Cycasin

Safety Data Sheet

Division of Occupational Health and Safety
National Institutes of Health

WARNING!

This compound is absorbed through the intestinal tract and transplacentally. It is toxic and carcinogenic. 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 water.

For eye exposure, irrigate immediately with large amounts of water. For ingestion, drink water. Induce vomiting. 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 water, then soap and water. Dispose of waste solutions and materials appropriately.

A. Background

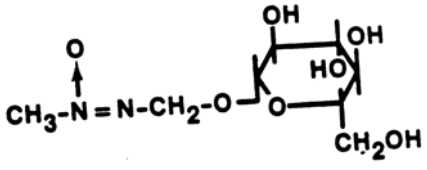
Cycasin is a crystalline, physically stable, water-soluble solid. It is a natural constituent of seeds and other portions of cycad plants. The biologically active portion of cyasin is its aglycone, methylazoxy-methanol (MAM). Because of physical instability of MAM, much experimental biological work has been carried out with its synthetic acetate derivative. Cycasin and MAM are highly toxic in humans and rodents (liver being the chief target organ) and carcinogenic in rodents. MAM, but not cycasin, is mutagenic in the Ames test, and both are teratogenic. There is no commercial use for these compounds.

B. Chemical and Physical Data

Cycasin

1. Chemical Abstract No.: 14901-08-7
2. Synonyms: Cykazine; B-D-Glucosyloxyazoxymethane; B-D-Glucosyloxyazoxymethase; Methylazoxymethanol-β-D-glycoside; (Methyl-ONN-azoxy)methyl-β-D-glucopyranoside; B-D-Glucopyranoside, (methyl-ONN-azoxy)methyl (9CI);
3. Molecular formula, weight and structure:

   $C_8H_{16}N_2O_7$; 253.23

4. Density: No data.

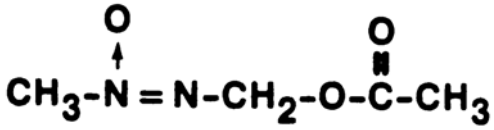
5. Absorption spectroscopy: UV absorption spectra have been reported (Riggs, 1956); λ (log ε)= 218 (3.86).
6. Volatility: No data; may be considered non-volatile for practical purposes.
7. Solubility: Very soluble in water and dilute ethanol; less soluble in absolute ethanol; insoluble in organic solvents.
8. Description, appearance: Colorless, long, needle-like crystals.
9. Boiling Point: No data.

   Melting Point: Reported as 144-145ºC with decomposition (Nishida et al., 1955) and 154ºC with decomposition (Riggs, 1956).
10. Stability: No quantitative data; easily hydrolyzed by treatment with alkali.
11. Chemical reactivity: Easily hydrolyzed, especially under alkaline conditions, to yield nitrogen, formaldehyde, and methanol among other products.
12. Flash point: No data.
13. Autoignition temperature: No data.
14. Explosive limits in air: No data.

MAM acetate (Kobayashi and Matsumoto, 1965)

1. Chemical Abstract No.: 592-62-1
2. Synonyms: MAMA; Methylazoxymethanol acetate; MAM Ac; Methylazoxymethyl acetate; MAM OAc; Methyl-(ONN)azoxy-methanol acetate; Methanol, (methyl-ONN-azoxy)-acetate (ester) (9CI).
3. Molecular formula, weight and structure:

   $C_4H_8N_2O_3$; 132.12



4. Density: No data
5. Absorption spectroscopy: UV: λ (log ε)= 215 (3.929).
6. Volatility: No data
7. Solubility: Slightly soluble in water and ether; completely miscible with chloroform.
8. Description, appearance: Colorless liquid.
9. Boiling point: 191ºC

   Melting point: No data.
10. Stability: Very stable in aqueous solution (a 10^{-4} M solution is unchanged after 30 minutes at 75ºC).
11. Chemical reactivity: Hydrolyzed by acids and alkali.
12. Flash point: No data.
13. Autoignition temperature: No data.
14. Explosive limits in air: No data.

C. Fire, Explosion, and Reactivity Hazard Data

1. Cycasin and MAM acetate do not require special fire-fighting procedures or equipment and do not present unusual fire and explosion hazards.
2. No conditions contributing to instability have been reported.
3. No incompatibilities are known.
4. Cycasin and MAM acetate do not require non-spark equipment.

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 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 cycasin.

1. Chemical inactivation: No validated method reported.
2. Decontamination: Turn off equipment that could be affected by cycasin or the materials used for cleanup. If more than 1 g has been spilled or if there is any uncertainty regarding the procedures to be followed for decontamination, call the NIH Fire Department (dial 911) for assistance. Wash surfaces with copious quantities of water. Glassware should be rinsed (in a hood) with water, followed by soap and water. Animal cages should be washed with water.
3. Disposal: No waste streams containing cycasin shall be disposed of in sinks or general refuse. Surplus cycasin or chemical waste streams contaminated with cycasin shall be handled as hazardous chemical waste and disposed of in accordance with the NIH chemical waste disposal system. Non-chemical waste (e.g., animal carcasses and bedding) containing cycasin 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 cycasin 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 cycasin shall be handled as potentially infectious waste and packages 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 cycasin shall be handled in accordance with the NIH radioactive waste disposal system.
4. Storage: Store in glass containers (no particular precautions necessary).

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

1. Sampling: No data.
2. Separation and analysis: GC has been employed for the estimation of cycasin in cycad flour, after trimethylsylylation of the extracted material (Wells et al., 1968). Other methods for the same purpose are extraction and column chromatography (Nishida et al., 1955) or paper chromatography (Nishida et al., 1956) followed by colorimetric analysis based on reduction of ferricyanide by the reducing sugars. The working limit of detection of these methods is about 50 mg. A colorimetric method that is based on liberation of formaldehyde from cycasin, other methylazoxyglycosides, and MAM, and its estimation with chromotropic acid, has been reported (Matsumoto and Strong, 1963).

