SUMMARY OF DATA FOR CHEMICAL SELECTION

Dietary Supplements Containing Ephedrine Alkaloids

BASIS OF NOMINATION

Dietary supplements containing ephedrine alkaloids from herbal sources (ephedra) are brought to the attention of the CSWG as part of an ongoing National Institutes of Health (NIH) Office of Dietary Supplements (ODS) research initiative on the safety and efficacy of these products. This initiative was recommended by the Senate Committee on Appropriations in fiscal year (FY) 2001. In its Report making FY 2002 appropriations for the Department of Health and Human Services, the Committee expressed pleasure that the ODS followed through on its FY 2001 recommendation and urged the ODS to continue and expand this important effort. The nomination of ephedra-containing supplements to the National Toxicology Program (NTP) is part of the expanded effort by the ODS.

As the Senate Committee noted, the number of Americans taking dietary supplements containing ephedra has risen dramatically. Dietary supplements containing herbal ephedra (chiefly *Ephedra sinica* Stapf.) are widely used as diet aids and athletic performance enhancers, with over 3 billion servings[1] sold each year for weight loss alone. The active ingredients in these supplements are primarily ephedrine and pseudoephedrine with small amounts of other ephedrine alkaloids usually present. Ephedra-based diet aids often contain other stimulants, such as caffeine (guarana), as well as salicin, diuretics, and cathartics. Ephedra-containing performance enhancers often contain caffeine and other ingredients, such as chromium picolinate or *l*-carnitine, and may be consumed with other performance enhancers, such as androstenedione and creatine monohydrate.

The ephedrine alkaloids are sympathomimetic amines, a class of compounds with fairly well characterized short to medium term biological activities. Specific combinations of synthetic ephedrine and caffeine have been used as prescription drugs in Europe, and were formerly available in the US until they were withdrawn by the FDA in 1992 for reasons of safety. Very little is known

[1]Dose or portion, in this case, is referred to as a "serving" because diet aids are defined as foods.

about the possible interactions between herbal ephedra and biologically active compounds in the other plant species that are constituents of many dietary supplements. Some agents, often components of herbal preparations at far greater concentrations than the ephedrine alkaloids, may profoundly influence the toxicity attributed to ephedra supplements.

Limited information suggests that ephedrine may cause fetal heart defects, a matter that needs further examination since dietary supplements containing ephedrine alkaloids are readily available even though the manufacturers caution against their use during pregnancy.

Ephedrine sulfate and caffeine, tested separately, were both negative for carcinogenic activity. The National Cancer Institute (NCI), through the Chemical Selection Working Group (CSWG), recently submitted several other ingredients commonly found in combination with ephedrine alkaloid sports supplements to the National Toxicology Program for toxicological testing. These include chromium picolinate, androstenedione, and yohimbe bark extract.

INPUT FROM GOVERNMENT AGENCIES/INDUSTRY:

Dr. Paul Coates, Director of the NIH Office of Dietary Supplements, provided information on existing studies of ephedra.

SELECTION STATUS:

Action By CSWG: 12/11/01

Studies requested:

- Reproductive and developmental toxicity studies

- Comparative toxicological evaluation using ephedra, ephedra and natural caffeine products, and other combinations of ephedra/herbal remedies with an emphasis on the heart and central nervous system

Priority: High

Rationale/Remarks:

- Herbal ephedrine alkaloids (ma huang) combined with other herbal products are widely used as diet aids and for performance enhancement.

- Concern that naturally occurring non-ephedrine alkaloid constituents of *Ephedra* spp. may have toxicologic significance.

- Concern that the herbal combinations used as dietary supplements may possess toxicity not seen from administration of synthetic ephedrine/caffeine combinations.

- Emphasize testing of products/combinations actually marketed to consumers as dietary supplements.

- Priority for testing should be given to combinations of herbals used as diet aids.

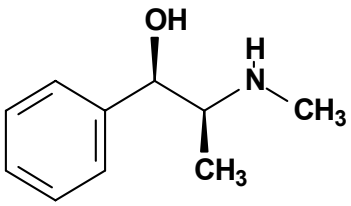
- Involve the NIH Office of Dietary Supplements and the FDA in study design of to ensure that the testing performed addresses their concerns.

