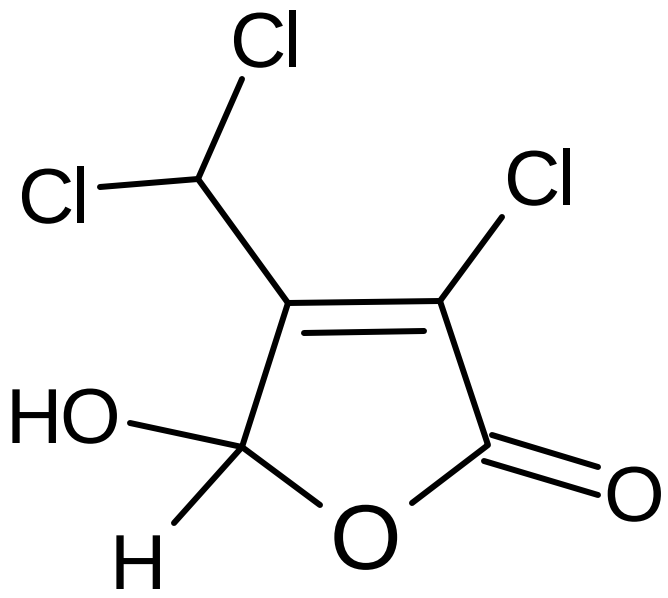
3-chloro-4-(dichloromethyl)-5-hydroxy-2(*5H*)-furanone (MX, Mutagen X)



Molecular Weight: 217.4 CAS Reg. No.: 77439-76-0

Occurrence of MX

- MX is a chlorination disinfection byproduct
  - forms from the reaction of chlorine with humic acids in raw water
- Levels in drinking water are low, ranging from 2 to 67 ng/L

Carcinogenicity of MX

- **Carcinogenicity in humans:**
  - **No direct data**
  - **Chlorinated water consumption has been associated with increases in cancers of the bladder and other sites in several studies.**
- **Carcinogenicity in experimental animals:**
  - **Drinking water studies in rats (Komulainen *et al.*, 1997)**
  - **MX has not been tested in mice.**

Carcinogenicity of MX (continued)

- **Authoritative body activity**
  - NTP has completed 13-week studies of MX in rats and mice, and carcinogenicity studies in rats and mice are scheduled.
  - US EPA Office of Water has recently developed a quantitative cancer assessment for MX, which is undergoing internal review.
  - IARC, FDA, NIOSH have not evaluated MX.

Male Wistar rats -- MX in drinking water for 104 weeks (Komulainen *et al.*, 1997; 2000)

Tissue	MX, mg/kg-d				p-value
	Control	0.4	1.3	5.0	(trend)
Liver					
carcinoma	0/50	0/50	2/50	1/50	0.1605
adenoma	0/50	1/50	2/50	4/50	0.0142
adenoma or carcinoma	0/50	1/50	3/50	5/50*	0.0066
cholangioma	0/50	0/50	1/50	4/50	0.0009
Adrenal gland					
cortical adenoma	5/50	2/50	7/50	14/50*	0.0001
Thyroid gland					
follicular carcinoma	0/49	1/50	9/50*	27/49*	<0.0001
follicular adenoma	2/49	20/50*	34/50*	21/49*	0.0045
follicular adenoma or carcinoma	2/49	20/50*	38/50*	44/49*	<0.0001

* Fisher Exact Test, $p \leq 0.05$

Tumors in male rats (continued)

Additional tumor sites, significant only by trend test

- basal cell skin tumors
- benign lung tumors
- Langerhans' cell tumors of the pancreas

Female Wistar rats -- MX in drinking water for 104 weeks (Komulainen *et al.*, 1997; 2000)

Tissue	MX, mg/kg-d				p-value
	Control	0.6	1.9	6.6	(trend)
Mammary gland					
adenocarcinoma	3/50	2/50	5/50	11/50*	0.0012
fibroadenoma	23/50	25/50	32/50	34/50*	0.0090
adenoma	0/50	0/50	3/50	1/50	0.1641
adenoma or adenocarcinoma	3/50	2/50	7/50	12/50*	0.0008
Liver					
carcinoma	1/50	1/50	3/50	0/50	0.7011
adenoma	1/50	1/50	1/50	10/50*	<0.0001
adenoma or carcinoma	2/50	2/50	4/50	10/50*	0.0001
cholangiocarcinoma	1/50	0/50	0/50	2/50	0.0828
cholangioma	0/50	4/50	10/50*	33/50*	<0.0001
cholangioma or cholangiocarcinoma	1/50	4/50	10/50*	34/50*	<0.0001
Adrenal gland cortical adenoma	5/50	10/50	12/50	16/50*	0.0098
Thyroid gland					
follicular carcinoma	1/50	3/49	6/50	22/50*	<0.0001
follicular adenoma	4/50	16/49*	36/50*	36/50*	<0.0001
follicular adenoma or carcinoma	5/50	18/49*	38/50*	47/50*	<0.0001

* Fisher Exact Test, $p \leq 0.05$

Tumors in female rats (continued)

Additional tumor sites, significant only by trend test

- thyroid follicular C-cell adenoma
- lymphoma and leukemia (combined)

Genotoxicity of MX

MX is a direct acting mutagen and clastogen.

