (1987) reported that MX was a direct-acting mutagen in *S. typhimurium* strains TA1535, TA1538, TA92, TA100, and TA102, with the greatest response (~13,000 revertants/nmol) observed in TA100. The TA100 response was 6-19 fold higher than in TA98 and TA102 and 100 to 500 fold higher than in TA1535, TA1538 and TA92. Addition of S-9 from livers of Aroclor 1254 treated rats along with NADPH cofactors led to a 90% reduction in TA100 mutagenicity. The mutagenicity of MX in TA100 was also noted by Kronberg and Vartiainen (1988) and LaLonde *et al*. (1991). MX was estimated to account for a mean value of 30% of the mutagenicity of drinking water (Meier, 1990; Kronberg & Vartiainen, 1988). The mutagenicity of MX was also decreased in the presence of bovine serum albumin (BSA), leading to the conclusion that MX mostly bound reversibly to BSA, while a minor portion bound irreversibly (Haataja *et al.*, 1991). A microsomal fraction (S-9) from human placenta also decreased the mutagenicity of MX, but the effect was less than that of S-9 from rat liver (Tuomisto & Vartiainen, 1990).

In *E. coli* WP2s (uvrA155, trp E65), addition of sulfhydryl compounds (cysteine, cysteamine, glutathione, dithiothreitol, and 2-mercaptoethanol) to the medium decreased the mutagenicity of TA100 in a dose-dependent fashion until at the higher doses there was no mutagenic effect. Various antioxidants had no such action (Watanabe *et al.*, 1994).

In Chinese hamster ovary cells (CHO), MX reduced the mitotic index, but with S-9 present much higher levels of MX were required to show an effect. MX caused structural chromosomal damage, mostly chromatid deletions in the CHO cells (Meier *et al.*, 1987). In CHO cells, MX treatment led to a dose-related increase in mutant frequency at the hprt locus to 6-thioguanine (6-TG) resistance (Jansson & Hyttinen, 1994). MX led to induction of 6-TG forward mutation in V79 cells when tested in Hanks balanced salt solution, but not when tested in serum-free minimal essential medium (Matsumura *et al.*, 1994). In the V79 cell system, MX led to DNA damage as measured by alkaline elution and it induced sister-chromatid exchanges (SCE), but there was no effect on hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) mutation induction (Brunborg *et al.*, 1991). MX induced strand breaks in human CCRF-CEM (human lymphblastoid line) cells and in primary rat hepatocytes (Chang *et al.*, 1991). It caused DNA damage in rat hepatocytes and testicular cells (Brunborg *et al.*, 1990; 1991), and induced SCEs and chromosomal aberrations in cultured rat peripheral lymphocytes (Jansson *et al.*, 1993). In rats given gavage doses of MX 5 days a week for 14-18 weeks, there were dose-related increases in SCEs of the peripheral lymphocytes (Jansson *et al.*, 1993). However, it did not induce micronuclei in mouse bone marrow when given at doses of 22.5-90 mg/kg/day (Meier *et al.*, 1987). Moreover, when various nucleosides and nucleotides were reacted with MX for 21 hours, HPLC analysis showed no stable adducts of MX, only those from degradation products of MX (Alhonen-Raatesalmi & Hemminki, 1991).

Metabolism:
The pharmacokinetics of MX, given as a single 14C-labeled dose, either orally or intravenously at three dose levels, was followed for periods up to six days. Levels of the label in blood were decidedly higher after iv injection than after the gavage dose and were still appreciable at 150 hours after dosing. However, by 72 hours after an oral dose, levels in blood were under the detection limit. Radioactivity initially was high in kidneys, gastro-intestinal tract and urinary bladder, but the levels decreased appreciably by 6 hours. Radioactivity in urine appeared rapidly with approximately 35% of the dose thus excreted in 72 hours, while 50% appeared in the feces over that time. Only 0.03-0.07% of the material excreted in urine was MX. There was no apparent degradation to respiratory CO2 (Komulainen *et al.*, 1992). These results corroborated the study by Ringhand *et al.* (1989) which had followed tissue distribution and excretion in rats over a 48 hour period. At that time only 3-4% remained in the organs, 1 % was in blood, and 2% in gastrointestinal contents. The percentages excreted in urine or feces amounted to 34% or 47%, respectively. When added to whole blood of rats, MX bound to plasma (42%), erythrocytes (26%), and 32% to hemoglobin. It increased methemoglobin in the erythrocytes but did not cause overt oxidative damage (Risto *et al.*, 1993).