CHEMICAL IDENTIFICATION

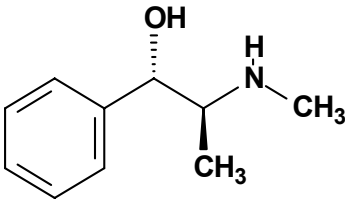
CAS Registry Number:	Ephedrine alkaloids: none *l*-Ephedrine; (-)-ephedrine: 299-42-3 *d*-Pseudoephedrine; (+)-pseudoephedrine: 90-82-4
CAS Name:	None
Synonyms and Trade Names:	Chinese Ephedra, Chinese Jointfir, herbal ephedra, ma huang
Structural Class:	Botanical mixture

Structure, Molecular Formula and Molecular Weight:

This information is provided for the main active ingredients in ephedrine alkaloid supplements.



l-Ephedrine



d-Pseudoephedrine

$C_{10}H_{15}NO$ Mol. wt.: 165.23

Chemical and Physical Properties: The information provided in Table 1 includes the main active ingredients in ephedrine alkaloid supplements and some of their common salts.

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

Table 1. Chemical & Physical Properties of Some Ephedrine Alkaloids.

d,l-Ephedrine	Crystals; mp 79EC; sol in water, alcohol, ether, chloroform, oils
l-Ephedrine	Waxy solid, crystals, or granules; soapy feel; mp 34EC (absorption of water raises mp to 40EC; sol in water, alcohol, ether, chloroform, oils
d,l-Ephedrine hydrochloride	Crystals; mp 187-188EC; sol in water, alcohol, insol in ether
l-Ephedrine hydrochloride	Orthorhombic needles; affected by light; mp 216-220EC; sol in water, alcohol, insol in ether or chloroform
d,l-Pseudoephedrine	Crystals, mp 118EC
(+)-Pseudoephedrine	Rhombic tablets from ether; mp 119EC; sparingly sol in water, sol in alcohol or ether
(+)-Pseudoephedrine hydrochloride	Needles; mp 181-182EC; sol in water, alcohol, chloroform

Source: Merck, 2000

Technical Products and Impurities: In the United States, ephedrine alkaloid dietary supplements contain herbal ephedra extracts, not synthetic ephedrine even though ephedrine is the principal active ingredient. Pure or synthetic ephedrine is considered to be a drug, not a dietary supplement; in fact, a company was recently fined, and all of its product seized, for "spiking" its ma huang-containing extracts with synthetic ephedrine (Coates, 2001).

The most commonly used herbal ephedra, ma huang contains total ephedrine alkaloids at 0.5-2.5% by weight; ephedrine accounts for 30-90% of these alkaloids (Lee *et al.*, 2000). Because of the variation in content, ma huang is mostly used as an extract, concentrated to about 6% alkaloids (American Herbal Products Association, 1999). The alkaloid pattern can often lead to identification of the species, the geographical area where it grew and the climatic conditions, while atypical patterns can suggest that a ma huang sample is "fake", made from pharmaceutical raw materials (American Herbal Products Association, 1999).

Twenty commercially available diet aids containing ephedrine alkaloids were tested by HPLC procedures. Ephedrine and pseudoephedrine in a 2:1 ratio accounted for 96-98% of the ephedrine alkaloid content. Norephedrine (phenylpropanolamine) content was less than 2% of the total ephedrine alkaloid content. Additional ephedrine alkaloids, (+)-norpseudoephedrine (cathine), a Schedule IV controlled substance, and methylephedrine have been identified in

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

small amounts in ma huang and in extracts prepared from ma huang (CANTOX, 2000; Gurley *et al.*, 2000; Kalix, 1991; Lwanami *et al.*, 1985).

Among the 20 dietary supplements examined, ephedrine alkaloid content varied considerably suggesting that industry standards are often not met by individual supplements. Total alkaloid content ranged from 0.0 to 18.5 mg per dosage unit. Ranges for (-)-ephedrine and (+)-pseudoephedrine were 1.1-15.3 mg and 0.2-9.5 mg, respectively. Significant lot-to-lot variations in alkaloid content, from 44 to 260% of label claim, were also observed. Half the products exhibited discrepancies between the label claim for ephedrine alkaloid content and actual alkaloid content in excess of 20% (CANTOX, 2000; Gurley *et al*., 2000).

