

SUMMARY OF DATA FOR CHEMICAL SELECTION

Milk Thistle Extract / Silymarin / Silybin

84604-20-6 / 65666-07-1 / 22888-70-6

August 1998

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BASIS OF NOMINATION TO THE CSWG

Milk thistle extract is presented to the CSWG as part of a review of botanicals being used as dietary supplements in the United States. Alternative herbal medicines are projected to be a \$5 billion market by the turn of the century. Milk thistle extract is consistently one of the more widely used alternative medicines.

A review of the available literature suggests that milk thistle extract has beneficial effects on the liver, helping reverse the damage caused by organic solvent exposure or alcoholism. The active flavolignan isomers in milk thistle extract, termed silymarin, also appear to possess anticarcinogenic activity. In contrast, no reports of severe side effects from the use of milk thistle extract or silymarin were reported in the available literature. However, many reports of beneficial effects may be from intravenous administration of a dosage form not available in the US. The lack of reports on side effects for dietary supplements in use in the US may simply indicate a lack of bioavailability of the active ingredients.

INPUT FROM GOVERNMENT AGENCIES/INDUSTRY

The American Botanical Council provided a report on milk thistle from their *Botanical Series* publications.

SELECTION STATUS

ACTION BY CSWG: 9/16/98

Studies requested:

- Toxicological characterization (90-day) including reproductive testing
- Metabolism studies
- Genotoxicity

Priority: Moderate

Rationale/Remarks

- Significant human exposure
- Popular dietary supplement thought to have beneficial effects on the liver
- Metabolism studies needed to resolve questions regarding bioavailability of orally administered milk thistle extract
- Examined as a hepatoprotectant, but limited information on safety
- NCI will conduct mouse lymphoma assay
- Consideration for further testing pending evaluation of results of 90-day toxicity study

CHEMICAL IDENTIFICATION

Milk Thistle Extract

CAS Registry Number: 84604-20-6

Chemical Abstracts
Service Name: *Silybum marianum*
extract

Synonyms and Trade
Names: Lady's thistle extract

Structural Class: Botanical mixture;
phytopharmaceutical

Silymarin

CAS Registry Number: 65666-07-1

Chemical Abstracts
Service Name: Silymarin

Synonyms and Trade
Names: Apihepar; Laragon;
Legalon; Pluropon;
Silarine; Silepar;
Silirex; Silliver; Silmar

Structural Class: Mixture of
flavonolignans
including silidianin,
silicristin, and the major
compound, silybin

Silybin

CAS
Registry
Number: 22888-70-6

Chemical
Abstracts
Service
Name: 4H-1-Benzopyran-4-one, 2-[2,3-
dihydro-3-(4-hydroxy-
3-methoxyphenyl)-2-(hydroxymethyl)
-1,4-benzodioxin-6-yl]-2,3-dihydro-
3,5,7-trihydroxy-, [2R[2 α -3 β , 6(2R*,
3R*)]]- (9 CI)

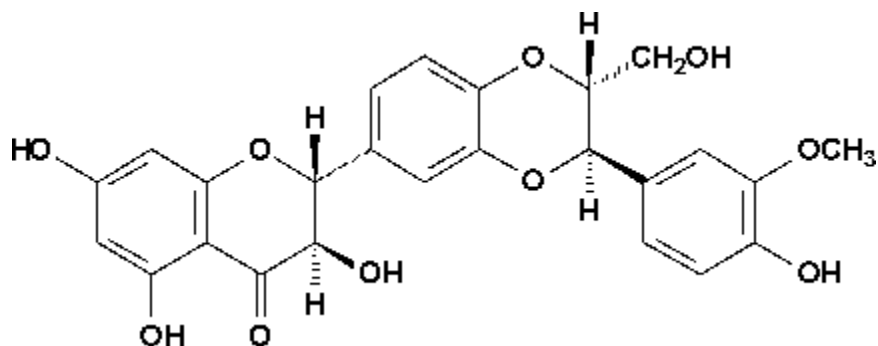
Synonyms
and Trade
Names: 4-Chromanone, 3,5,7-trihydroxy-
2-[3-(4-hydroxy-
3-methoxyphenyl)-2-
hydroxymethyl)-1,4-benzodioxan-
6-yl]-;

silibinin; silibinine; silybum
substance E; silymarin I

Structural

Class: Flavonolignan

Structure, Molecular Formula and Molecular Weight:



