Common Name: **Malachite Green Chloride;** Acryl Brilliant Green; Aniline Green; Aizen Malachite Green; Astra Malachite Green; Basic Green 4; Benzal Green; China Green; C.I. Basic Green 4; Diamond Green; Victoria Green; Fast Green.

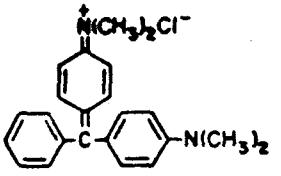
Malachite Green Oxalate; C.I. Basic Green 4; C.I. Basic Green 4, oxalate.

Chemical Name: **Malachite Green Chloride;** Methanaminium, N-(4-((4-(dimethylamino)phenyl)phenylmethylene)-2,5-cyclohexadien-1-ylidene)-N-methyl-, chloride.

Malachite Green Oxalate; Methanaminium, N-(4-((4-(dimethylamino)phenyl)phenylmethylene)-2,5-cyclohexadien-1-ylidene)-N-methyl-, ethanedioate.

CAS No: **Malachite Green Chloride** (569-64-2)
Malachite Green Oxalate (18015-76-4)

Chemical Structure:



Production:

No. of U.S. manufacturers; No information.

Production volumes; (1972) > 4.54×10^2 Kg. (1975) 1.45×10^5 Kg.

No. of pounds imported; (1972) 9.6×10^4 Kg. (1975) 3.03×10^4 Kg.

Use: Major and Minor Uses:

Malachite green has several diverse uses. It has been used as an antibacterial and antifungal agent, as a dye for cloth and leather, as an histological stain, as an intestinal anthelmintic and as a wound disinfectant (Nelson, 1974). Although malachite green is not approved for use on any aquatic animal species, in fish hatcheries, malachite green has been widely used as an antifungal agent for the treatment of fungal infections in brood fish and fish eggs (Nelson, 1974). It is considered by many in the industry as the most efficacious agent for combatting fungal infections in aquatic species (Schnick, 1988). In a 1989 review, Meyer and Schnick presented an extensive table of potential antifungal agents, all of which were compared to the activity of malachite green as the standard. Out of the 187 compounds and formulations evaluated in this review it was concluded that none equaled malachite green for animal safety and efficacy. Malachite green is also effective against certain external protozoan and bacterial infections (Nelson, 1974). Because of its effectiveness, this chemical is considered to have a very high probability of abuse. There is an especially strong potential for diversion from domestic nonfood fish use into the food fish arena. The extent of malachite green use in various categories of food fish

(for example, fingerlings, juveniles and adults) currently is unclear.

Malachite green may be applied as an aqueous solution in tanks, troughs, raceways and ponds for fixed or extended treatments; used as a dip or applied topically (Nelson, 1974). It has the potential for a profound effect on aquatic ecology. Since the aquaculture industry is growing rapidly, it is difficult to estimate the number of facilities which could benefit from malachite green or the number that may be using it already. There are at least 100 Federal and State fish hatcheries throughout the country as well as an unknown number of commercial facilities. As recently as April of this year, The Center for Veterinary Medicine received inquiries from the State of Alaska Department of Fish and Game requesting the legal options for some 14 hatcheries within the state currently using malachite green on a routine basis. The 1992 catalog of a major distributor of aquaculture drugs offers malachite green for sale in units up to 100 lb barrels, albeit with a note that the compound is not for use in food animals.

Occupational Exposure:

Because of the various modes of administration, the use of this product could result in significant occupational exposure for workers in the aquaculture facilities.

Environmental Exposure:

The effluent from the aquaculture facilities could enter the water supply resulting in exposure of the general public through recreational activities and drinking water. Finally, the use of malachite green in food fish could result in human consumption of malachite green residues.

Regulatory Status:

Who regulates; Malachite green is not approved for use as a drug in food or non-food animals. Its use is restricted to state and federal hatcheries that hold special Investigational New Animal Drug permits from the FDA. No occupational exposure limits have been established by OSHA, ACGIH, or NIOSH. The chloride is subject to SARA Section 313 Annual Toxic Chemical Release reporting.