Structure/Activity Relationships:
The available literature was screened for any structural analogs of MX. A total of 11 MX analogs have been identified, the structure-activity relationships regarding their relative mutagenicity in the Ames Salmonella

typhimurium TA100 assay (LaLonde *et al.*, 1991b, 1991c) is summarized in Table 1. These MX structural analogs all possess the 2(5H)-furanone skeleton, and MX is found to be the most potent mutagen among this family of compounds. The effect on mutagenicity of the different substituents (i.e., chlorine atom, hydroxy group, and hydrogen atom) on the 2(5H)-furanone can be summarized as follows:

1) All the compounds (1 - 6 & 11) that possessed at least one chlorine atom at C-6 had positive mutagenicity. Compounds 8 -10 which lack a C-6 chlorine atom gave negative results.

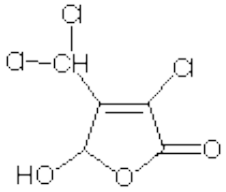
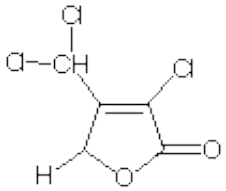
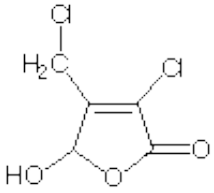
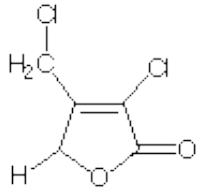
2) The magnitude of mutagenicity reduction for a given type of replacement ranged from 10-to 100-fold. In general, replacement of the hydroxy group at C-5 or the C-6 chlorine atom with a hydrogen atom resulted in a 10-fold diminished mutagenicity (see comparison of MX to compound 2 or MX to compound 3). However, replacement of the C-3 chlorine with a hydrogen atom caused a 100-fold reduction in mutagenicity (see comparison of MX to compound 11).

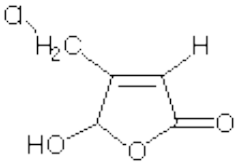
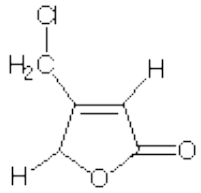
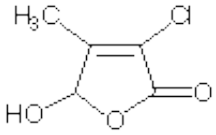
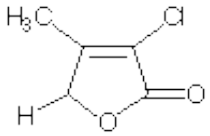
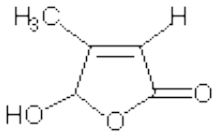
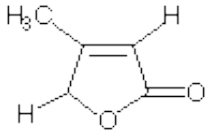
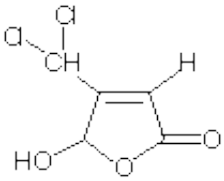
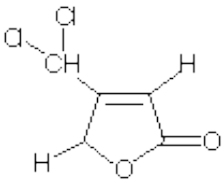
3) With a single chlorine atom attached to C-6 (compounds 3 - 6), the C-5 **OH-by-H** (compound 3 to compound 4) or C-3 **Cl-by-H** (compound 3 to compound 5) replacement resulted in a same order of 100-fold reduction on mutagenicity, respectively, but these are 10-fold less than that resulting from the C-6 **Cl-by-H** replacement (compound 3 to compound 7, which shows a 1000-fold reduction in mutagenicity). This means the C-6 **Cl-by-H** replacement has a greater effect on reducing mutagenicity than any other single replacement on this group of compounds.

4) Comparison of MX to compound 7 (a double chlorine-atom replacements at C-6), shows a 104-fold reduction on mutagenicity, which was the same order of magnitude as the product of a single C-6 Cl-by-H replacement from MX (10-fold) and a second replacement in compound 3 (103-fold).

5) Removal of all chlorine atoms from MX resulted in a reduction of the mutagenicity to roughly 10 rev/mmol (see compound 9) (Prival & Dunkel, 1989).