Silybin

$C_{25}H_{22}O_{10}$ Mol. wt.: 482.4

Chemical and Physical Properties (Silybin):

Melting Point: 158° C (anhydrous substance); 167° C (monohydrate) (Budavari, 1996)

Solubility: Practically insoluble in water; soluble in acetone, ethyl acetate, methanol, ethanol; sparingly soluble in chloroform (Budavari, 1996)

Milk thistle (*Silybum marianum*), a member of the Aster family, is a tall herb with large prickly white-veined green leaves and a reddish-purple flower that ends in sharp spines. Milk thistle fruits (often erroneously referred to as seeds) contain up to 6 percent silymarin, the active flavonoid constituent. Silymarin's principal components are silybin, silycristin, and silydianin. The primary investigational focus has been on silybin, which is the most biologically active. A number of other flavonolignans have also been found in the seeds including dehydrosilybin, desoxysilycristin, desoxysilydianin, silandrin, silybinome, silyhermin, and neosilyhermin. In addition apigenin, silybonol, and myristic,

palmitic, stearic, and oleic acids have been reported (Foster, 1991; Awang, 1993; Brown, 1996; Grauds, 1996).

Technical Products and Impurities: Milk thistle is available at health food stores and pharmacies as well as through direct-mail companies. Extracts are supplied as capsules, tablets, liquids, powders and creams. Some of these milk thistle preparations are sold in combination formulas with other herbs. In Europe, a water-soluble silybin compound is available for use in intravenous infusion treatments (Ferenci *et al.*, 1989; Foster, 1991; Awang, 1993).

Capsules. Capsules range in strength from 100 to 250 mg and most are standardized to contain 80 percent silymarin. Herbal Resources offers milk thistle extract in 150 mg capsules standardized to contain 70 percent silymarin (as silybin). Solgar's Vegicaps contain 100 mg of milk thistle extract (standardized to 80% silymarin) in a base of milk thistle herb and seed powder (Herbal Resources, Inc., 1995a; Global Nutrients, 1997; Smart Basics, 1997; Ripplecreek, 1998; Solgar Herbal Supplements, 1998). Time release capsules of milk thistle extract are also available (Dialog, 1998a).

Tablets. Source Natural's Silymarin 80 tablets are 210 mg standardized to deliver 168 mg of the three flavonolignans silybin, silydianin and silycristin. In addition, the tablets contain 50 mg of whole milk thistle seed (Dialog, 1998a).

Liquid. A blend of the liquid extracts of milk thistle mature seed (20%); dandelion root, leaf and flower (20%), Oregon grape root (20%), artichoke leaf (16%), beet leaf (16%), and fennel seed (8%) is available from Herbal Resources (Herbal Resources, Inc. 1995b).

Powder. Each 5 ml of a tonic powder from the Women's Health Advisory Service contains milk thistle (330 mg), psyllium husk powder (924 mg), dandelion root powder (330 mg), globe artichoke (165 mg), taurine (165 mg), and slippery elm bark (82.5 mg) (Women's Health Advisory Service, 1997).

Cream. A moisturizing cream, available from Abra Therapeutic Skin Care, contains milk thistle, green tea, and white willowbark; Derma E produces a skin lightening cream which combines milk thistle, licorice and vitamin C (Dialog, 1998a).

Silymarin is available from Aldrich and Sigma. The Aldrich product is a mixture of toxifolin, silicristin, silidianin, silybin A, silybin B, isosilybin A, and isosilybin B. The Sigma product is described as a mixture of anti-hepatotoxic flavonolignans from the fruit of *Silybum marianum*. Sigma also supplies silybin (Aldrich Chemical Co., Inc., 1996; Sigma, 1998).

EXPOSURE INFORMATION

Production and Producers: *Silybum marianum*, commonly known as milk thistle, is distributed throughout Southern Europe, Australia, North and South

American, and parts of Asia (Ikram *et al.*, 1984). Although milk thistle has been used medicinally for over 2000 years, the active hepatoprotective constituent, silymarin, was first isolated from the seeds (fruit) in 1968 (Brown, 1996).