Most ephedra product labels indicate the amount of ephedra herb or extract contained in each dosage form; however, not all make a claim of ephedrine alkaloid content (CANTOX, 2000).

Dietary supplements containing ephedrine alkaloids generally contain a host of other botanical ingredients. Kola nut and guarana are used as natural caffeine sources; St. John's Wort, white willow bark (salicin), diuretics, or cathartics are also often included (CANTOX, 2000). Performance enhancers may include chromium picolinate or *l*-carnitine (Karch, 2000). Yohimbine is sometimes added (Bucci, 2000).

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

EXPOSURE INFORMATION

Production and Producers: Dietary supplements containing botanical sources of ephedrine alkaloids are marketed for weight loss and increased energy. These products are available in a variety of forms, including pills, powders, liquid drops, and teas (GAO, 1999).

Herbal ephedra products are sold via traditional retail chains (drug stores, health food stores, etc.), mail-order catalogs, and direct sales distribution networks. Industry sources estimate that there are over 500,000-750,000 independent distributors of dietary supplements that contain herbal ephedra (Pape *et al.*, 1999).

Most companies marketing herbal ephedra are privately held and do not report sales and earnings figures to the public. Nevertheless, retail sales of these dietary supplement products are conservatively estimated to exceed $1.5 - $2 billion per year (Pape *et al.*, 1999).

In testimony to the Office on Women's Health, industry sources estimated that 3 billion doses of ephedra-containing products are sold in the United States annually. This figure appears to be low. The American Herbal Products Association contacted 42 companies known to distribute dietary supplements containing ephedrine alkaloids. The 13 companies that responded reported aggregate figures of 3,086,041,072 servings sold in 1999. Metabolife International reported that projected sales of one ephedrine alkaloid weight-loss product, Metabolife 356®, were nearly 2 billion caplets in that same year (Anon., 1999; CANTOX, 2000; Office on Women's Health, 2000).

Use Pattern: Ma huang is derived from *Ephedra sinica* Stapf, *E. intermedia* Schrenk et C.A. Mey, and *E. equisetina* Bunge (Ephedraceae). North American and Central American types are alkaloid free (American Herbal Products Association, 1999).

A traditional Chinese medicine, it has been used for about 5000 years to treat asthma, nose and lung congestion, and fever with anhidrosis. Ma huang's traditional use to treat cough and respiratory infections may be related to 4-quinolone-2-carboxylic acid

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

(transtorine) which inhibits the growth of several common bacteria. Large quantities of ma huang are now used as a source of ephedrine in dietary supplements formulated to promote weight loss. Ma huang products sold for weight control often contain other stimulants, such as caffeine, which may have synergistic effects and increase the potential for adverse effects (American Herbal Products Association, 1999; Botanical Dermatology Database, 2001; FDA, 1993; Office on Women's Health, 2000; Karch, 2000; Lee *et al.*, 2000).

The best selling herbal weight-loss product is Metabolife 356® (Anon., 1999). According to its manufacturer, Metabolife 356® contains vitamin E, magnesium chelate, zinc chelate, chromium picolinate, guarana (seed), ma huang extract, bee pollen, Siberian ginseng (root), ginger root, lecithin, bovine complex, damiana (leaf), sarsaparilla root, goldenseal, nettle leaf, gotu kola, spirulina, Royal jelly, citric acid, glycine, caffeine, croscarmellose sodium, protein hydrolysate, silica, modified cellulose, magnesium stearate, dextrin, dextrose, sodium carboxymethylcellulose, sodium citrate, and ascorbic acid (Metabolife, 2001). The exact amounts of each ingredient are proprietary, but each tablet contains 12 mg of ephedrine alkaloids and 40 mg of caffeine alkaloids (Metabolife, 2001).

Ephedrine alkaloid/caffeine products are used by some athletes to enhance physical performance, especially bodybuilders who want to maximize body fat loss while maintaining muscle mass (Bucci, 2000). Ephedra is often taken along with other sports nutrition supplements, especially androstenedione and creatine monohydrate (Consumers Union, 2001).

l-Ephedrine sulfate has FDA approval as an over-the-counter (OTC) medication for use in asthma patients. An $\alpha + \beta$ adrenergic agonist, ephedrine functions as a bronchodilator. Isolated and synthesized in 1885, ephedrine became a first-line drug against asthma in the 1930s, although this no longer appears to be an important use of ephedrine. Unlike the other alkaloid drugs, ephedrine is also a potent central nervous system (CNS) stimulant that has been used as a drug of abuse, much like methamphetamine (McEvoy, 2000;

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

Karch, 2000; Office on Women's Health, 2000).

