SUMMARY OF DATA FOR CHEMICAL SELECTION

S-Adenosylmethionine
29908-03-0

BASIS OF NOMINATION TO THE CSWG

S-Adenosylmethionine (SAMe) is brought to the attention of the CSWG because of the rapid increase in its use as a dietary supplement since being introduced into the US market in 1999. In the US, SAMe is marketed for improved mobility and mood enhancement. However, SAMe has a long history in other countries as a prescription drug for the treatment of various liver diseases, pain associated with arthritis, and depression. In the US, depression affects 19 million persons each year, and 40 million people suffer from chronic joint pain. If only a small fraction of these individuals decides to use SAMe to treat their illnesses, large numbers of consumers will be exposed.

SAMe is a physiological methyl donor involved in enzymatic transmethylation reactions. It is present in all mammalian systems. A considerable body of information suggests that hypomethylation caused by insufficient concentrations of methyl donors may be associated with epigenetic changes leading to carcinogenesis. Lipotrope-deficient (methyl-deficient) diets have been associated with liver cancer in laboratory animals, including those not administered an initiating carcinogen. Some information also suggests that lipotrope-rich diets containing methionine, choline, and betaine prevent the development of cancers in laboratory rodents administered initiating carcinogens. Conversely, limited information associates methionine excesses with colon or gastric cancer. Whether adverse effects occur from the long-term administration of SAMe is unknown.

INPUT FROM GOVERNMENT AGENCIES/INDUSTRY

Dr. John Walker, Executive Director of the TSCA Interagency Testing Committee (ITC), Environmental Protection Agency (EPA), indicated that the ITC has deferred action on SAMe.

SELECTION STATUS

ACTION BY CSWG: 12/16/99

Studies requested:

Subchronic (90-day) studies specifically designed for SAMe which will take into account exogenous vs endogenous metabolism, genotoxicity and cell transformation, and DNA alkylation (C-5 and N-7 positions)

Priority: High

Rationale/Remarks:

Widespread exposure to consumers from its use as a popular dietary supplement recently introduced into the United States

Used to treat osteoarthritis, depression, and liver diseases

SAMe is a physiological methyl donor present in all mammals

Hypomethylation is associated with epigenetic changes leading to carcinogenesis; whether high doses of exogeneously administered SAMe can cause adverse effects from methylation of DNA bases is not known

Not genotoxic in several assays

CHEMICAL IDENTIFICATION

CAS Registry Number: 29908-03-0

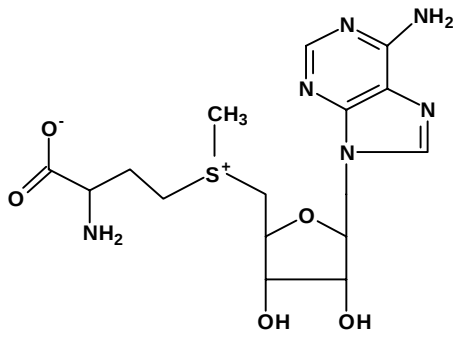
Chemical Abstract Service Name: Adenosine, 5'-[(3-amino-3-carboxypropyl) methyl-sulfonio]-5'-deoxy-, inner salt, (3*S*)- (9CI)

Adenosine, 5'-((L-3-amino-3-carboxypropyl) methyl-sulfonio)-5'-deoxy-, hydroxide, inner salt (8CI)

Synonyms and Trade Names: S-Adenosylmethionine; active methionine; ademetionine; adenosyl-L-methionine; methioninyladenylate; AdoMet; Donamet; SAMe, Sam-e; S. Amet Disulfate-ditosylate salt: Gumbaral, Samyr

Structural Class: Amino acid derivative

Structure, Molecular Formula, and Molecular Weight:



$C_{15}H_{22}N_6O_5S$ Mol. wt.: 398.49

Chemical and Physical Properties:

Description: No information found in the available literature

Properties: Unstable at room temperature (NCI, 1993; Czap, 1999)

Technical Products and Impurities: The following salts of S-adenosyl-L-methionine (SAMe) are available from Sigma-Aldrich: S-adenosyl-L-methionine chloride salt (~70%) [CAS No. 24346-00-7]; S-adenosyl-L-methionine iodide salt (~80%) [CAS No. 3493-13-8]; S-

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adenosyl-L-methionine *p*-toluenesulfonate salt (~90%) [CAS No. 86562-85-8] (Sigma-Aldrich, 1999). SAMe salts can quickly degrade upon exposure to heat or moisture, presenting problems for animal or clinical testing (NCI, 1993; Czap, 1999). The disulfate-ditosylate and butanedisulfonate salts, forms of SAMe used in dietary supplements, are more stable than SAMe, although handling during manufacture remains an issue (Cowley & Underwood, 1999; Czap, 1999).

Some SAMe products introduced as dietary supplements are prepared as relatively stable pharmaceutical grade, enteric coated dosage systems. However, SAMe products vary widely in price and quality (Cowley & Underwood, 1999; Czap, 1999).

Use Pattern: The therapeutic categories for SAMe are as an anti-inflammatory and in the treatment of liver disease (Merck, 1997). In the US, SAMe is marketed as a dietary supplement used for improved joint mobility and mood enhancement (Sauer, 1999).