The total synthesis of silybin, which constitutes about 60 percent of the silymarin complex, has been reported (Tanaka *et al.*, 1985; Budavari, 1996; Boigk *et al.*, 1997). Tanaka and coworkers (1985) achieved synthesis via a key intermediate, 3-(4-hydroxy-3-methoxyphenyl)-2-hydroxymethyl-1,4-benzodioxan-6-carbaldehyde. This aldehyde was converted to the methoxymethyl ether which was condensed with an acetophenone derivative to yield the chalcone. Oxidation of the chalcone with alkaline hydrogen peroxide followed by treatment of the resulting epoxide with hydrochloric acid in methanol afforded racemic silybin in 63 percent yield.

Milk thistle extract is available from Acta Pharmacal, American Ingredients, Inc., Anmar International, Ltd., Ashland Chemical Co., Belmont Chemicals, Inc., Bio-Botanica, Inc., China Tech, Inc., G.C.I. Nutrients, Marcor Development Corp., Mini Star International, Inc., Motherland Herb-Pharm, Inc., Pharmline, Inc., QBI (Quality Botanicals Ingredients, Inc.), RIA International, Schweizerhall, Inc., F.H. Taussig, Inc., and P.L. Thomas & Co., Inc. (McCoy, 1997).

Silymarin is available from Amax Industries, Inc., CPB International, Inc., DNP International Co., Inc., Kaden Biochemicals GMBH, M.M.P., Inc., Marcor Development Corp., Maypro Industries, Inc., Mini Star International, Inc., Motherland Herb-Pharm, Inc., Pharmline, Inc., and F.H. Taussig, Inc. (McCoy, 1997).

For the 12-month period ending April 1998, milk thistle formulas were among the strongest sellers in natural product stores with a growth rate of 83.2 percent (Scimone & Scimone, 1998).

In the 6-month period from October 1997 to April 1998, the Port Import/Export Reporting Service (PIERS) reported milk thistle seed and silymarin imports of 73,082 and 27,645 pounds, respectively (Dialog, 1998b).

Neither milk thistle nor silymarin are listed in the EPA's Toxic Substances Control Act (TSCA) Inventory.

Use Pattern: Milk thistle has a long history of European cultivation for food. Young leaves can be used in salads and as a substitute for spinach; stalks can be eaten like asparagus; roots are eaten like salsify; and boiled flower heads used like artichoke. Milk thistle fruit has also been used as a coffee substitute (Awang, 1993).

Various preparations of milk thistle, especially the seeds, have been used medicinally for over 2000 years. It was taken as a tonic, demulcent, anti-depressant, and stimulant for milk production in nursing mothers. In

homeopathy, a tincture of the fruits is used to treat bronchitis, cough, gallstones, hemorrhage, jaundice, peritonitis, uterine congestion, and varicose veins. Its use as a liver-protecting agent dates to early Greek references. The plant is not mentioned in most American works on medicinal plants until the end of the nineteenth century (Foster, 1991; Awang, 1993).

Currently the most important medicinal application of milk thistle is its use as an hepatoprotectant and as supportive treatment of chronic inflammatory liver disorders such as cirrhosis, hepatitis, and fatty infiltration due to alcohol and toxic chemicals. It has also been used in the treatment of liver damage by poisonous mushrooms. Following the isolation of silymarin and the development of standardized extracts in 1968, over 200 clinical studies involving over 4,000 patients have been completed with milk thistle extracts. Modern clinical research on the hepatobiliary effects of milk thistle began in Germany under the guidance of Madaus AG over thirty years ago (Foster, 1991; Awang, 1993; Brown, 1996).

For the treatment of liver disease (hepatitis, cirrhosis, toxin damage), the recommended dose of milk thistle extract is 420 mg (silymarin) a day taken in three divided doses. Suggested treatment periods range from 4 weeks to 9 months. When milk thistle is used as a nutritional supplement or for preventive purposes, 210 to 280 mg (silymarin) a day is recommended (Brown, 1996; Takao Co., 1996).

Human Exposure: The primary exposure of humans to milk thistle occurs through its use as an herbal supplement. Approximately one third of the US adult population or approximately 60 million consumers, have increasingly used alternative pharmaceutical preparations to prevent or treat illnesses (Tanaka, 1997).

