

Safety Data Sheet

Methotrexate

Division of Safety
National Institutes
of Health



WARNING!

THIS COMPOUND IS ACUTELY TOXIC, TERATOGENIC, AND EMBRYOTOXIC. IT IS READILY ABSORBED THROUGH THE SKIN, INTESTINAL TRACT, AND TRANSPLACENTALLY. AVOID FORMATION AND BREATHING OF AEROSOLS.

LABORATORY OPERATIONS SHOULD BE CONDUCTED IN A FUME HOOD, GLOVE BOX, OR VENTILATED CABINET.

AVOID SKIN CONTACT: IF EXPOSED, WASH WITH SOAP AND COLD WATER. AVOID RUBBING OF SKIN OR INCREASING ITS TEMPERATURE.

FOR EYE EXPOSURE, IRRIGATE IMMEDIATELY WITH LARGE AMOUNT OF WATER. FOR INGESTION, INDUCE VOMITING. REFER FOR GASTRIC LAVAGE. FOR INHALATION, REMOVE VICTIM PROMPTLY TO CLEAN AIR. ADMINISTER RESCUE BREATHING IF NECESSARY. REFER TO PHYSICIAN.

IN CASE OF LABORATORY SPILL, WEAR PROTECTIVE CLOTHING DURING CLEANUP. AVOID SKIN CONTACT OR BREATHING OF AEROSOLS. WASH DOWN AREA WITH SOAP AND WATER. SEE CASTEGNARO ET AL. (1985) FOR DETAILS. DISPOSE OF WASTE SOLUTIONS AND MATERIALS APPROPRIATELY.

A. Background

Methotrexate (MTX) is a bright yellow-orange powder or crystalline compound, stable in pure form and in neutral solution but unstable in excess acid or alkali or under ultraviolet light. It is acutely toxic in all mammalian species tested (LD50 in the 5-100 mg/kg range), teratogenic, and embryotoxic, but apparently not mutagenic; carcinogenicity has not been demonstrated. MTX is an antineoplastic inhibitor of folic acid metabolism, used in the treatment of lymphoblastic leukemia, osteogenic sarcoma, choriosarcomas, and various carcinomas of the testis, lung, cervix, etc. Intrathecal injection of MTX has also been used in the treatment of meningeal

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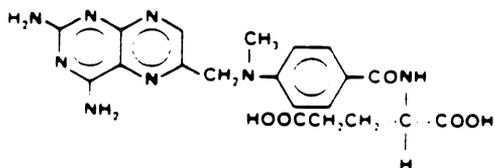
leukemias and lymphomas. MTX has also been used in the treatment of psoriasis, usually in topical application. Treatment of cancer patients with high doses of MTX may result in kidney toxicity but this can be prevented ("leucovorin rescue"). The main mechanism of MTX action is the strong (but reversible) inhibition of dihydrofolate reductase (DHFR), resulting in prevention of one-carbon transfer which is vital in DNA, RNA, and protein synthesis.

General reviews include: Bleyer (1978), Evans (1980), IARC (1981), Chabner (1982), Zaharko and Dedrick (1984), and Jackson (1984).

B. Chemical and Physical Data

Note on purity. MTX free acid powder for clinical use usually consists of the monohydrate. Many commercial samples of MTX contain several impurities of synthetic origin, sometimes amounting to 10-15% of material; those which have been identified have little or no DHFR inhibitory action and therefore probably no positive or negative therapeutic effect (Jordan et al., 1977; Chabner, 1982; Przybylski et al., 1982). However, it has more recently been noted that many commercial samples contain significant amounts of D-MTX (MTX containing D- instead of L-glutamic acid) which is a good enzyme inhibitor but a poor inhibitor of cell growth (Lee et al., 1974; Cramer et al., 1984).