Although doctors in 14 countries have prescribed SAMe for two decades, SAMe was not available in the US until recently.[1] In the first quarter of 1999, BASF introduced SAMe as its first entry into the US dietary supplement market. Under the brand name "Sam-e", the BASF product is being marketed exclusively by Pharmavite, GNC, and NBTY (Sauer, 1999). Considerable publicity has benefitted the launch of "Sam-e" supplements; as of July 1999, *Newsweek* reported that Pharmavite's Nature Made brand ranked 25th among the 13,000 supplements sold in grocery and drugstores. US retailers also import tosylate salts for manufacture of SAMe supplements. A few of these retailers are Life Extension Foundation, NutraLife Health Products, Solgar Vitamin and Herb Co., Natrol, and Great Earth Companies (Cowley & Underwood, 1999; Czap, 1999; Sauer, 1999).

[1]Information on production, use, and human exposure from standard sources such as the Port Import/Export Reporting Service, EPA's Toxic Substances Control Act Inventory, the National Occupational Exposure Survey, and the US International Trade Commission's *Synthetic Organic Chemicals, US Production and Sales, 1983-1993*, would not reflect recent usage of SAMe, so information from these sources is not presented.

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In Europe, Russia, and China, SAMe is prescribed for the treatment of various liver diseases (Mazzanti *et al.*, 1979; Manzillo *et al.*, 1992; Cowley & Underwood, 1999). According to Almasio and coworkers, exogenous SAMe undergoes liver first-pass metabolism and significantly restores the glutathione depletion observed in patients with chronic liver disease. Moreover, exogenous SAMe may increase trans-sulfuration reactions, which seem to play a role in activating cholestatic estrogen metabolites and bile salts (Almasio *et al.*, 1990). Some studies suggest that malnourished patients with cirrhosis may have an acquired metabolic block in the conversion of methionine to SAMe (Chawla *et al.*, 1990).

Animal studies and clinical trials in humans have shown that SAMe, administered orally or by injection, alleviates signs and/or symptoms of liver disease caused by alcohol (humans, rats, and baboons) (Micali *et al.*, 1983; Feo *et al.*, 1986; Lieber *et al.*, 1990); toxic chemicals, including carbon tetrachloride (rats) (Varela-Moreiras *et al.*, 1995) and hexachlorobenzene (rats) (Cantoni *et al.*, 1990); nonsteroidal anti-inflammatory drugs (NSAIDs), including acetaminophen (mice) (Bray *et al.*, 1992); and cyclosporin A (rats) (Galán *et al.*, 1999). SAMe also alleviated estrogen-induced liver problems (*e.g.*, cholestasis associated with pregnancy) (Almasio *et al.*, 1990; Frezza & Terpin, 1992; Osman *et al.*, 1993; Floreani *et al.*, 1996) and hepatic necrosis in rats from methyl deficient diets (Chawla *et al.*, 1998).

SAMe is critical for manufacturing joint cartilage and for maintaining neural cell membrane function (Vibrant Life, 1999). People who suffered from osteoarthritis, rheumatoid arthritis, fibromylagia, joint injuries, and osteoporosis have been treated successfully with SAMe (Glorioso *et al.*, 1985; Marcolongo *et al.*, 1985; DiPadova, 1987; König, 1987; Maccagno *et al.*, 1987; Vetter, 1987).

A dozen European clinical trials involving more than 22,000 patients have found SAMe to be effective for treatment of joint pain and inflammation from arthritis. Although

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gastrointestinal distress is reported as a side effect of SAMe, these effects appear to be much less severe than the gastric side effects from arthritis-strength doses of NSAIDs. In the US, nearly a third of the 40 million persons with chronic joint pain use drugs like aspirin and ibuprofen. Some 103,000 Americans are hospitalized annually for NSAID-induced ulcers, and 16,500 die (Cowley & Underwood, 1999). All persons with arthritis pain are potential candidates for SAMe use, particularly those who have experienced severe side effects from NSAIDs.

SAMe was first developed as a pharmaceutical by an Italian firm in the early 1970s. To date, it remains one of the most widely prescribed antidepressants in Italy. Impairment in methylation has been implicated in the etiology of depression, dementia, and demyelination of nerves. It has been hypothesized that the antidepressant effects of SAMe may result from its role as a methyl donor to biogenic amines which influence neurotransmitter metabolism, and from its role in the methylation of membrane phospholipids which modify membrane fluidity and receptor function (Bottiglieri & Hyland, 1994; Cestaro, 1994; Cowley & Underwood, 1999).

The antidepressant effects of SAMe were first suggested by Pinzello and Andreoli (1972). Since then, researchers have published some 40 open and double-blinded studies evaluating the efficacy of SAMe supplements for the treatment of depressive disorders in roughly 1,400 subjects. Several studies have shown that SAMe can produce clinical improvement in depressed subjects as effectively as classic tricyclic antidepressants. SAMe also demonstrated antidepressant activity in several predictive models in mice and rats (Baldessarini, 1987; De Leo, 1987; Kagan *et al.*, 1990; Rosenbaum *et al.*, 1990; Czyrak *et al.*, 1992; Bressa, 1994; Benelli *et al.*, 1999; Cowley and Underwood, 1999).