Table 1. Ames Salmonella Typhimurium TA100 Mutagenicity Comparisons of MX Structural Analogs

Compound	Structure	CAS No.	Mutagenicity
MX		[77439-76-0]	+ (103-104 rev/nmol)
2		[122551-89-7]	+ (1/10 of MX)
3		[125974-08-5]	+ (1/10 of MX)
4		[125974-01-8]	+ (1/1000 of MX) (10o-fold decreased from 3)

5		[125974-06-3]	+ (1/1000 of MX) (10o-fold decreased from 3)
6		[125973-99-1]	+ (1/10,000 of MX) (10-fold decreased from 5)
7		[112309-61-2]	+ (1/10,000 of MX) (1000-fold decreased from 3)
8		[134705-35-4]	-
9		[40834-42-2]	- (10 revertants/mmol); *(non-mutagenicity)
10		[6124-79-4]	-
11		[125974-07-4]	+ (1/100 of MX)
12		[125974-00-7]	erratic result

REFERENCES

Alkonen-Raatesalmi, A., and Hemminki, K. (1991) Assay for nucleoside and nucleotide binding of a potent mutagen, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone. Toxicology Letter 56, 167-172

Andrews, R.C., S.A. Daignault, C. Laverdure and P.M. Huck (1988) Occurrence of the mutagenic compound MX in Drinking Water and its Removal by Activated Carbon. Paper presented at the National Annual Conference of the American Water Works Association, Los Angeles, CA.

Brunborg, G., Holme, J.A., Søderlund, E.J., Hongslo, J.K., Vartiainen, T., Lötjönen, S., and Becher, G. (1991) Genotoxic effects of the drinking water mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone (MX) in mammalian cells in vitro and in rats in vivo. Mutation Research, 260, 55-64

Brunborg, G., Holme, J.A., Sderlund, E.J., and Dybing, E. (1990) Organ-specific genotoxic effects of chemicals: the use of alkaline elution to detect DNA damage in various organs of *in vivo*-exposed animals. Prog Clin Biol Res 340D, 43-52

Bull, R. J. and F. C. Kopfler (1991) Formation and Occurrence of Disinfection Byproducts in *Health Effects of Disinfectants and Disinfection By-Products*, American Water Works Research Association, Denver.

Chang, L.W., Daniel, F.B., and DeAngelo, A.B. (1991) DNA strand breaks induced in cultured human and rodent cells by chlorohydroxy-furanones-mutagens isolated from drinking water. Teratogenesis, Carcinogenesis, and Mutagenesis 11:103-114

Daniel, F.B., Robinson, M., Olson, G.R., and Stober, J.A., and Page, N.P., Toxicological Studies on MX, a disinfection by-product, *J. AWWA*, March 1994.

Daniel, F.B., Robinson, M., Olson, G.R., Stober, J.A., and Page, N.P. (1994) Toxicological studies on MX, a disinfection by-product. J. Am. Water Works Assoc. Vol 86, ISS3, 103-11

Daniel, F.B., Olson, G.R., and Stober, J.A. (1991) Induction of gastrointestinal tract nuclear anomalies in B6C3F1 mice by 3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone and 3,4-(dichloro)-5-hydroxy-2[5H]-furanone, mutagenic byproducts of chlorine disinfection. Environ. Mol. Mutagen. 17, 32-39

Dayan, A.D. (1993) Carcinogenicity and drinking water. Pharmacol Toxicol. 72(suppl. 1), S108-115

Furihata, C., Yamashita, M., Kinae, N., and Matsushima, T. (1992) Genotoxicity and cell proliferative activity of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone [MX] in rat glandular stomach. Wat. Sci. Tech. 25(11):341-345

Haataja, L., Vartiainen, T., Lampelo, S., Lötjönen, S., and Tuomisto, J. (1991) Binding of the strong bacterial mutagen, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) to bovine serum albumin. Toxicology Letters 59, 187-195

Holmbom, B., R. Voss, R. Mortimer and A. Wong (1984) Fractionation, Isolation and Characterization of Ames Mutagenic Compounds in Fraft Chlorination Efluents, *Env. Sci. Tech*., **18**: 333-337.

Horth, H., M. Fielding, T. Gibson, H.A. James and H. Ross (1989) Identification of Mutagens in Drinking Water. EC 9105 SLD. Water Research Center, P.O. Box 16, Medmenham, Marlow, Bucks, SL7 2HD, United Kingdom.