1. Chemical Abstract No.: 59-05-2
2. Synonyms: Amethopterin; 4-amino-10-methylfolic acid; 4-amino-N¹⁰-methylpteroylglutamic acid; glutamic acid, N-[p-[[[(2,4-diamino-6-pteridinyl)methyl]-methylamino]benzoyl]-, L(+);^A methylaminopterin; CI-14377; NSC-740; R9985; NCI-CO4671.
3. Chemical structure and molecular weight:



$C_{20}H_{22}N_8O_5$; 454.5

Structures of major metabolites:

- a. 7-hydroxy-MTX;
- b. 2,4-diamino-N¹⁰-methylpteroic acid (DAMPA; MTX minus glutamic acid;

^AChemical Abstract name, used for listing in 5th Decennial Index and subsequently.

c. 4-amino-4-deoxy-N¹⁰-methylpterotic acid (ADPA; DAMPA minus C²-NH₂).

Other related compounds:

Folic acid: C⁴-OH instead of C⁴-NH₂; N¹⁰-H instead of N¹⁰-CH₃
Leucovorin (folinic acid, citrovorum factor): C⁴-OH; N⁵-CHO and saturated B-ring

Density: No data.

Absorption spectroscopy: Ultraviolet spectra in acid and alkali have been published (Seeger et al., 1949; Poe, 1977). Typical data are (λ_{max} , $\epsilon \times 10^{-3}$): in 0.1 N HCl: 307, 21.6; 243, 18.1 in 0.1 N NaOH: 370, 7.45; 302, 24.8; 258, 24.7 (Chamberlin et al., 1976). Other data deal with mass spectra (Przybylski et al., 1982) and fluorescent emission and absorption (Udenfriend et al., 1957).

Optical rotation: Stated as $[\alpha]_{589}^{21} = 20.4^\circ$ in 0.1 N NaOH (Chamberlin et al., 1976). It is assumed that this value refers to pure L-MTX (see Note on purity, above).

Volatility: No data; may be regarded as essentially non-volatile.

Solubility: Water solubility is strongly pH-dependent (1 mg/ml at pH 5.7, 10 mg/ml at pH 6.9) (Evans, 1980); more soluble in dilute alkali hydroxide or carbonate. Practically insoluble in ethanol, chloroform, and ether.

Description: Bright yellow-orange odorless powder or crystals, usually in the form of the monohydrate. Values for acid dissociation constants have been published (Poe, 1977; Schilsky 1983). MTX in solution at physiological pH is nearly completely ionized.

Boiling point: No data; melting point: 185-204°C as the monohydrate with decomposition.

Stability: MTX in neutral solution has high heat stability; in fact, such solutions can be boiled without degradation (Zaharko and Dedrick, 1984), a method used in total tissue analysis to free MTX from protein linkage. Solutions in 5% dextrose, used for parenteral injection, are stable (less than 10% change) in glass or plastic containers for 24 hours at room temperature (Benvenuto et al., 1981).^A The kinetics and mechanism of thermal and photolytic decomposition of MTX have been investigated (Chatterji and Gallelli, 1978; Hansen et al., 1983).

Irradiation of MTX in oxygenated solution leads to a number of photodynamic products which may be responsible for photosensitization in patients on high dose MTX therapy (Chahidi et al. 1983).

12. Chemical reactivity: MTX is subject to the usual reactions which may be expected from a molecule of this type (hydrolysis, oxidation, substitution on the benzene and heterocyclic rings, etc.). The most important biological reactions include combination with DHFR, hydroxylation at C⁷ by liver aldehyde oxidase, and combination with additional glutamic acid residues these will be discussed in F, below.
13. Flash point: No data.
14. Autoignition temperature: No data.
15. Explosive limits in air: No data.

C. Fire Explosion, and Reactivity Hazard Data

1. MTX is likely to be inactivated under conditions of fire. Fire-fighting personnel should wear protective clothing and face masks.
2. Flammability is likely to be low.
3. Conditions contributing to instability are acid, alkali, elevated temperatures, and prolonged exposure to ultraviolet light.
4. Hazardous decomposition products under conditions of fire are likely to include nitrogen oxides.

D. Operational Procedures

The NIH Guidelines for the Laboratory Use of Chemical Carcinogens describe operational practices to be followed when potentially carcinogenic chemicals are used in NIH laboratories. The NIH Guidelines should be consulted to identify the proper use conditions required and specific controls to be implemented during normal and complex operations or manipulations involving MTX.