SAMe has also been effective in treating depression secondary to chronic diseases including Parkinson's disease, rheumatoid arthritis, fibromyalgia, and liver disease including alcoholism (Carney *et al.*, 1987; Carrieri *et al.*, 1990; Jacobsen *et al.*, 1991; Volkmann *et*

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al., 1997).

Depression is an important public health problem expected to affect more than 19 million adults in the US in 1999. According to the National Institute of Mental Health (NIMH), nearly two out of three depressed people do not seek treatment for their depression, causing many to be unnecessarily incapacitated for weeks or months. The NIMH estimates that the cost of depression to the US in 1990 was between $30 and $44 billion (NIMH, 1999).

SAMe has not been more effective than prescription antidepressants, but it is clearly less toxic than the tricyclics and MAO inhibitors. While newer antidepressants are less dangerous than the tricyclics and MAO inhibitors, their side effects still are more severe than the side effects observed with SAMe. Until large clinical trials confirm the results seen from the limited European studies, however, it is unlikely that American doctors will recommend SAMe to severely depressed persons (Cowley & Underwood, 1999).

SAMe has potential uses for inhibition of blood platelet aggregation (De la Cruz *et al.*, 1997) and for treatment of pancreatitis (Scott *et al.*, 1992; Bilton *et al.*, 1994), abnormal circulating lipoprotein particles (Owen *et al.*, 1992), neurological complications of AIDs (Castagna *et al.*, 1995; Tan & Guiloff, 1998), and hereditary spherocytosis (a congenital hemolytic anemia associated with alterations in erythrocyte membrane proteins) (Maggio *et al.*, 1994). SAMe has also been evaluated for treatment of attention deficit-hyperactive disorder in adults (Shekim *et al.*, 1990). Parenteral and oral SAMe administration has been described as effective in the treatment of Gilbert's syndrome, a familial defect of bilirubin metabolism (Bombardieri *et al.*, 1985).

Human Exposure: Doses of SAMe administered to humans for treatment of depression range from 45 to 1600 mg/day for a duration of up to 42 days; SAMe has been administered intravenously (iv), intramuscularly (im), or perorally (po) (Bressa, 1994). In treating

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cholestasis of pregnancy, the usual dose is 800 mg/day for 20 days (Schenker *et al.*, 1998). To relieve the symptoms of pruritus, other cholestasis patients have been administered SAMe at iv doses of 800 mg/day or oral regimens of 1.6 g/day for two weeks (Almasio *et al.*, 1990). Nonpregnant women with abnormal sensitivity to estrogens have been administered oral doses of SAMe at 600 or 800 mg/day to protect against estrogen-induced hepatotoxicity (Almasio *et al.*, 1990). Osteoarthritis patients have received SAMe (Gumbaral) at 400 mg/day (po) for up to several years (Berger & Nowak, 1987; König, 1987).

Environmental Occurrence: SAMe is found in almost every tissue in the body in humans and other mammals. SAMe is formed by an enzyme (methionine S-adenosyltransferase (MAT)) catalyzed reaction between methionine and ATP (Osman *et al.*, 1993; LEF Magazine, 1997). No information on any other environmental occurrence of SAMe was identified in the available literature.

Regulatory Status: No standards or guidelines have been set by NIOSH or OSHA for occupational exposure to or workplace allowable levels of SAMe. SAMe was not on the American Conference of Governmental Industrial Hygienists (ACGIH) list of compounds for which recommendations for a threshold limit value (TLV) or biological exposure index (BEI) are made.

Since 1994, dietary supplements have been regulated under the Dietary Supplement Health and Education Act (DSHEA). 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).Neither SAMe nor any of its salts are approved drugs in the US.

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EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

Hypomethylation and Hypermethylation as Factors in Carcinogenesis: The activation of oncogenes and deactivation of tumor suppressor genes are important factors in carcinogenesis that can result from two types of molecular change, a genetic alteration resulting from a mutation or a modification in gene expression resulting from epigenetic changes (Duker, 1998).

Epigenetic changes are often related to the locations and functions of 5-methylcytosine present in mammalian DNA. 5-Methylcytosine comprises <1 percent of the base content of the human genome, and is mostly confined to "CpG" islands associated with the 5' regions upstream of genes involved in vital cell functions. The introduction of 5-methylcytosine into mammalian DNA differs from that of the four canonical coding bases adenine, guanine, cytosine, and thymine. Cytosines at "CpG" loci are enzymatically methylated at the 5-carbon by methyltransferases, with SAMe serving as the methyl group donor. Therefore, levels of DNA methylation depend at least partly on available cellular SAMe (Duker, 1998).

Loss of DNA 5-methylcytosine at sites 5' to the gene, or "hypomethylation" (*e.g.*, from reduced levels of SAMe or L-methionine, or folate deficiency) usually results in gene activation, subsequent transcription, and synthesis of biologically active protein from the messenger RNA. Since activation of cellular oncogenes is associated with neoplasia, reduction of DNA 5-methylcytosine may also be associated with neoplasia (Duker, 1998). Experimental evidence on DNA hypomethylation is discussed in the section on "Other Biological Effects."