Meier, J.R., W.F. Blazak and R.B. Knohl (1987b) Mutagenic and Clastogenic properties of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furano ne, a potent bacterial mutagen in drinking water. *Environ. Molec. Mutagen.*, **10**: 411-424.

Ishiguro, Y., LaLonde, R.T., Dence, C. W., and Santodonato, J. (1987) Mutagenicity of chlorine-substituted furanones and their inactivation by reaction with nuclephiles. *Environ. Toxicol. Chem.* **6**, 935-946.

Jansson, K., Hyttinen, J.M.T. (1994) Induction of gene mutation in mammalian cells by 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX, a chlorine disinfection by-product in drinking water. Mutation Res. 322, 129-132

Jansson, K., Mäki-Paakkanen, J., Vaittinen, S.-L., Vartiainen, T., Komulainen, H., and Tuomisto, J. (1993) Cytogenetic effects of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) in rat peripheral lymphocytes in vitro and in vivo. Mutation Res. 229, 25-28

Koivusalo, M., Jaakkola, J.J.K., Vartiainen, T., Hakulinen, T., Karjalainen, S., Pukkala, E., and Tuomisto, J. (1994) Drinking water mutagenicity and gastrointestinal and urinary tract cancers: An ecological study in Finland, *American Journal of Public Health*, **84**: xx-yy.

Komulainen, H., Vaittinen, S.-L., Vartiainen, T., Lötjönen, Paronen, P., and Tuomisto, J. (1992) Pharmacokinetics in rat of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), a drinking water mutagen, after a single dose. Pharmacology & Toxicology 70:424-428

Komulainen, H., Kuuskonen, H., Kosma, V-L, Lötjönen, Vartiainen, T. (1994) Toxic effects and excretion in urine of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) in the rat after a single oral dose. Arch Toxicol 68:398-400

Kronberg, L. and T. Vartiainen (1988) Ames Mutagenicity and Concentration of the Strong Mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone and of its Geometric Isomer (E)-2-chloro-3-(dichloromethyl)-4-oxobutenoic acid in Chlorine-treated Tap Waters. *Mutation Res*., **206**:177-182.

LaLonde, R. T., Cook, G. P., Perakyla, H., and Lin, Bu. (1991c) Structure-activity relationship of bacterial mutagens related to 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone: An emphasis on the effect of stepwise removal of chlorine from the dichloromethyl group. *Chem. Res. Toxicol.* **4**, 540-545.

LaLonde, R. T., Cook, G. P., Perakyla, H., and Dence, C. W. (1991b) Effect on mutagenicity of the stepwise removal of hydroxyl group and chlorine atoms from 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone: 13C NMR chemical shifts as determinants of mutagenicity. *Chem. Res. Toxicol*. **4**, 35-40.

LaLonde, R. T., Cook, G. P., Perakyla, H., Dence, C. W., and Babish, J. G. (1991a) Salmonella typhimurium (TA100) mutagenicity of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone and its open- and closed-ring analogs. *Environ. Mol. Mutagen.* **17**, 40-48.

Matsumura, H., Watanabe, M., Matsumoto, K., Ohta, T. (1994) 3-Chloro-4-(Dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) induces gene mutations and inhibits metabolic cooperation in cultured Chinese hamster cells. J. Toxicol. Environ. Health 43:65-72

Meier, J.R., Blazak, W.F., and Knohl R.B. (1987) Mutagenic and clastogenic properties of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone: a potent bacterial mutagen in drinking water. Environ. Mol. Mutagen 10, 411-424

Meier, J.R. (1990) Mutagens in chlorinated water. Pro Clin Biol Res 340E:11-9

Meier, J.R., R.B. Knohl, W.E. Coleman, H.P. Ringhand, J.W. Munch, W.H. Kaylor, R.B. Streicher and F.C. Kopfler (1987a) Studies on the potent bacterial mutagen, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone: Aqueous stability, XAD recovery and analytical determination in drinking water and in chlorinated humic acid solution. *Mutation Res*., **189**: 363-373.