How listed (CERs); No information.

Mutagenicity/Genetic Toxicology:

In vitro;

Prokaryotic; No mutations were detected in Salmonella typhimurium strains TA 100, TA 1535, or TA 1537 with or without S-9 being added, but in TA 98 there was evidence of mutagenesis, only after S-9 activation (Clemmensen et al., 1984). Ferguson and Baguley (1988) reported negative findings in TA 100, TA 1537 and TA 98 with S-9 being added. An increase in DNA damage was detected in a pol A^- (polymerase deficient) strain of E. coli by Rosenkranz and Carr (1971).

Eukaryotic; Numerous chromosome defects were reported after treatment of trout eggs with therapeutic concentrations of malachite green (Lieder, 1961).

Malachite green also was positive as an inducer of a respiration-deficient mutation in yeast (Nagai, 1959) and induced chromosome damage in CHO cells (Au and Hsu, 1979).

In vivo;

The micronucleus test on mice was negative (Clemmensen et al., 1984) as was the mouse spot test (Styles and Penman, 1985).

Toxicological Effects:

Animals;

Acute; The oral LD_{50} of the chloride is 80 mg/kg in mice (OHS13533, 1990). The oral LD_{50} of the oxalate in mice is approximately 50 mg/kg, in rats it has been estimated as 275 mg/kg (Clemmensen, et al, 1984). Both may cause eye irritation (OHS13533, 1990; OHS60321 1991), but a 20% suspension of the oxalate applied to the skin of rats or guinea pigs caused no visible signs of irritation (Clemmensen, et al, 1984). Acute toxicity of malachite green in rats apparently is related to an effect on the cytochrome c respiratory system, uncoupling oxidative phosphorylation (Werth and Boiteux, 1967). In dogs malachite green acts directly on the renal tubule to cause an increase in electrolyte excretion and water which indirectly alters hemodynamics (Lavender and Pullman, 1964).

Subchronic; Repeated dosing of rats with the oxalate at levels of 10, 100, or 1000 ppm for 228 days caused no clinical signs other than hyperactivity at the highest level and caused weight gain reduction and reduced food intake at this level also. In the highest dosed females there was an increase in lymphocytes and a decrease in neutrophils. In males dosed at this level there was an increase in plasma urea (Clemmensen, et al, 1984).

Chronic/carcinogenic; Werth (1958) reported an increased number of tumors in several generations of untreated offspring from a study in which the ancesters had been treated with malachite green. The interpretation of these results is uncertain. Nevertheless, Fernandes et al (1991) have reported evidence suggesting that malachite green may be more potent as a promoter of liver carcinogenesis than phenobarbital. Treatment of rats with 25 ppm malachite green in the drinking water for 2.5 months enhanced the development of GGT-positive foci induced by diethylnitrosamine (DEN). Malachite green or DEN alone induced clear and eosinophilic foci. Further studies will be required to determine whether the effect of malachite green was a syncarcinogenic or strictly promoting effect.

Human;

Acute;

The chloride may cause severe eye irritation or damage (OHS13533, 1990). A single occupational exposure may induce sensitization reactions (Nelson, 1974).

Subchronic;

No data available.

Chronic/carcinogenic;

The chloride may cause conjunctivitis or dermatitis (OHS13533, 1990).

Reproductive/Developmental Effects:

Animals;

Malachite green is teratogenic to rabbits at doses of 5, 10, and 20 mg/kg, causing visceral and skeletal anomalies (Nelson, 1974). Inheritable malformations thought to have been induced by malachite green in rats have been reported by Werth and Hirth (1968).

Human;

No data found.

Immunotoxicology:

Animals;

No data found.

Human;

No data found.