The DNA of tumor cells has also been found to be "hypermethylated" (*i.e.*, contains a higher 5-methylcytosine content than normal cells). Hypermethylation could be associated with carcinogenesis by silencing tumor suppressor genes or by marking chromosomes for deletion (Duker, 1998). SAMe has shown chemopreventive activity when administered

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to laboratory animals following their exposure to an initiator (see "Other Biological Effects"). The consequences of hypermethylation caused by introduction of exogenous SAMe have not been fully explored, however; whether the orally administered SAMe found in dietary supplements causes hypermethylation is uncertain.

DNA-methylcytosine involvement in carcinogenesis can also occur through genetic mechanisms. Restriction sites containing CpG show a frequency of polymorphism in human DNA far higher than expected. Many mutations in the tumor suppressor p53 gene are G:C to A:T transition mutations at CpG sites. Methylation of cytosine can cause an increase in potential for mutations at such loci by an order of magnitude (Duker, 1998).

Human Data: No epidemiological studies or case reports investigating the association of exposure to exogenously administered SAMe and cancer risk in humans were identified in the available literature.

5-Methylcytosines have been implicated as sites of procarcinogenic mutations and may also be associated with carcinogenesis through epigenetic mechanisms. High expression of DNA methyltransferase is a characteristic of human neoplastic cells, especially in colon cancer progression (Duker, 1998). These findings suggest that DNA methylation, and therefore available cellular SAMe may have a role in human carcinogenesis.

In clinical trials of limited duration, tolerance to SAMe has been described as good with a low incidence of serious side effects (*Anon.*, 1988). Occasional cases of mania have been reported as a side effect of administration of SAMe to patients with depression (Carney *et al.*, 1987; Kagan *et al.*, 1990; Rosenbaum *et al.*, 1990).

The largest clinical trial of SAMe to date was a non-controlled study involving 20,641 osteoarthritis patients administered 1200 mg/day in the first week, 800 mg in the second week, and 400 mg in the third week. Moderate or severe side effects, mostly related to the

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GI tract, were reported by 21 percent of the patients participating in the trial. In 5 percent of the patients therapy was stopped prematurely because of adverse effects (Berger & Nowak, 1987).

A multicenter open trial in 108 patients with osteoarthritis of the knee, hip, and spine is of particular interest because of the long-term administration of SAMe. Patients received SAMe for 24 months (600 mg/day for two weeks, then 400 mg/day) at which time 97 patients were still in the study. SAMe showed good clinical effectiveness in treating the symptoms of osteoarthritis and was well tolerated. During the first 18 months, nonspecific side effects occurred in 20 patients, but therapy was not stopped (König, 1987).

Animal Data: No 2-year carcinogenicity studies of SAMe were identified in the available literature. The LD_{50} values for SAMe (disulfate-ditosylate salt) are given in Table 1.

Table 1. Acute toxicity values for SAMe

Species	Route of Administration	LD_{50} (mg/kg)
mouse	oral	>6000
mouse	ip	2500
mouse	iv	560
rat	iv	>2000

Source: NLM, 1999

SAMe (64 µmol/kg, 6x/day, im) administered to rats for six months did not cause any change in body weight relative to controls (Pascale *et al.*, 1992; NCI, 1993).

Short-Term Tests: SAMe did not induce genotoxicity in any of the following *in vitro* test systems with or without metabolic activation: mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 at concentrations up to 2 mg/plate; sister chromatid exchanges (SCE) in Chinese hamster ovary cells (CHO) at concentrations up to

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1 mg/tube; DNA repair in *E. coli* polA+/- strains at concentrations up to 500 µg/well; and increases in the incidence of chromosome aberrations in cultured human lymphocytes at concentrations up to 1 mg/ml. *In vivo* treatment of adult Chinese hamsters with SAMe at 166-1500 mg/kg intraperitoneally (ip) did not induce chromosomal aberrations in bone marrow cells, and treatment of male Sprague-Dawley rats with SAMe (100 mg/kg a day subcutaneously [sc] for 10 days or single ip injection of 1500 mg/kg) did not produce measurable abnormal methylated bases in DNA (Pezzoli *et al.*, 1987).

A slight but significant increase of mutation frequencies was observed in *E. coli* by Näslund and coworkers (1983) when SAMe was administered. SAMe is an essential metabolite in yeast, and mutations causing a lack of SAMe synthetase are lethal unless SAMe is provided in the medium. This has not been established for *E. coli* because the cell is impermeable to SAMe (Newman *et al.*, 1998).

Incubation of DNA with SAMe in a neutral aqueous solution led to base modification with formation of small amounts of 7-methylguanine and 3-methyladenine. It was estimated that SAMe at an intracellular concentration of 4×10^{-5} M caused DNA alkylation at a level expected from exposure of cells to 2×10^{-8} M methyl methanesulfonate (Rydberg & Lindahl, 1982).

Mammalian small molecule methyltransferases appear to have originated from a common ancestral protein designed to bind SAMe. Eukaryotic and prokaryotic methyltransferases that methylate DNA bases have their own sequence motifs different from those of mammalian enzymes, and probably constitute distinct classes of enzymes (Fujioka, 1992). Thus, the results of short-terms tests designed to measure the genotoxicity of SAMe may have little relevance to human risk from exposure to exogenous sources of SAMe.