It should be emphasised that this data sheet and the NIH Guidelines are intended as starting points for the implementation of good laboratory practices when using this compound. The practices and

^ASee, however, note on adsorbability under conditions of analysis (Section E, below).

procedures described in the following sections pertain to the National Institutes of Health and may not be universally applicable to other institutions. Administrators and/or researchers at other institutions should modify the following items as needed to reflect their individual management system and current occupational and environmental regulations.

Solutions of MTX penetrate various glove materials (Slevin et al., 1984). This factor should be taken into account when handling MTX.

1. Chemical inactivation: Validated methods have been reported (Castegnaro et al., 1985).
2. Decontamination: Turn off equipment that could be affected by MTX or the materials used for cleanup. If there is any uncertainty regarding the procedures to be followed for decontamination, call the NIH Fire Department (dial 116) for assistance. Consult Castegnaro et al. (1985) for details concerning decontamination of surfaces, glassware, and animal cages.
3. Disposal: It may be possible to decontaminate waste streams containing MTX before disposal. For details, see Castegnaro et al. (1985). No waste streams containing MTX shall be disposed of in sinks or general refuse. Surplus MTX or chemical waste streams contaminated with MTX shall be handled as hazardous chemical waste and disposed of in accordance with the NIH chemical waste disposal system. Nonchemical waste (e.g., animal carcasses and bedding) containing MTX shall be handled and packaged for incineration in accordance with the NIH medical-pathological waste disposal system. Potentially infectious waste (e.g., tissue cultures) containing MTX shall be disinfected by heat using a standard autoclave treatment and packaged for incineration, as above. Burnable waste (e.g., absorbent bench top liners) minimally contaminated with MTX shall be handled as potentially infectious waste and packaged for incineration, as above. Absorbent materials (e.g., associated with spill cleanup) grossly contaminated shall be handled in accordance with the chemical waste disposal system. Radioactive waste containing MTX shall be handled in accordance with the NIH radioactive waste disposal system.
4. Storage: Store solid MTX and its solutions in dark-colored, tightly closed containers, preferably under refrigeration. Avoid exposure to light and moisture. Store working quantities of MTX and its solutions in an explosion-safe refrigerator in the work area.

Monitoring and Measurement Procedures Including Direct Field Measurements and Sampling for Subsequent Laboratory Analysis

Notes:

- a. While adsorption of MTX from solution on glass or plastic appears to be negligible when dealing with MTX concentrations such as those used for parenteral administration, it becomes considerable for very low concentrations encountered in preparation of samples for analysis, particularly when water-soluble organic solvents are used. This also includes adsorption on syringes used in HPLC injection, and may be a source of variability in analytical results (Chen and Chiou, 1982a).
 - b. Analytical procedures in use through 1979 have been summarized (Chamberlin et al., 1976; IARC, 1981).
1. Sampling: Methods for preparation of biological samples have been reviewed (Stout et al., 1985). The two main approaches to deproteinization are:
 - a. precipitation of protein with perchloric acid (Watson et al., 1978), acetonitrile (Chen and Chiou, 1981), or boiling (Krakower and Kamen, 1983) followed by extraction; and
 - b. the use (mainly in combination with HPLC) of precolumns (Tony et al., 1980; Breithaupt et al., 1982; So et al., 1985).
 2. Analysis: Critical reviews of various analytical procedures applicable to biological materials have been published (Overdijk et al., 1975; Falk et al., 1976; Breithaupt et al., 1982; Zaharko and Dedrick, 1984). Earlier methods include microbiological, fluorometric, and radiolabeling analysis; these methods generally suffer from lack of sensitivity or specificity, or require prolonged time for assay. They have been largely superseded by one of the following three types of analysis:
 - a. enzymatic: Based on inhibition of DHFR in presence of NADPH₂ (Kamen et al., 1976; Falk et al., 1976). The lower limit of sensitivity in serum is 10 µg MTX per liter. This method is fast and simple, but suffers from variable cross-reactions with MTX metabolites and indigenous folic acid derivatives in biological samples. Interference by the latter group of substances has been eliminated by combination of DHFR inhibition with radioassay (Arons et al., 1975).
 - b. immunoassay: This method is specific in the presence of folate analogs, except in very high concentrations, and has sensitivities of the order of 100 pg MTX (Raso and Schreiber, 1975; Loeffler et al., 1976; Paxton and Rowell, 1977). It has been fully automated for clinical use (Kamel