Meier, J.R., DeAngelo, A.B., Daniel, F.B., Schenck, K.M., Doerger, J.U. (1990) Genotoxic and carcinogenic properties of chlorinated furanones: important by-products of water chlorination. Available from National Technical Information Service, Springfield, VA as NTIS/PB90-119223, 22 p

Moller, H. (1992) Occurrence of carcinogens in the external environment epidemiological investigations. Pharmacol. Toxicol. 72(suppl. 1), S39-45

Padmapriya, A.A., Just, G., and Lewis, N.G. (1985) Synthesis of 3-Chloro-4-dichloromethyl-5-hydroxy-2-5H-furanone, a potent mutagen, *Can. J. Chem.* **63**, 828-832.

Vartiainen, T., K. Heiskanen, and S. Lotjonen (1991) Analysis and some chemical properties of MX, (3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone), the potent drinking water mutagen, *Fresenius'J. Anal. Chem*., **340**: 230-233.

Prival, M. J., and Dunkel, V. C. (1989) Reevaluation of the mutagenicity and carcinogenicity of chemicals previously identified as "false positives" in the Salmonella typhimurium mutagenicity assay. *Environ. Mol. Mutagen* **13**, 1-24.

Ringhand, H.P., Meier, J.R., Coleman, W.E., Schenck, K.M., Kaylor, W.H., Munch, J.W., and Robinson, M. (1990) Studies on MX: a potent mutagenic by-product of the chlorination of drinking water and kraft pulp mill effluents. Trace Subst. Environ. Health Vol. 23, 269-76

Ringhand, H.P., Kaylor, W.H., Miller, R.G., and Hopfler, F.C. (1989) Synthesis of 3-14C-3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone and its use in a tissue distribution study in the rat. Chemosphere 18 (11-12), 2229-2236

Risto, L., Vartiainen, T., and Komulainen, H. (1993) Formation of methemoglobin by 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone, MX, in rat erythrocytes in vitro. Toxicology Letter 68, 325-332

Tuomisto, J., and Vartiainen, T. (1989) Genotoxicity of drinking waters. International Agency for Research on Cancer, No. 104. Complex Mixtures and Cancer Risk; Workshop, ESPOO, Finland, May 14-17, 1989. X+441P. International Agency for Research on Cancer: Lyon, France. Illus. Maps. Paper. ISBN 92-832-2104-4.; 0(0), 307-313

Watanabe, M., Kobayashi, H., Ohta, T. (1994) Rapid inactivation of 3-chloro-4-dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), 2 potent mutagen in chlorinated drinking water, by sulfhydryl compounds. Mutation Res. 312, 131-138

BASIS OF NOMINATION TO THE CSWG

MX, a potent mutagen, has been identified in chlorine-treated drinking water and in chlorinated effluents of Kraft pulp mills. It has been found in drinking waters in Finland, Great Britain, Japan and the United States. *In vitro* tests have shown that MX is a strong mutagen in bacterial systems. MX exhibits direct-acting mutagenicity in the Ames assay with a strong response being induced in the strain TA 100. It could account for 30-60 percent of the mutagenicity of chlorinated drinking water. There is evidence of MX-induced toxicity and forestomach lesions in rats (Daniel et al., 1994). MX is clastogenic to cultured Chinese hamster ovary cells when there is no S-9. MX induces DNA strand breaks in a cultured lymphoblastoid human cell line.

INPUT FROM GOVERNMENT AGENCIES/INDUSTRY

The absence of cancer bioassay data on a number of chlorine-treatment by-products, of which MX is one, led the American Water Works Association Research Foundation to nominate MX for carcinogenesis bioassays to the NTP (R.P. McHugh to V. Fung, March 4, 1991). The National Association of Water Companies supported the nomination (C.A. Buescher to V. Fung, April 17, 1991).

A February 8, 1994 NIEHS/EPA Drinking Water Research meeting identified MX as an important candidate for the evaluation of toxicity/carcinogenicity (R. Melnick memo, February 23, 1994). The recommendation was forwarded to the NIEHS Chemical Nominations Committee (R. Melnick memo, February 24, 1994). The NIEHS Chemical Nominations Committee recommended the nomination of MX for future studies by the NTP (R. Melnick memo, September 9, 1994).

MX is currently being studied in a full-range two-year bioassay in rats at the Finland National Public Health Institute, Division of Environmental Health (Memo from J. Tuomisto to S. Stasiewicz, June 2, 1994).