Metabolism: SAMe is involved in three major biochemical pathways in the mammalian body: (1) transmethylation reactions in which the methyl group acceptors include a variety of low

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molecular weight compounds, proteins, hormones, phospholipids, DNA, and RNA; (2) trans-sulfuration reactions that form glutathione (GSH) and sulfated compounds *via* homocysteine and cysteine; and (3) the aminopropylation reaction where it is the source of the propylamino group in the biosynthesis of the polyamines, spermidine and spermine. As an intact molecule, it regulates the distribution of folate cofactors *in vivo* (Poirier *et al.*, 1990; Clarke, 1993; NCI, 1993; Scott *et al.*, 1994).

The demethylated product from mammalian methyltransferase reactions involving SAMe is S-adenosylhomocysteine (SAH) which is rapidly metabolized to homocysteine. SAH is a potent competitive inhibitor of most methylation reactions, and the ratio of SAMe to SAH is said to regulate the activity of methyltransferase reactions (Bottiglieri *et al.*, 1994).

Homocysteine, produced entirely from the methylation cycle, can undergo remethylation to methionine, or undergo condensation with serine to form cystathionine. In the latter reaction, homocysteine is committed to the trans-sulfuration pathway, leading to the formation of glutathione. SAMe regulates the fate of homocysteine. An increase in SAMe concentration inhibits 5,10-methylenetetrahydrofolate reductase, resulting in a decrease in the flow through the methionine synthetase pathway and a diversion of homocysteine metabolism toward the trans-sulfuration pathway. This dual regulatory mechanism suggests that normally the steady state concentrations of the components of the methylation cycle are carefully regulated to maintain levels of SAMe (Bottigglieri *et al.*, 1994).

In mammals, every tissue can synthesize SAMe, employ it for transmethylation, hydrolyze SAH, and remethylate homocysteine. Trans-sulfuration to catabolize homocysteine occurs only in liver, kidney, small intestine and pancreas. The liver has a unique isoenzyme, methionine adenosyltransferase (MAT) that allows the utilization of excess methionine for the continued synthesis of SAMe (Finkelstein, 1998).

Administration of high concentrations of SAMe from exogenous sources has increased the

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levels of SAMe in the human body (Bilton *et al.*, 1994). In depressed patients, injected SAMe (toluenesulfonate salt) disappeared from plasma with a half life of approximately 100 minutes (Bottiglieri & Hyland, 1994). It remains to be established whether SAMe can be taken up intact by cells (Chiang *et al.*, 1996).

Elevated plasma homocysteine levels are associated with various forms of vascular disease. When oral SAMe (400 mg) was administered to humans, both S-adenosylhomocysteine and 5-methyltetrahydrofolate showed significant transient increases; homocysteine and methionine levels did not change (Loehrer *et al.*, 1997).

Figure 1 represents SAMe metabolism as adapted from Bottiglieri and coworkers (1994).

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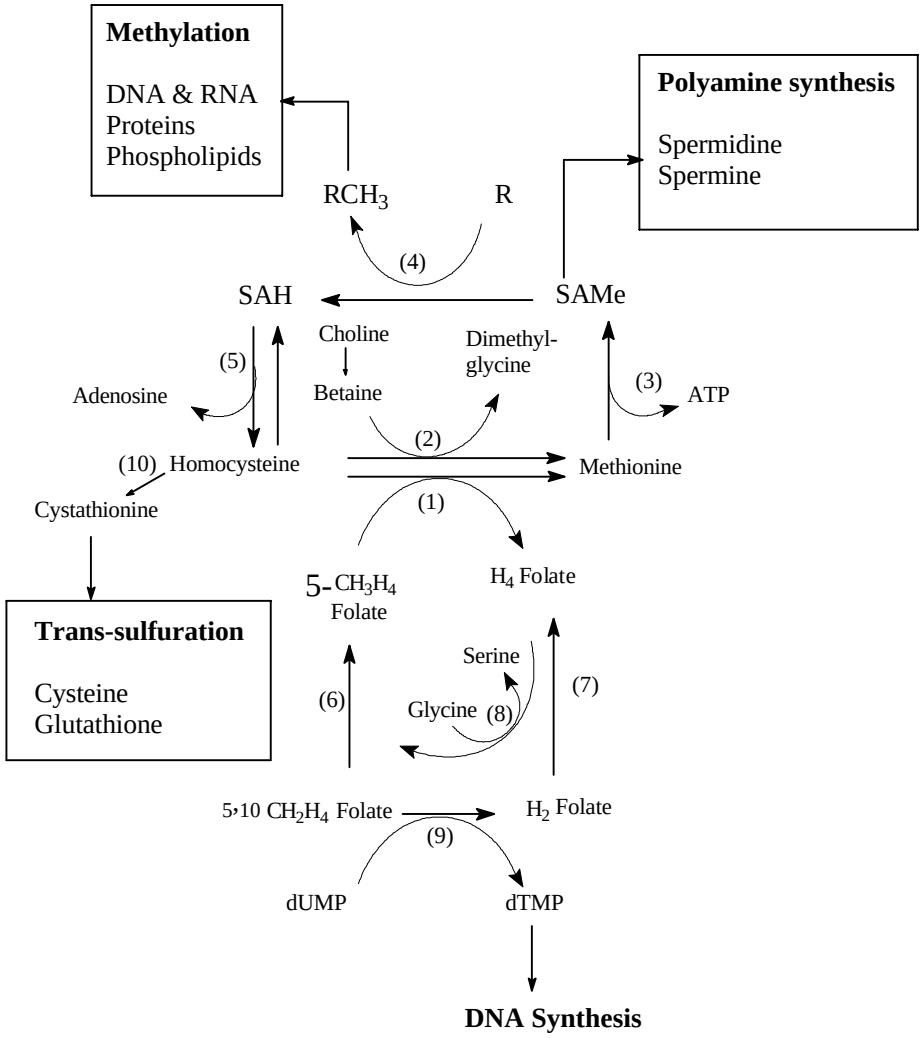
Figure 1. Relationship between the folate one-carbon cycle and SAMe metabolism