et al., 1980; Oellerich et al., 1980). There appears to have been no investigation whether there is interference from MTX metabolites.

- c. high-pressure liquid chromatography: With the emergence of the necessity for measuring concentrations of the MTX metabolite, 7-OH-MTX, in addition to MTX because of its clinical importance, this method has become the one of choice. Simultaneous measurement of both compounds has been described (Watson et al., 1978; Tony et al., 1980; Cohen et al., 1980; Chen and Chiou, 1981; Buice and Sidhu, 1982; So et al., 1985). Either ultraviolet or fluorescence detectors are used. Sensitivity limits are of the order of 20 ng/ml (Stout et al., 1985).

Biological Effects (Animal and Human)

Absorption: MTX is absorbed and produces biological effects after parenteral (intravenous, intraperitoneal) injection and by ingestion. Absorption by this route is virtually complete in man at low dosages (30 mg/m² or less) (Shen and Azarnoff, 1978), but incomplete at higher dosages. In studies of the treatment of psoriasis with MTX it has also been demonstrated that MTX is absorbed through human and mouse skin in vitro (Newbold and Stoughton, 1972; Wallace et al., 1978) and this has been confirmed in mice (Ball et al., 1982). However, systemic effects of MTX administered by this route have not been demonstrated.

Distribution and pharmacokinetics: Parenterally administered MTX is partially bound to serum protein and then distributed, with major amounts appearing in the intestine, urine, and liver in several species (Henderson et al., 1965a,b). There is prolonged retention in the liver and kidney of rat (Scheufler et al., 1981) and rabbit (Chen and Chiou, 1982b). Elimination from plasma has been described as triphasic in the rat, with terminal half-life of 4.2 hours after intravenous MTX (Scheufler et al., 1981) and as "multi-exponential" in man (Shen and Azarnoff, 1978); however, these results are somewhat indeterminate because of extensive intestinal metabolism of MTX leading to high plasma concentrations of metabolite(s). MTX is actively transported across cell membranes; the mechanism of this reaction has been reviewed (Goldman and Matherly, 1985). Pharmacokinetic data for the rabbit (Iven et al., 1985) and man (Bleyer, 1978; Shen and Azarnoff, 1978) have been published. It is interesting to note that while the blood-brain barrier is not readily crossed by MTX, there is a considerable uptake into solid brain and meningeal tumors (Shapiro et al., 1979).

Metabolism and excretion: MTX undergoes both anabolic and catabolic reactions in animals and man. The former consist of addition of further glutamic acid residues to the one already

present in the molecule, with the formation of MTX polyglutamates. The total number of these glutamic acid residues appears to be at least seven. The MTX polyglutamates inhibit DHFR as well as does MTX, and have been detected in skin and other organs of animals after parenteral MTX treatment (Krakower and Kamen, 1983; Zimmerman et al., 1984). Among catabolic products of MTX metabolism, the most important one appears to be 7-hydroxy MTX, produced by the action of liver aldehyde oxidase as well as by intestinal bacterial enzymes during enterohepatic circulation (Bleyer, 1978). The activity of this enzyme is highly species-specific, being high in the rabbit, moderate in the guinea pig, and low in the mouse and rat (Johns et al., 1966). Because of its lower aqueous solubility in acidic media as compared with MTX, 7-OH-MTX is believed to be mainly responsible for the nephrotoxicity of MTX (Zaharko and Dedrick, 1984). This oxidation is rapid, and as a result the concentration of 7-OH-MTX exceeds that of MTX in animal cells shortly after exposure in vitro (Fabre et al., 1985) and in vivo (Erttmann et al., 1985). Other catabolic products include DAMPA and ADPA (for identification see B3) which are considerably less toxic than MTX (Valerino, 1972). It has not been determined whether these metabolites are the result of intracellular metabolism of MTX or of action by bacterial enzymes and subsequent partial reabsorption. Excretion is mostly in the form of unchanged MTX or 7-OH-MTX in the urine, with minimal amounts in the feces.