There is potential for worker exposure to milk thistle during the growing, harvesting and processing of the plants. For the purposes of quantifying the costs of food labeling regulations, the FDA (1997) estimated that there were 250 herbal/botanical firms; the number of firms producing milk thistle products was not identified

No listing was found for milk thistle or silymarin in the National Occupational Exposure Survey (NOES), which was conducted by the National Institute for Occupational Safety and Health (NIOSH) between 1981 and 1983.

Environmental Occurrence: Milk thistle (*Silybum marianum*), a member of the Aster family, is a widespread wayside herb of uncultivated ground and wastelands throughout much of Europe. The plant is naturalized in the Eastern United States, California, and South America (Foster, 1991).

Regulatory Status: Since 1994, dietary supplements have been regulated under the Dietary Supplement Health and Education Act (DSHEA). For dietary supplements on the market prior to October 15, 1994, the DSHEA requires no proof of safety in order for them to remain on the market. The labeling

requirements for supplements allow warnings and dosage recommendations as well as substantiated "structure or function" claims. All claims must prominently note that they have not been evaluated by the FDA, and they must bear the statement "This product is not intended to diagnose, treat, cure, or prevent any disease" (Croom & Walker, 1995).

EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

Human Data: No epidemiological studies or case reports investigating the association of exposure to milk thistle extract and cancer risks in humans were identified in the available literature.

Adverse effects are rarely found in human studies with milk thistle extracts. A mild laxative effect has been observed in isolated cases and mild allergic reactions have been reported (Foster, 1991, Awang, 1993; Brown, 1996).

Numerous studies, mostly German, have examined the effectiveness of silymarin as a treatment for liver disorders and as an hepatoprotectant.

Ferenci and coworkers (1989) conducted a prospective, double blind, randomized study to determine whether silymarin improved the prognosis of patients with cirrhosis of the liver. Eighty-seven patients (46 alcoholic, 41 non-alcoholic) received 140 mg of silymarin orally three times a day; 83 patients (45 alcoholic, 38 non-alcoholic) received a placebo. The mean duration of treatment was 41 months. Long-term treatment with silymarin reduced the mortality of patients with cirrhosis (4-year survival, 58% vs. 39%, $P = 0.036$). The beneficial effect of silymarin was more pronounced in alcoholic cirrhosis although the researchers estimated that 60 percent of the patients with alcoholic cirrhosis continued to consume appreciable quantities of alcohol during the study. No side effects of drug treatment were observed.

The hepatoprotective effects of silymarin in workers exposed to organic solvents were evaluated by Szilard and coworkers (1988). Forty-nine workers who had been exposed to toluene and/or xylene for 5 to 20 years were selected for the study based on abnormal liver function tests (increased Aspartate and Alanine-aminotransferase, AST and ALT) and/or abnormal hematological values (low platelet count, leukocytosis, relative lymphocytosis). Thirty of these workers were treated with 140 mg of Legalon® (silymarin) orally 3 times a day for one month. The remaining 19 workers received no treatment. The liver function tests and platelet counts improved significantly with silymarin treatment. The leukocytosis and relative lymphocytosis showed a nonsignificant tendency of improvement.

Additional clinical studies reviewed by Foster (1991), Awang (1993), and Brown (1996) indicate that silymarin and silybin are effective in the treatment of viral hepatitis and Amanita mushroom poisoning.

Animal Data: The LD_{50} of orally administered silymarin in mice is > 1600 mg/kg (NLM, 1998).

Animal experiments have been reported to demonstrate the safety of milk thistle seed extracts with even large doses being virtually free of adverse or embryotoxic effects (Foster, 1991; Awang, 1993).

No 2-year carcinogenicity studies of milk thistle extract were identified in the available literature. Several protocols have been used to examine the tumor inhibiting-potential of silymarin.

Mammary gland. Silymarin was tested for prevention of 7, 12-dimethylbenz(a)anthracene (DMBA)-induced lesions in a Balb mouse mammary gland organ culture assay. Mammary glands were exposed to 2 mg of DMBA/ml for 24 hours followed by a 5-day exposure to 25 ng/ml of 7,12-tetradecanoylphorbol-13-acetate (TPA); 1 μ M silymarin was added to the medium for 4 days beginning prior to carcinogen treatment (anti-initiator) or for 15 days beginning 5 days after carcinogen treatment (antipromoter). Silymarin was an effective inhibitor when administered during the promotional phase with a lesion incidence of 33 percent vs 80 percent in controls. It was ineffective as an anti-initiating agent (Mehta & Moon, 1991).