In the past, ephedrine was used to treat Stokes-Adams attacks with complete heart block and as a CNS stimulant in narcolepsy and depressive states. Ephedrine has been used to treat the hypotension that may occur with spinal anesthesia (McEvoy, 2000).

As an alternative for the structurally related illegal drug, Ecstasy (4-methyl-2-dimethoxy-amphetamine, MDMA), ephedra supplements are sometimes sold outside usual channels of commerce and marketed specifically to young adults to achieve a legal high (FDA, 2000). These products may be spiked with synthetic ephedrine alkaloids and combined with other stimulants such as caffeine (Bucci, 2000). One of these products, Cloud 9, was labeled as containing 650 mg of Indonesian ma huang extract per capsule with a recommended dose of 1-2 capsules 30-35 minutes before activity (Blumenthal & King, 1996).

The FDA considers Herbal Ecstasy to be a street drug alternative, not a dietary supplement. Since street drug alternatives have a potential for abuse, FDA considers them to be unapproved new drugs and misbranded drugs under sections 505 and 502of the Federal Food, Drug, and Cosmetic Act (FDA, 2000; US Department of Health and Human Services, 1996).

Human Exposure: The two main groups that use ephedra products are young to middle age, overweight individuals, and young individuals engaged in exercise programs (Office on Women's Health, 2000).

Results of the National Health and Nutrition Examination Survey (NHANES) in 1999 indicate that an estimated 61% of US adults are either overweight or obese. In adults aged 20-74 years, obesity has nearly doubled from approximately 15% in 1980 to 27% in 1999 (CDC, 2001). According to industry sources, Americans spend more than $30 billion a year on diet-related products and services related to obesity treatment (Office on Women's Health, 2000).

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

Ephedra supplement labels warn customers not to use herbal ephedra for more than 12 weeks. An industry spokesperson testified before the Office on Women's Health that this warning label is needed to comply with state laws and is not regarded by the industry as scientifically based (Office of Women's Health, 2000). On the other hand, there have been no published studies in which subjects have been given ephedra for longer than 12 weeks (Coates, 2001). Metabolife International advertises a "preferred customer" program that ships Metabolife 356® automatically every month without a specified time limit (Metabolife, 2001).

A typical ephedra supplement for weight loss contains approximately 12 mg of ephedrine alkaloids per oral serving with a maximum daily intake of 96 mg recommended. Some products include as much as 20 mg of ephedra per dose, but those products recommend consumption of no more than 100 mg/day (Office on Women's Health, 2000).

No information was found in the available literature on the number of persons taking ephedrine alkaloid diet aids. If 3 billion servings are taken at a rate of 8 per day for 12 weeks in the course of one year, then over 370,000 persons would be exposed annually for purposes of weight loss alone.

According to Nutrition Business Journal, 4% of American adults have taken a sports supplement at least once and 1.2 million use them regularly. According to a 1999 survey by Blue Cross/Blue Shield, 6% of youths 15-16 and 8% of those 17-18 have taken sports supplements; the vast majority of users were male. Although ephedra was mentioned as one of the most popular supplements, estimates of the actual number of persons taking ephedra supplements for performance enhancement were not provided. Ephedra is often taken along with other sports nutrition supplements, e.g., androstenedione and creatine monohydrate (Consumers Union, 2001; Council for Responsible Nutrition, 2001).

Ripped Fuel by Twin Labs is a popular supplement used by bodybuilders that contains ma huang, guarana, *l*-carnitine, and chromium picolinate. Another popular product is AST Research's EPH 833, composed mainly of ma huang. The ephedrine content of this

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

type of product ranges anywhere from 12-80 mg per serving (Karch, 2000).

The recommended dosage for *l*-ephedrine sulfate as a bronchodilator is 25 mg (as ephedrine) with a maximum daily dosage of 150 mg (McEvoy, 2000; Office on Women's Health, 2000). The number of persons exposed to ephedrine through this and other medical uses is believed to be small.