(1) methionine synthetase; (2) betaine:homocysteine methyltransferase; (3) methionine adenosyltransferase; (4) R-methyl transferase; (5) SAH hydrolase; (6) 5,10-methylenetetrahydrofolate reductase; (7) dihydrofolate reductase; (8) serine hydroxymethyltransferase; (9) thymidylate synthetase; (10) cystathionine-β synthetase. Abbreviations: 5,10 CH_2H_4 Folate = 5,10-methylenetetrahydrofolate; 5- CH_3H_4 Folate = 5 methyltetrahydrofolate; dTMP = deoxythymidine monophosphate; dUMP - deoxyuridine monophosphate; H_2 Folate = dihydrofolate; H_4 Folate = tetrahydrofolate (adapted from Bottiglieri *et al.*, 1994).

Other Biological Effects:

Hypomethylation from SAMe deficiency. Disorders that inhibit methylation can cause clinical sequella ranging from structural abnormalities such as myelopathy to functional abnormalities such as depression. Methylation can be disrupted by an inadequate supply

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of methionine synthetase (following vitamin B_{12} deficiency or folate deficiency), SAMe synthetase (due to ethanol), or SAH hydrolase (for unknown reasons) or inhibited by limiting the availability of SAMe or by elevating levels of the inhibitor SAH (Scott *et al.*, 1994).

The polyamine biosynthetic enzyme ornithine decarboxylase (ODC) is induced in many preneoplastic systems. Its level in the liver of methyl deficient rats is inversely proportional to the hepatic content of SAMe. The depletion of SAMe in treated L1210 cells or in the livers of rats treated with an initiation/promotion regimen also results in marked increases in ODC and polyamine levels. These changes could be reversed by multiple injections of exogenous SAMe. If a definitive role in carcinogenesis can be ascribed to altered polyamine metabolism, then methyl deprivation may exert its activity in part through this mechanism (Poirier *et al.*, 1990).

It is well established that dietary methyl deprivation results in decreased SAMe levels in the livers of rodents. For example, administering an amino acid defined methyl deficient diet to rats for 1 to 4 weeks also led to DNA hypomethylation that could be reversed by placing the animals on a methyl adequate diet for 1-2 weeks. The discovery of an association between DNA hypomethylation and decreased hepatic SAMe led to the proposal that increased oncogene expression caused by such hypomethylation was a causative factor in hepatocarcinogenesis by methyl deficency. More recent results have shown that feeding an amino acid defined methyl deficient diet for 1 to 32 weeks resulted in the hypomethylation of the c-H-*ras*, c-K-*ras*, and c-*fos* protooncogenes (Poirier, 1994).

The molecular biological studies conducted to date on hepatocarcinogenesis by dietary methyl deprivation indicate that while hypomethylation and increased expression of c-H-*ras*, c-K-*ras*, and c-*fos* have all been observed, their role in carcinogenesis, in the absence of an initiating agent, remains to be established. On the other hand, the presence of activated *myc* and mutated p53 genes in the tumors of animals fed methyl deficient diets

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without any further carcinogen treatment suggests that such changes are likely to play a causative role in carcinogenesis. Other mechanisms besides enhanced oncogene expression caused by gene hypomethylation may be responsible. The evidence currently available indicates that in humans, folate deprivation may contribute strongly to methyl insufficiency (Poirier, 1994).

Anticarcinogenic Effects. The administration of SAMe and its precursors L-methionine, choline, and betaine inhibits the development of preneoplastic and neoplastic lesions in rats subjected to various initiation/promotion treatments (Pascale *et al.*, 1993). The chemo-preventive effect of SAMe correlates with inhibition of polyamine synthesis, DNA methylation, and decreases in expression of c-*myc*, c-Ha-*ras* and c-Ki-*ras* genes in preneoplastic liver lesions (Simile *et al.*, 1996). Six hepatocarcinogenic stressors whose activities were inhibited by methyl donors decreased the hepatic content of SAMe or the ratio of SAMe to SAH in the liver when chronically fed to rats (Poirier, 1994).