Chemical Disposition:

Animals;

Disposition and metabolism studies have been carried out by the National Fisheries Research Center at La Crosse, Wisconsin. They have examined uptake and elimination of Malachite Green in rainbow trout eggs and fry and in adults. Under simulated use conditions (at 1 mg/liter). The concentration of MG continued to increase in eggs or fry during the exposure period to a maximum of 300.22 ng/g tissue. Withdrawal of treatment resulted in an estimated half-life of 14.32 days. Leuco malachite green was the predominant metabolite found. Adult trout were exposed to 1 mg/liter MG for one hour and examined immediately or 5 days after exposure. In samples taken immediately MG residues amounted to an equivalent of 1 ug/g tissue, 28.8% as the parent compound and 45.4% as leuco malachite green. At 5 days total residue amounted to 0.37 ug/g, with 2.6% parent compound and 58.7% leuco MG. Detoxication of MG to leuco MG by channel catfish (Poe & Wilson, 1983) and rats (Werth & Boiteux, 1968) has also been reported.

Human;

No data found.

Structure/Activity: Studies of chemicals with similar structure.

Genotoxicity;

Prokaryotic;

Increases in DNA damage due to gentian violet, crystal violet and methyl violet were detected in a pol A⁻ (polymerase deficient) strain of E. coli by Rosenkranz and Carr (1971). Structure function relationships among the triphenylmethane dyes are discussed by Combs and Haveland-Smith (1982). Leuco gentian violet and leuco pentamethylpararosaniline, important metabolites of gentian violet, both have been reported to be mutagenic (Hass et al, 1986) in S. typhimurium strain TA98.

Eukaryotic;

Crystal violet, ethyl violet, methyl violet, pararosaniline, rosanaline, victoria blue B and Victoria blue 4R were all positive as inducers of a respiration-deficient mutation in yeast (Nagai, 1959), and brilliant crystal blue, crystal violet, and gentian violet all induced chromosome damage in CHO cells (Au and Hsu, 1979). See Combs and Haveland-Smith (1982) for a discussion of structure function relationships among the triphenylmethane dyes.

Animals;

Among structurally similar compounds benzyl violet 4B has been clearly shown to be carcinogenic in the rat (Ikeda et al., 1974), and gentian violet was found to induce hepatocellular carcinomas and Harderian gland adenomas in both sexes and reticulum cell sarcomas of the urinary bladder, ovaries, uterus and vagina in female B6C3F1 mice (Littlefield et al., 1985). In a related study gentian violet gave no carcinogenic response in a 3 generation study in male and female Fischer 344 rats (Littlefield, 1988). Leuco gentian violet and leuco pentamethylpararosaniline were important metabolites of gentian violet found in liver, kidney, muscle and fat of B6C3F1 mice and F344 rats. These leuco metabolites were in very high levels, particularly in fat (McDonald, 1989) and both have been reported to be mutagenic (Hass et al, 1986) in S. typhimurium strain TA98.

Human;

No data found.

Recommendations:

Combination of metabolism, reproduction/development, genotoxicity and carcinogenesis studies.

Priority:

High.

References:

Au W., and Hsu, T. (1979) Studies on the clastogenic effects of biologic stains and dyes. Environ. Mutagen. 1: 27-35.

Clemmensen, S., Jensen, J., Jensen, N., Meyer, O. Olsen, P., and Wurtzen, G.

(1984) Toxicological studies on malachite green: A triphenylmethane dye. Arch. Toxicol. 56: 43-45.

Combs, R., and Haveland-Smith, R. (1982) A review of the genotoxicity of food, drug and cosmetic colours and other azo, triphenylmethane and xanthene dyes. Mutat. Res. 98: 101-248.

Ferguson, L., and Baguley, B. (1988) Verapamil as a co-mutagen in the Salmonella/mammalian microsome mutagenicity test. Mutat. Res. 209: 57-62.

Fernandes, C., Lalitha, V., and Rao, K. (1991) Enhancing effect of malachite green on the development of hepatic pre-neoplastic lesions induced by N-nitrosodiethylamine in rats. Carcinogenesis 12: 839-845.

Hass, B.S., Heflich, R.H., and McDonald, J.J. (1986) Evaluation of the mutagenicity of crystal violet and its metagolites in Salmonella typhimurium and Escherichia coli. Environmental Mutagenesis 8: 36.