Environmental Occurrence: *Ephedra sinica* Stapf is one of 40 species in the *Ephedraceae* (Joint-Pine) family that occurs in northern and southern warm temperate regions. A perennial shrub indigenous to China, its dried young stems, harvested in fall, are used to produce ephedra alkaloids. Ephedra alkaloids can be derived from other species in the *Ephedracea* family as well, notably *E. equisentina* and *E. gerardiana* Wall (Pakistani ephedra). The alkaloid content and the relative quantities of different alkaloids vary widely among the various species; generally, the roots, berries, and seeds contain no alkaloids. *Ephedra sinica* Stapf is a natural source of the clinically important isomeric alkaloids, (-)-ephedrine and (+)-pseudoephedrine. These compounds differ only in their stereochemistry (Botanical Dermatology Database, 2001; Duke & Beckstrom-Sternberg, 2001; Karch, 2000; Nutritionfocus.com, 1999).

Regulatory Status: Since 1994, dietary supplements have been regulated under the Dietary Supplement Health and Education Act (DSHEA). The DSHEA requires no proof of safety for dietary supplements on the market prior to October 15, 1994. Labeling requirements for dietary supplements allow warnings and dosage recommendation 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" (FDA, 1995).

FDA has taken several actions to respond to a perceived public heath concern related to ephedrine alkaloid supplements. In 1994, the Center for Food Safety and Applied Nutrition issued a medical bulletin outlining potential adverse reactions from dietary supplements containing ephedrine alkaloids. In 1995, the FDA warned consumers not

to purchase or consume Nature's Nutrition Formula One products labeled as containing ma huang and kola nut because the product posed a risk to consumers' health. In October of that same year, FDA convened a special workgroup to address ephedrine alkaloid dietary supplements. In August 1996, FDA's Food Advisory Committee (FAC) was asked to provide opinions on specific ways to address ephedrine alkaloid supplements. Over half the members concluded that no safe level for ephedrine alkaloids could be identified and recommended that these products be removed from the market (FDA,1995; GAO, 1999).

In June 1997, FDA published a proposed rule *Dietary Supplements Containing Ephedrine Alkaloids* (62 FR107) defining 8 mg as the amount of ephedrine alkaloids in a serving of dietary supplement above which the product would be deemed adulterated. Among other things, the proposed rule restricted daily dosages to 24 mg or less, required warning labels that product should not be used for more than 7 days, and prohibited use of ephedrine alkaloids with ingredients having a known stimulant effect (e.g., caffeine). FDA's proposed rule was extremely controversial, leading to a GAO audit which found the FDA's position to be deficient on a number of points. Portions of the proposal were withdrawn on April 3, 2000, and the FDA held public hearings to collect more information. To date a final rule has not been issued (FDA, 1997; GAO, 1999).

Ephedrine is listed in and regulated by the 1988 UN Convention Against Illicit Traffic in Narcotic Drugs and Psychotropic Substances (Pape *et al.*, 1999). Its use by athletes is banned by the National Collegiate Athletic Association, the International Olympic Committee, and recently, by the National Football League (Wilner, 2001; Vardigan, 2001).

Since ephedrine became the precursor of choice for making methamphetamine, federal regulators have severely restricted its bulk sales (Karch, 2000). The complex matrix of ingredients present in herbal ephedra, however, is not conducive to easy conversion to methamphetamine (Pape *et al.*, 1999).

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

To date, 28 states have taken action to restrict ephedrine use (Leikin & Klein, 1000). Under Ohio law, the maximum recommended dosage of ephedrine for a healthy adult is 100 mg in a 24-hour period for not more than 12 weeks (Metabolife, 2001). Texas law requires the following statement: "This product has ephedrine group alkaloids in the form of herbal extracts and may cause serious adverse health effects"(Metabolife, 2001).

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

EVIDENCE FOR POSSIBLE TOXICOLOGIC ACTIVITY

Human Data on Ephedrine and Ephedrine Alkaloid Supplements: No epidemiological studies or case reports investigating the association between cancer and the ingestion of ephedrine or dietary supplements containing ephedrine alkaloids, either alone or in combination with caffeine, diuretics and cathartics, or herbal remedies to protect the heart, were identified in the available literature.