Colon/Small Intestine. Silymarin significantly ($P < 0.05$) inhibited the number of 1,2-dimethylhydrazine (DMH)-induced colon tumors in male Sprague-Dawley rats. Tumors of the small intestine were not significantly different. Silymarin was administered at 0.1 percent in the diet for 32 weeks beginning 2 weeks prior to the administration of 20 mg/kg of DMH by gavage once a week for 15 weeks. The number of animals with colon tumors was 11/16 in silymarin-treated rats vs. 19/22 in controls. The total numbers of colon adenocarcinomas were 60 and 132 in the silymarin and controls groups, respectively (Gershbein, 1994).

Skin. The protective effect of silymarin against photocarcinogenesis was assessed in the SKH-1 hairless mouse model. Female mice were subjected to the following: UVB-induced tumor initiation followed by TPA-mediated promotion; DMBA tumor initiation followed by UVB-mediated tumor promotion; or UVB-induced complete carcinogenesis. Forty mice were used in each protocol and were divided into control and treatment groups. Silymarin was applied topically at 9 mg before UVB exposure. In the protocol with UVB-initiation, silymarin treatment reduced tumor incidence by 20 percent and tumor multiplicity by 67 percent; tumor volume showed a 66 percent reduction. These differences were not significant. In the protocol with UVB-promotion, silymarin reduced tumor incidence 40 percent ($P < 0.003$) and tumor multiplicity 78 percent ($P < 0.0001$); tumor volume reduction was 90 percent ($P < 0.003$). The strongest inhibition was seen in the protocol with UVB-induced complete carcinogenesis. Silymarin reduced tumor incidence 75 percent ($P < 0.0001$) and tumor multiplicity 92 percent ($P < 0.0001$); tumor volume reduction was 97 percent ($P < 0.0001$). No signs of toxicity, body weight loss, or mortality were observed following silymarin treatment in all three protocols (Katiyar *et al.*, 1997).

Silymarin was also an effective inhibitor of DMBA-initiated and TPA- or okadaic acid (OA)-promoted skin tumors in female Sencar mice. Topical application of 6 mg of silymarin 30 minutes prior to each TPA treatment of DMBA-initiated mouse skin resulted in almost complete protection in terms of tumor incidence (85%, $P < 0.0001$) as well as multiplicity (94%, $P < 0.0001$). In the OA-promotion study, application of 6 mg of silymarin 30 minutes prior to OA treatment in DMBA-initiated mouse skin resulted in complete protection against tumorigenicity (Zi *et al.*, 1997).

Agarwal and coworkers (1996) examined the stage specificity of silymarin against phorbol ester-type tumor promoters as well as its effect against benzoyl peroxide (BPO), a nonphorbol ester-type promoter. Application of silymarin prior to that of TPA in stage I or mezerein in stage II in DMBA-initiated Sencar mouse skin, resulted in highly significant protection in stage I where silymarin showed 74 percent and 92 percent reductions in tumor incidence and multiplicity, respectively; the protective effect of silymarin was 26 percent in

the stage II protocol. Application of silymarin prior to BPO in DMBA-initiated mouse skin, also showed significant protection in terms of both tumor incidence (70%) and multiplicity (67%).

Short-Term Tests: The effect of silymarin on DNA damage in human cells has been investigated in the comet assay. When tested at doses up to 1000 &M, silymarin induced DNA strand breakage in epithelial cells (HeLa). It was negative in colon cells (Caco-2) and liver cells (HepG2). At doses up to 100 µM silymarin was also negative in human lymphocytes however, genotoxicity became evident at 1000 &M. Silymarin inhibited cell growth in HeLa cells at 250 µM; no cytotoxicity was indicated even at the highest doses tested. Silymarin did not increase the level of damaged pyrimidine bases above the endogenous level in Hela cells, indicating that it did not induce oxidative damage to DNA (Duthie *et al.*, 1997).