Toxic effects: The acute intraperitoneal LD50 of single doses of MTX is 94 and 6-25 mg/kg in mice and rats, respectively. When this dose was divided into five daily doses the corresponding toxicities were 9.7 and 5.6 mg/kg total dose. Death occurred in 3-7 days with no symptoms during the first day but development of anorexia, diarrhea, and weight loss. The oral LD50 in rats is 180 mg/kg and the intravenous LD50 in the dog around 50 mg/kg. The maximum tolerated dose in man is stated to be 80-900 mg/m² without and 900-30,000 mg/m² with "citrovorum rescue" therapy (Bleyer, 1978).

Toxic effects in animals and patients have been summarized (IARC, 1981); they include action on the hematopoietic system (leucopenia, reticulocytopenia, platelet decrease), myelosuppression, gastrointestinal tract (mucositis, ulceration), liver, and kidney. In the latter organ renal failure is due to precipitation of MTX or 7-OH-MTX in the tubules under the usual acidic conditions but can be prevented by alkalinization (administration of sodium bicarbonate during MTX treatment) (Stoller et al., 1975). Intrathecal treatment of meningeal tumors is often accompanied by neurotoxic effects (Bleyer, 1978). Occasionally an acute pneumonitis with eosinophilia has been noted during prolonged treatment with MTX for skin disorders (Whitcomb et al., 1972).

The chief mechanism of toxic action of MTX is the stoichiometric, competitive, and reversible inhibition of dihydrofolate reductase (DHFR), reviewed by Jackson and Grindey (1984). A schematic drawing of the combination of MTX with the active site of this enzyme has been published (Roth, 1986). The inhibition results in depletion of 1-carbon carrying tetrahydrofolate cofactors, important in the synthesis of thymidilic and inosinic acids which are necessary for DNA and RNA synthesis, respectively. While this binding is normally very tight (MTX-DHFR complexes remain for many months in animal tissues), under conditions of massive MTX therapy it can be reversed by simultaneous administration of leucovorin. It should be noted that animals survived with no gross abnormalities when liver and kidney DHFR was completely inhibited, which might indicate that in these organs DHFR is either physiologically not essential or is resynthesized. In vitro evidence indicates that accumulation of dihydro- and oxidized MTX polyglutamates may contribute to the cytotoxic effects of MTX (Baggott et al., 1986).

5. Carcinogenic effects: All pertinent information has been described in detail (IARC, 1981). There is no evidence of carcinogenicity in all animal species studied due to MTX administration.
6. Mutagenic and teratogenic effects: Most studies have shown MTX to be not mutagenic in the Ames test (Benedict et al., 1977; Simmon, 1979, and others); a more recent publication has presented evidence that in somatic cells of *Drosophila* MTX is "recombinogenic and most probably mutagenic" (Würgler et al., 1983) but this has not been confirmed. MTX is embryotoxic in man, rat, rhesus monkey, and rabbit (reviewed by Skalko, 1974) and produces malformations in the cat (Khera, 1976) and in vitro in rat embryos (Schmid, 1984).

Emergency Treatment

1. Skin and eye exposure: For skin exposure, remove contaminated clothing and wash skin with soap and water. Since MTX is readily absorbed through the skin, avoid rubbing of skin or increasing its temperature. For eye exposure, irrigate immediately with sodium bicarbonate solution, followed by copious quantities of running water for at least 15 minutes. Obtain ophthalmological evaluation.
2. Ingestion: Induce vomiting. Refer for gastric lavage.
3. Inhalation: Remove victim promptly to clean air. Administer rescue breathing if necessary.
4. Refer to physician.

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