Ephedrine alkaloids belong to a class of chemicals called the *sympathomimetic amines*. The biological activities of individual ephedrine alkaloids are well characterized, but the effects of combinations of these compounds, such as are found in dietary supplements, are less well known (CANTOX, 2000).

In the US, sympathomimetic poisoning is a common toxicologic emergency. The 1999 annual report of the American Association of Poison Control Centers' Toxic Exposure Surveillance System noted 29,023 sympathomimetic exposures, of which 16,599 were referred to a health care facility and 67 resulted in fatality. Toxicity typically presents with classic sympathomimetic signs and symptoms that include tachycardia, hypertension, diaphoresis, hyperthermia, agitation, and combativeness (Kolecki, 2001).

In persons taking caffeine and ephedrine (often with other ingredients) for weight loss, adverse events appear to reach and remain at placebo levels after 4 to 12 weeks, but data from randomized trials are available only up to 6 months. Since obesity requires chronic treatment, controlled clinical trials of longer duration may be needed to assess whether the benefits outweigh risk, even for synthetic product combinations (Greenway, 2001).

Overview of the Individual Ephedrine Alkaloids as Sympathomimetic Amines.

Ephedrine: Ephedrine stimulates both α- and β-adrenergic receptors. Stimulation of α_1-receptors produces contraction of vascular smooth muscle, increased contractile force of the heart, and arrhythmias, as well as glycogenolysis, gluconeogenesis, and hyperpolarization and relaxation of intestinal smooth muscle. Stimulation of α_2-receptors decreases insulin secretion, platelet aggregation, and the release of norepinephrine from

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

the nerve terminals, but causes contraction of the vascular smooth muscle. Stimulation of β_1-receptors increases force and rate of contraction of the heart, increased velocity of conduction through the atrioventricular node, and increased renin secretion. The primary effects from stimulation of β_2-adrenergic receptors with therapeutic doses of ephedrine are relaxation of the smooth muscle of the blood vessels and bronchi. Stimulation of β_3-adrenergic receptors causes lipolysis. In addition to its direct sympathomimetic effects, ephedrine can release norepinephrine from its storage sites (Mack, 1997).

With prolonged use or frequent dosing, ephedrine depletes norepinephrine stores in sympathetic nerve endings. After several doses of ephedrine are administered, hypotension may result from direct cardiac depression and vasodilation. Ephedrine has CNS stimulating effects similar to those of amphetamines, but less pronounced (McEvoy, 2000).

Pseudoephedrine: Pseudoephedrine acts directly on α-adrenergic receptors in the respiratory tract mucosa producing vasoconstriction which results in shrinkage of swollen nasal mucous membranes, reduction of tissue hyperemia, edema, and nasal congestion and an increase in nasal airway patency. Drainage of sinus secretions is increased. Like ephedrine, pseudoephedrine has an indirect effect by releasing norepinephrine from its storage sites. Pseudoephedrine acts on β-adrenergic receptors to a lesser degree than ephedrine. Pseudoephedrine may relax bronchial smooth muscle by stimulation of β_2-adrenergic receptors. However, substantial bronchodilation following oral administration has not been demonstrated consistently. Usual doses of pseudoephedrine generally produce negligible effect on blood pressure in normotensive patients. Pseudoephedrine may increase the irritability of the heart muscle and may alter the rhythmic function of the ventricles, especially in large doses (McEvoy, 2000).

Norephedrine: d,l-Norephedrine hydrochloride, better known as phenylpropanolamine hydrochloride (PPA), indirectly stimulates both α- and β-adrenergic receptors by releasing norepinephrine from its storage sites. Like pseudoephedrine, PPA produces vasoconstriction of the bronchial mucosa. PPA increases heart rate, force of contraction,

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

and cardiac output and excitability and causes CNS stimulation which may result in nervousness, restlessness, insomnia, dizziness, and headache. In some patients, severe reactions including headache, chest tightness, greatly elevated blood pressure, ventricular or atrial premature contractions, and paroxysms of ventricular and atrial tachycardia have occurred with therapeutic doses. Excessive doses may produce heart attack, stroke, intracranial bleeding, parenchyma cerebral hemorrhage, and death (McEvoy, 2000).