Simile and coworkers (1996) examined the effects of SAMe administration on hepatocarcinogenesis promoted with thiobenzamide (TB) and initiated by diethyl-nitrosamine (DEN). DEN-initiated male F344 rats were subjected to two TB cycles and SAMe (384 umol/kg/day, im) was given between these cycles. Many γ-glutamyl transpeptidase (GGT)-positive lesions developed in initiated rats after the first TB cycle. They decreased in number after TB withdrawal, with partial recovery after the second TB cycle. Liver ornithine decarboxylase (ODC) activity and c-*myc* and c-H-*ras* mRNAs increased during the TB cycles and returned to normal after TB withdrawal. Number and size of GGT-positive lesions, DNA synthesis of GGT-positive cells, liver ODC activity, and c-*myc* and c-H-*ras* mRNA levels decreased as a consequence of SAMe treatment during the first TB cycle. The recovery of these parameters induced by a second TB cycle was prevented by SAMe treatment. These results suggested to the authors that SAMe causes a persistent decrease in growth capacity of preneoplastic liver lesions in rats subjected to a DEN/TB protocol (Simile *et al.*, 1996).

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Persistent (neoplastic) nodules (PN) were measured in the livers of male Wistar rats initiated with DEN and receiving SAMe im at intervals up to 20 weeks after initiation. SAMe caused a dose-dependent decrease in number and surface area of GGT-positive foci and in labeling index (LI) of focal cells. SAMe liver contents, SAMe toSAH ratio, and overall methylation of liver DNA were low during the development of GGT-positive foci, but SAMe administration caused a dose-dependent recovery of DNA methylation. A high ODC activity found in the liver during the development of preneoplastic foci was inhibited by SAMe treatment (Pascale *et al.*, 1991).

The administration of SAMe (384 umol/kg/day) caused 77 percent and 42 percent decreases in the percentage of GGT-positive and GST-P-positive lesions, respectively, in DEN-initiated rats. A 46 percent decrease in labeling index of GGT-positive foci was also observed in SAMe-treated DEN-initiated rats. These changes were associated with decreases in liver pyruvate kinase, lactate dehydrogenase, and glycerol-3-phosphate dehydrogenase. SAMe did not affect these enzymatic activities in normal and uninitiated controls. The decrease in DNA synthesis observed in SAMe-treated rats was paralleled by a partial reversion of carbohydrate metabolic features to those present in normal liver (Gerbracht *et al.*, 1993).

To determine if SAMe treatment prevents formation of preneoplastic and neoplastic liver lesions or merely causes a delay in their development, Pascale and coworkers (1992) subjected male Wistar rats to initiation with DEN, followed by phenobarbital, and then im injections of SAMe (34 µmol/kg/day) for 24 weeks. In SAMe-treated rats, a decrease in the incidence of PNs was found 6, 14, and 24-28 months after initiation. At the end of SAMe treatment the number of PNs per rat liver, nodule diameter, and labeling and mitotic indices of nodular cells decreased considerably. Nodule diameter started to increase rapidly again 8 months after SAMe treatment had ceased. Fourteen and 24-28 months after initiation, hepatocellular carcinoma incidences were 11 of 12 and 10 of 10 in control rats, respectively, but only 1 of 12 and 2 of 11 in SAMe-treated rats.

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Reproductive Effects. Several clinical trials of pregnant women, congenitally hypersensitive to the estrogen load produced by the placenta, have shown the effectiveness of iv administration of SAMe for treatment of intrahepatic cholestasis of pregnancy and associated pruritus. Dosages were 200 to 800 mg/d for 10 to 30 days administered intravenously. SAMe also exerted a beneficial effect on the fetal complications of this syndrome, *i.e.*, premature labor and low-birth weight. Adverse reactions were not observed and newborns had normal Apgar scores (Almasio *et al.*, 1990; Bonfirraro *et al.*, 1990; Coltorti *et al.*, 1990; Catalino *et al.*, 1992).

Schenker and coworkers (1998) investigated the transfer of SAMe across the term, normal human placenta. SAMe was transferred slowly, similarly to passively transported L-glucose. A nonenzymatic breakdown of SAMe to at least one other derivative occurred.

SAMe has also been examined for embryofetal toxicity in rats and rabbits and for peri- and post-natal toxicity in rats. Dosages were 100 to 400 mg/kg/d in rats (sc or iv) and 10-40 mg/kg/d in rabbits (iv). SAMe did not produce adverse effects upon any of the reproductive parameters examined and there was no indication that treatment adversely affected litters, including incidences of malformations, anomalies, and skeletal variants. Adverse effects on the parents noted at 400 mg/kg/d included local tissue reaction, retardation of body weight gain, histopathological changes in the female rat kidney (sc studies only), and some rigidity and dyspnea (iv studies only). At 200 mg/kg/d (sc), some histopathological changes to the female kidney were also observed (Cozens *et al.*, 1988).

Mammalian development is dependent on DNA methyltransferase and its product 5-methylcytosine (5MC) to help establish, define, or stabilize the various cell types that constitute the developing embryo. In mammals, 5MC is involved in a major epigenetic mechanism with some 5MC patterns being inherited epigenetically. Little is known about how maternal dietary methyl supplements affect epigenetic regulation of the developing mammalian embryo or whether high levels of methyl supplements are toxic. It is possible

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that such supplements affect gene expression and 5MC levels in adults and that the level of gene-specific 5MC in young mammals could affect their adult health and longevity (Wolff *et al.*, 1998).