F. Biological Effects (Animal and Human)

1. Absorption: Cycasin and MAM are rapidly absorbed from the gastrointestinal tract. Absorption through intact skin is unlikely, but carcinogenic effects have been noted after application of cycad extracts to mouse skin ulcerated with croton oil.
2. Distribution: No data.
3. Metabolism and excretion: Cycasin is not toxic but is activated by microbial β-glucosidases in the intestinal tract to yield MAM, which is responsible for the toxic and carcinogenic effects (as "proximate carcinogen"). MAM is further hydrolyzed to water, formaldehyde, and the "ultimate carcinogen," methylcarbonium ion, which methylates nucleic acids and proteins in vivo and intro (Magee et al., 1976; Miller and Miller, 1976; Clark, 1976). Cycasin is almost totally excreted unchanged in urine when not subject to intestinal enzymes (intraperitoneal injection or ingestion by germ-free rats) and otherwise partially excreted in unchanged form; no metabolites have been identified. It is also transmitted to offspring through the placenta and mother's milk in rats.
4. Toxic effects: The acute oral LD50 of cycasin is highly species dependent, ranging from less than 20 and 30 mg/kg in the guinea pig and rabbit to 250, 500, and 562 mg/kg in the hamster, mouse, and rat, respectively (Hirono, 1972). This variability may be partially related to the activity levels of intestinal glucosidases. For comparison, the oral LD50 of MAM acetate in the rat is 90 mg/kg. Ingestion of cycasin by the rat produces hepatotoxic effects within 24 hours, including glycogen, RNA, and phospholipid depletion; cellular necrosis; and hemorrhage (Williams and Laqueur, 1965). Central nervous system effects, mainly confined to cerebellar involvement in cattle and rodents, include hind limb paralysis. In humans, ingestion of insufficiently treated (presoaked) cycad four has resulted in similar hepatotoxicity and neurogenic effects, and the symptoms include, after a latent period of 12-24 hours, nausea and vomiting without fever or diarrhea, followed by death 20 hours later. The high incidence of amyotrophic lateral sclerosis (ALS) among natives of Guam has been ascribed to ingestion of improperly prepared cycad meal.
5. Carcinogenic effects: Oral administration of cycasin to animals produces tumors whose incidence and location are as species dependent as are the toxic effects. Rats show predominantly tumors of the kidney and large intestine; mice, hepatocellular and lung carcinomas; and hamsters, intrahepatic bile duct carcinomas. Rabbits and guinea pigs appear to be resistant to tumor production (Hirono et al., 1971, 1972). There is no evidence for carcinogenicity of cycasin in humans.
6. Mutagenic and teratogenic effects: MAM, but not cycasin, is mutagenic in the Ames test; both are mutagenic in the host-mediated assay in mice. Teratogenic effects are found in the offspring of rats and hamsters dosed parenterally with cycasin during gestation.

G. Emergency Treatment

1. Skin and eye exposure: For skin exposure, remove contaminated clothing and wash skin with soap and water. For eye exposure, irrigate immediately with copious quantities for running water for at least 156 minutes.
2. Ingestion: Drink plenty of water. Induce vomiting.
3. Inhalation: Remove victim promptly to clean air. Administer rescue breathing if necessary
4. Refer to physician.

H. References

Clark, A.M. 1976. Naturally occurring mutagens. Mutat Res 32:361-374.

Hirono, I., K. Hayashi, H. Mori, and T. Miwa. 1971. Carcinogenic effects of cycasin in Syrian golden hamsters and the transplantability of induced tumors. Cancer Res 31:283-287.

Hirono, I. 1972. Carcinogenicity and neurotoxicity of cycasins with special reference to species difference. Fed Proc 31:1493-1497.

Kobayashi, A., and H. Matsumoto. 1965. Studies on methlazoxymethanol, the agylcone of cycasin. Arch Biochem Biophys 110:373-380.

Magee, P.N., R. Montesano, and R. Preussmann. 1976. N-nitroso compounds and related carcinogens. Pages 596-605 in C.E. Searle, ed. Chemical Carcinogens. American Chemical Society, Washington, DC.

Matsumoto, H., and F.M. Strong. 1963. The occurrence of methylazoxymethanol in Cycas circinalis L. Arch Biochem Biophys 101:299-310.

Miller, J.A., and E.C. Miller. 1976. Carcinogens occurring naturally in foods. Fed Proc 35:1316-1321.

Nishida, K., A. Kobayashi, and T. Nagahama. 1955. Studies on cycasin, a new toxic glycoside of Cycas revoluto Thunb. I. Isolation and the structure of cycasin. Bull Agr Chem Soc Japan 19:77-84.

Nishida, K., A. Kobayshi, and T. Nagahama. 1956. Studies on cycasin, a new toxic glycoside of Cycas revoluto Thunb. V. Quantitative determination of cycasin in cycasin seeds. Bull Agr Chem Soc Japan 20:74-76.

Riggs, N.V. 1956. Glucosyloxyazoxymethane, a constituent of the seeds of Cycas circinalis L. Chem and Ind, No. 35, p. 926.

Wells, W.W., M.G. Wang, W. Bolzer, and O. Mickelsen. 1968. Gas-liquid chromatographic analysis of cycasin in cycad flour. Anal Biochem 25:325-329.

Williams, J.N., and G.L. Laqueur. 1965. Responses of liver nucleic acids and lipids in rats fed Cycas circinalis L. endosperm or cycasin. Proc Soc Exp Biol Med 118:1-4.