A recently published study of 702 patients and 1376 control subjects suggested that PPA in appetite suppressants, and possibly in cough and cold remedies, is an independent risk factor for hemorrhagic stroke in women (Kernan *et al.*, 2000). Early in 2001, FDA issued a public health advisory asking companies to stop marketing products containing PPA (Coates, 2001).

Case Reports of Toxicity from Ingestion of Ephedrine or Ephedrine Alkaloid Supplements.

Toxicity of ephedrine occurs mostly in the cardiovascular and central nervous systems and includes hypertension, dysrhythmias, myocardial infarction, seizures, anxiety, psychosis, stroke, and death (Powell *et al.*, 1998).

Case reports describing toxicity from the ingestion of ephedrine alkaloid supplements or ephedrine diet pills have been reported in the literature. Examples include:

- Vasculitis and hypersensitivity myocarditis in a 39-year-old man who ingested Herbalife, a ma huang product (1-3 tablets twice daily for 3 months) (Zaacks *et al.*, 1999).

- Cardiovascular toxicity in a 21-year-old man who ingested herbal ecstasy taken with alcohol and a marijuana cigarette. Caffeine may have contributed to the toxicity observed (Zahn *et al.,* 1999).

- Death of a 23-year-old body builder who consumed Ripped Fuel "according to the stated directions" once or twice a day for >6 months. Autopsy revealed myocardial necrosis with healing of 1-2 weeks, mild fibrosis, and moderate myocyte hypertrophy and vascular congestion (Theoharides, 1997).

- Cerebral infarct in a 33-year-old sportsman consuming high doses of ma huang extract and creatine monohydrate (Vahedi *et al.*, 2000).

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

- Thalamic infarct (stroke) in a 37-year-old man who ingested street-purchased "speed" containing ephedrine for weight loss (Bruno *et al.*, 1993).

- Hemorrhagic stroke in a 19-year-old woman with a history of anorexia/bulemia and alcoholism after she ingested alcohol and 15-18 tablets containing 25 mg ephedrine and 100 mg guaifenesin (Matthews *et al.*, 1997).

- Myocardial infarction in a previously healthy 19-year-old male bodybuilder after using tablets containing ephedra and caffeine (Traub *et al.*, 2001).

- Three deaths from non-prescription diet aids or stimulants; ephedrine concentrations in the blood were 3.5-20.5 mg/L; corresponding blood caffeine concentrations (130 to 344 mg/L) were sufficient to be lethal (Garriott *et al.,* 1985).

- Hepatitis in a previously well 33-year-old woman shortly after taking a Chinese medicine product containing ma huang (Nadir *et al.*, 1996).

Cardiomyopathy has been reported in individuals who have taken high doses of ephedrine for prolonged durations, in one case 80 25-mg tablets daily for 8 years (Gualtieri & Harris, 1996). In another case, a 35-year old man developed cardiomyopathy following many years of ephedrine use at doses sometimes exceeding 400 mg/day for treatment of asthma (Van Mieghem *et al.*, 1978).

At the time of FDA's proposed rule, the FDA had received over 800 Adverse Event Reports (AERs) for products thought to contain ephedrine alkaloids, more AERS than for any other dietary supplement. FDA does not have the authority to require submission of reports of adverse events, and AERs are a passive monitoring tool that relies on voluntary reporting. Like all passive surveillance systems, AERs have certain limitations such as under-reporting and poor report quality (GAO, 1999).

An independent review of 276 AERs conducted for the Ephedra Education Council, a trade association, contended that the FDA database contained numerous inaccuracies, inconsistencies, and omissions (Ephedra Education Panel, 2000).

Because the FDA's use of AER data to support its proposed regulation of ephedrine-alkaloids was widely criticized, the FDA requested an independent review of the AER

reports. A total of 140 reports of adverse effects related to the use of dietary supplements containing ephedra alkaloids submitted to FDA between June 1, 1997, and March 31, 1999, were examined. Of these cases, 31% were judged as definitely or probably related to the use of supplements containing ephedra alkaloids, and 31% were deemed to be possibly related. Among the adverse events deemed definitely, probably, or possibly related, 47% involved cardiovascular symptoms and 18% involved the CNS. Hypertension was the single most frequent adverse effect (17 reports), followed by palpitations, tachycardia, or both (13), stroke (10); and seizures (7). Ten events resulted in death and 13 events produced permanent disability (Haller & Benowitz, 2000).