Structure-Activity Relationships: The concentration of SAMe available for methylation is dependent on the concentrations of other compounds in the methylation and trans-sulfuration pathways. Information for structure-activity analysis was sought by examining the literature to determine the effects of excesses and deficiencies of these compounds, in particular methionine. In addition, information was obtained on the carcinogenicity of an inhibitor of SAMe, ethionine. This information is presented below.

Methionine toxicity. Methionine is an essential amino acid which is required for protein synthesis as well as for the synthesis of choline, cysteine, glutathione, and taurine by the trans-sulfuration pathway (Chawla *et al.*, 1990).

Although it is nutritionally essential for all mammals, methionine possesses significant toxicity. Rats fed toxic levels of methionine accumulated methionine, taurine, and glutathione in all tissues measured, and developed a marked accumulation of SAMe and its catabolites in the liver. Thus, methionine toxicity is likely to be linked to hepatic accumulation of SAMe, resulting in liver dysfunction (Regina *et al.*, 1993).

Adult Wistar rats initiated with azoxymethane were fed diets enriched with 1 percent methionine. After 12 weeks, the methionine-supplemented diet had stimulated the turnover rate of ileal epithelial cells, indicating enhanced crypt cell proliferation. There was also a 2-fold increase in the number of aberrant hyperproliferative crypts and tumors in the colon. These data did not support the view of a chemopreventive effect of dietary methionine supplementation, but rather suggested that methionine promotes intestinal carcinogenesis (Duranton *et al.*, 1999).

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Slattery and coworkers (1997) observed a modest trend of increasing risk of colon cancer associated with methionine intake in a large retrospective population-based case control study of incident colon cancer. LaVeccia and coworkers (1994, 1997) found evidence from a large case control study to suggest that a diet rich in methionine, salt, and nitrite may be associated with increased gastric cancer risk.

Lipotrope-deficient (methyl-deficient) diets and liver cancer. Lipotropes are a group of nutrients that includes choline, methionine, folic acid, and vitamin B_{12}. These substances interact in the regulation of the intracellular supply and metabolism of methyl groups, particularly in the form of SAMe. Lipotrope-deficient diets cause extensive liver damage including fatty livers, induce cell turnover, and promote liver carcinogenesis in rats and certain strains of mice (Wainfan & Poirier, 1992; Pascale *et al.*, 1993).

In male Fischer 344 rats and $B6C3F_1$ mice, prolonged intake of methyl deficient diets, even without exposure to any known carcinogens, has been observed to result in the development of liver tumors (Wainfan & Poirier, 1992). The chronic feeding of a diet lacking both methionine and choline led to the formation of altered hepatic foci which continuously increased in volume but not in number, an observation consistent with the hypothesis that methyl deprivation is not a continuously initiating stress, but a promoter of preexisting initiated hepatocytes (Poirier, 1994).

Lipotrope-rich diets and chemoprevention. Various laboratories have indicated that lipotropic compounds, such as methionine, choline, and betaine, prevent the development of mammary gland, skin, and liver cancers induced in rats and mice by various carcinogens, with and without successive administration of a promoting agent. Chemoprevention of spontaneous thymic lymphoma has been observed in AKR/J mice fed a lipotrope-enriched diet (Pascale *et al.*, 1993).

Inhibition of methylation process by ethionine. Dietary ethionine administered chronically

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at appropriate concentrations is a complete hepatocarcinogen in rats and mice. Supplementation of ethionine-containing diets with methionine, choline, or betaine reduced ethionine-induced carcinogenesis in rats, suggesting that ethionine may exert its activity through perturbation of intracellular transmethylation functions (Allen & Poirier, 1997).

The metabolism of ethionine mimics that of methionine, apparently via the same enzymatic systems that metabolize methionine. The ethyl homolog of SAMe, S-adenosylethionine (SAE) is found in high concentrations in the livers of rats fed ethionine. SAE has been identified as an inhibitor of DNA transmethylase and is responsible for the inhibition of tRNA methylation. SAE functions as an ethyl donor in some reactions that normally use SAMe as a methyl donor. Thus, high levels of SAE, coupled with low levels of SAMe, may create an environment where normal methylation processes are suppressed or supplanted by ethylation (Allen & Poirier, 1997).

Further evidence to support a role for SAMe and methyl group insufficiency as a potential mechanism for ethionine carcinogenesis is as follows. Ethionine at 0.1 percent induced an 89 percent incidence (24/27) of hepatocellular carcinoma in male F344 rats. Adding phenobarbital to the 0.1 percent ethionine diet reduced the incidence of hepatocellular carcinoma to 36 percent (10/28). Dietary ethionine at 0.1 percent reduced the intracellular hepatic level of SAMe to <50 percent of that seen in control rats. Combinations of phenobarbital and 0.1 percent ethionine led to increases in hepatic levels of SAMe of 40-60 percent after 3 and 6 weeks of feeding compared to those seen in rats receiving 0.1 percent ethionine alone (Allen & Poirier, 1997).

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