Ikeda, Y., Horiuchi, S., Imoto, A., Kodama, Y., Aida, Y., and Kobayashi, K. (1974) Induction of mammary gland and skin tumours in female rats by the feeding of benzyl violet 4B. Toxicol. 2: 275-284.

Lavender, A., and Pullman, T. (1964) The renal actions of malachite green, a diruretic dye. J. Pharmacol. Exp. Ther. 146: 87-91. [CA]

Lieder, U. (1961) The effect of cancerogen and mutagen, malachite green, on the mitosis of fish and fish eggs. Naturwissenschaften 48: 437-438. [CA]

Littlefield, N., Blackwell, B-N., Hewitt, C., and Gaylor, D. (1985) Chronic toxicity and carcinogenicity studies of gentian violet in Mice. Fund. Appl. Toxicol. 5: 902-912.

Littlefield, N. (1988) NCTR Technical Report for Experiment Numbers 305, 354, 355. Three-Generation Reproduction and Toxicity Studies of Gentian Violet in Fischer 344 Rats. National Center for Toxicological Research, eJefferson, Arkansas.

McDonald, J. J. (1989) NCTR Technical Report for Experiment 302, 303. Metabolism of Gentian Violet in Fischer 344 Rats and B6C3F1 Mice. National Center for Toxicological Research, Jefferson, Arkansas.

Meyer, F.P., and Schnick, R.A. (1989) A review of chemicals used for control of fish diseases. Rev. Aquat. Sci. 1(4): 693-710.

Nagai, S. (1959) Induction of the respiration-deficient mutation in yeast by various synthetic dyes. Science 130: 1188-1189.

National Fisheries Research Center. Ege-Finch, M.K , Gengerich, W. H., Allen, J. L., and Meinertz, J. R. A simulated use-pattern study of malachite green uptake and elimination in rainbow trout (Salmo gairdneri) eggs and fry.

National Fisheries Research Center. Malachite green residues in muscle of adult rainbow trout treated with the chemical.

eggs and fry.

Nelson, C. (1974) A review of the literature on the use of malachite green in fisheries. U. S. Department of the Interior, Fish and Wildlife Service, Western Fish Disease Laboratory.

OHS13533 (1990) Occupational Health Services, Inc. Material Safety Data Sheet: Malachite Green Chloride.

OHS60321 (1991) Occupational Health Services, Inc. Material Safety Data OSheet: Malachite Green Oxalate.

Poe, W.E., and Wilson, R.P. (1983) Absorption of malachite green by channel catfish. Prog. Fish-Cult. 45: 228-229. [CA]

Rosenkranz, H. and Carr, H. (1971) Possible hazard in use of gentian violet. Brit. Med. J. 3: 702-703.

Schnick, R.A. (1988) The impetus to register new therapeutants for aquaculture. Progr. Fish Culturist 50: 190-196.

Styles, J., and Penman, M. (1985) The mouse spot test : Evaluation of its performance in identifying chemical mutagens and carcinogens. Mutat. Res. 154: 183-204.

Werth, G. (1958) Disturbances of the heredity pattern and formation of tumors by experimental tissue anoxia. Arzneimittel-Forsch. 8: 735-744. [CA]

Werth, G., and Boiteux, A. (1967) Biological effects of malachite green. II. The intoxication syndrome and erythrocyte oxygen saturation under the influence of the dye. Arzneim. Forsch. 17: 155-158. [CA]

Werth, G., and Boiteux, A. (1968) Toxicity of triphenylmethane dyes. Malachite green as a uncoupling agent of oxidative phosphorylation in vivo and in vitro. Archiv. fur Toxikol. 23: 82-103. [CA]

Werth, G., and Boiteux, A. (1968) Biological activity of malachite green. VI. Detoxication of malachite green by formation of leucomalachite green. Arzneim. Forsch. 18: 39-42. [CA]

Werth, G. and Hirth, R. (1968) Die wirkung von Thalidomid auf die Missbildungsrate eines durch malachitgrun genetisch geschadigten rattenstammes. Archiv fur Toxikol 23: 104-111.

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