~100 publications on the genotoxicity of MX

- **Bacterial assays**
  - **MX caused mutations in over 35 strains of bacteria. MX is one of the most potent mutagens ever tested in *S. typhimurium* TA100 and two other short-term assays**
- **Human and other mammalian cells *in vitro***
  - **MX caused mutations, chromosomal aberrations, sister chromatid exchanges, strand breaks, or unscheduled DNA synthesis**

Genotoxicity of MX (continued)

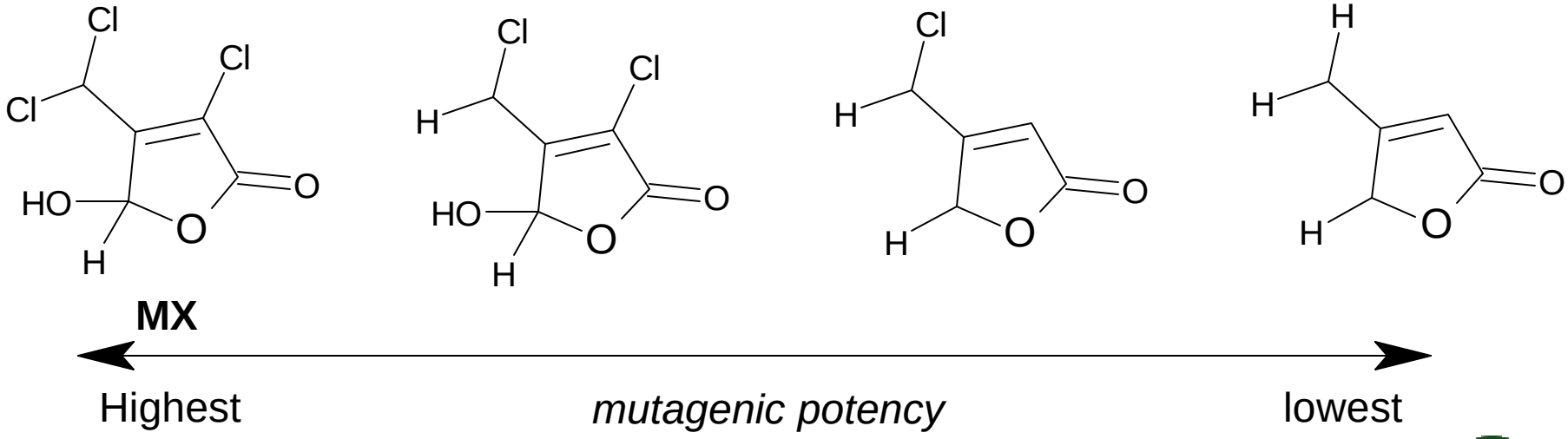
- **Rodents *in vivo***
  - **Oral or i.p. administration of MX to rodents induced strand breaks or alkali-labile sites, micronuclei or sister chromatid exchanges were observed in multiple tissues.**
  - **Some studies reported negative results which may be explained in part by tissue specificity and the time kinetics of MX-induced DNA damage and repair.**

Initiation/Promotion Studies

- **MX induced cellular proliferation in the stomach of Wistar rats**
  - **a site at which tumors were not observed in carcinogenicity studies in the same rat strain**
- **MX was reported to act as a tumor promoter in an initiation/promotion study of the glandular stomach in Wistar rats**

Structure-Activity Comparisons

- **Chlorinated or brominated furanones, structurally similar to MX**
  - **induced mutations in bacterial test systems.**
  - **Of the chlorinated furanones, only MX has been tested in cancer studies.**



Potential mechanisms of genotoxicity

- **DNA adduction**
  - 5 DNA adducts have been detected, *in vitro*
  - Based on studies of mutations induced by MX, guanine is expected to be a major site of adduction
    - no guanine adducts upon treatment of whole DNA with MX
- **Thermodynamic (non-covalent) mechanism**
  - MX has an unusually high reductive potential
  - MX may "pull" an electron from DNA, resulting in an ionized DNA base (e.g., $CG^{+\cdot}$)
  - Mutational hot spots correspond with ability to stabilize DNA free radical

Mechanism of MX-induced Thyroid Tumors

- **MX-induced thyroid tumors in rats are not likely due to proliferation resulting from thyroid hormone disruption.**
  - **No significant changes in TSH, T_4, T_3 were observed among short-term (1 wk or 3 wk) or long-term (104 wk) treatments with MX.**

MX: Summary

- **Animal evidence of carcinogenicity:**
  - Induction of tumors at multiple sites in both male and female rats following treatment via drinking water for 2 years
- **Other relevant evidence:**
  - Extensive genotoxicity evidence both *in vitro* and *in vivo*
  - Suggestive evidence that MX may induce cellular proliferation or promote tumors in some tissues