The comet assay has also been used to assess silymarin-induced DNA damage in human sperm. At doses of 100-500 µM, silymarin produced a positive response based on a reduction in percentage sperm head DNA. In combination with the food mutagens 3-amino-1-methyl-5H-pyrido (4,3-b)indole (Trp) and 2-amino-3-methylimidazo-(4,5-f) quinoline (IQ), 100-500 µ M silymarin produced a protective response (Anderson *et al.*, 1997).

Metabolism: The biliary excretion of silybin, the main active component of silymarin, was evaluated in nine cholecystectomy patients with T-tube drainage following single oral doses of silymarin (120 mg, expressed as silybin equivalents). The bile collected after silymarin intake contained silybin as well as isosilybin and very low levels of silydianin and silycristin. The amount of silybin recovered in bile in free and conjugated form within 48 hours accounted for 3 percent of the dose. Recovery of silycristin and silydianin accounted for 1 percent of the administered dose. Samples for the determination of silybin and other flavonolignans in plasma were obtained from 3 patients at 0, 1, 3, 5, and 9 hours following the administration of silymarin. Silybin was detected in 2 of the 1-hour samples at levels of 5 and 20 ng/ml, respectively. The authors noted that with T-tube drainage only a fraction of the bile is collected and they calculated that the actual biliary excretion of silybin could be as high as 12 percent of the silymarin dose. This study also examined the pharmacokinetics of Silipide, a silybin-phosphatidylcholine complex, which was developed to improve the oral bioavailability of silybin. There is no indication that this product is available in the US (Schandalik *et al.*, 1992).

Morazzoni and coworkers (1992) compared the bioavailability of silybin and Silipide in rats. After the administration of 200 mg/kg of silybin by gavage to Sprague-Dawley rats, the biliary and urinary excretion accounted for 0.001 percent and 0.032 percent of the dose, respectively. The plasma levels of both the unconjugated and total compound were below the detection limit. Silipide was shown to have higher bioavailability which was attributed to increased gastrointestinal absorption.

Other Biological Effects: A number of studies have examined the biochemical mechanisms of action of silymarin and silybin. Valenzuela and Garrido (1994) have reviewed findings from different *in vivo* and *in vitro* experimental models, such as whole animals, perfused organs, cell tissue homogenates and isolated nuclei. They note that three levels of action have been proposed for silymarin in experimental animals: 1) as an antioxidant, by scavenging prooxidant free radicals and by increasing the intracellular concentration of the

tripeptide glutathione; 2) regulatory action of the cellular membrane permeability and increase of its stability against xenobiotic injury; 3) at the nuclear level, by increasing the synthesis of ribosomal RNA by stimulating DNA polymerase I and by exerting a steroid-like regulatory action on DNA transcription.

Kim and coworkers (1994) demonstrated that silymarin and silybin are inhibitors of β -glucuronidases of intestinal bacteria, human feces, and rat microsomal fraction. The enzyme catalyzes the hydrolysis of β -glucuronides produced in the body, such as benzo(a)pyrene glucuronides, and of natural plant glucuronides such as glycyrrhizin. The authors speculated that silymarin and its components reduce the risk factor of colon cancer and protect the liver by inhibiting the hydrolysis to glucuronides of proximate metabolites.

Letteron and coworkers (1990) conducted a series of *in vivo* and *in vitro* studies in order to determine the mechanisms involved in the protective effects of silymarin against carbon tetrachloride (CCl₄)-induced hepatotoxicity. They concluded that silymarin prevents CCl₄-induced lipid peroxidation and hepatotoxicity in mice, firstly, by decreasing the metabolic activation of CCl₄ and secondly, by acting as a chain-breaking antioxidant.

In studies of the inhibition of mouse skin tumor promoters, silymarin was shown to significantly inhibit TPA-caused induction of ornithine decarboxylase (ODC) activity and mRNA expression in mouse epidermis. Topical application of silymarin on mouse skin prior to that of TPA or OA also resulted in highly significant to complete inhibition against both TPA- and OA-caused induction of tumor necrosis factor α (TNF α) mRNA expression. TNF α one of the inflammatory cytokines, has been shown to act as an endogenous tumor promoter and a central mediator of tumor promotion (Agarwal *et al.*, 1994; Zi *et al.*, 1997).

Structure Activity Relationships: Milk thistle consists of a complex mixture of natural products which does not lend itself to traditional structure activity analysis.

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