In a September 5, 2001 petition to the FDA to ban dietary supplements containing ephedra, Public Citizen contends that 1,398 of 3,308 AERs received by FDA between January 1993 and February 2000 were associated with ephedra and related substances. Among the 1,398 cases, there were 81 deaths, 32 heart attacks, 62 reports of cardiac arrhythmia, 91 reports of hypertension, 69 strokes, and 70 seizures. Public Citizen also noted an increase in adverse events associated with ephedra-containing supplements in data collected by the American Association of Poison Control Centers, which showed 211 events in 1997 vs 407 in 1999 (Public Citizen, 2001).

In a single study, nephrolithiasis (kidney stones) was reported to be associated with chronic ephedrine intake (Blau, 1998). In followup to a case of nephrolithiasis in a 27-year old man who had taken 4-12 energy supplement tablets containing 170 mg of ma huang extract daily for 11 months, it was determined that 106 specimens from a total of 166,466 stones archived at a single facility contained ephedrine metabolites (Powell *et al.*, 1998).

In many of the case reports and AERs, alternative explanations for the cause of the adverse event are possible, or additional factors were present that may have contributed to the toxicity. Thus, the role of ephedra remains disputed.

Effects of Ephedrine or Ephedra without Caffeine.

According to an industry spokesperson, an extensive literature review by Jewel and Binramache indicated that a single dose of 60 mg of ephedrine is required to cause a significant increase in blood pressure in healthy adults. The magnitude of this increase was 10-15 mm Hg (Office of Women's Health, 2000).

A short-term (2 day) randomized crossover study involving 10 subjects (5 male, 5 female) compared the effects of ingesting three dietary supplements containing ma huang vs 25 mg ephedrine capsules. Although the dietary supplements also contained other herbal ingredients, the adverse effects reported (tachycardia, anxiety, headache, irritability, insomnia, and loss of appetite) were typical of ephedrine (Gurley *et al*., 1998).

A second study examined the short-term effects of ingesting ma huang on heart rate and blood pressure in 12 normotensive subjects administered two doses (19.4 mg ephedrine, 4.9 mg pseudoephedrine, 1.2 mg methylephedrine) 9 hours apart. Six developed a statistically significant increase in heart rate and three had a nonsignificant increase. Four participants experienced statistically significant increases in systolic blood pressure, but two had significant decreases in diastolic blood pressure (White *et al.* 1997).

Using a double blind, placebo, crossover protocol, Kaats and Adelman (1994) demonstrated a significant loss of weight ($P<0.01$) and body fat ($P<0.001$) in 100 subjects administered a proprietary formulation that included ma huang over a 4-week period. No significant changes were observed in resting heart rates and blood pressure.[2]

In a controlled study with exposures for up to a year, the effects of an ephedrine bronchodilator on the cardiovascular system were measured (Wilson *et al.*, 1976). Baseline heart rate changed little in the ephedrine group. Both baseline systolic pressure and diastolic pressure fell progressively, particularly during the last 6 months. Study participants took ephedrine at 75 mg/day and terbutaline at 15 mg/day in 3 divided doses.

[2]Whether this formulation contains caffeine could not be determined; the study sponsor manufactures Lite Bites® which contain chromium picolinate and 22 vitamins and minerals (AMBI, Inc., 2001).

Controlled Studies of Ephedrine/Ephedra and Caffeine.

Ephedra-containing dietary supplement products reported to the FDA as causing adverse events have almost always contained large amounts of caffeine (150-300 mg per unit dose) in addition to ephedrine alkaloids (Bucci, 2000). Thus, the adverse events reported in controlled studies of such products are of interest. Several key papers are described below. Additional information is contained in CANTOX (2000).

Single doses of placebo or ephedrine + caffeine (E+C) (10/200 mg; 20/100 mg; 20/200 mg) were administered to six healthy, lean subjects. The 3-hour post-intake increase in systolic blood pressure after all three combinations average 5-7 mm Hg more than placebo ($P<0.01$). Diastolic blood pressure was increased by approximately 4 mm Hg more than placebo only in the 10/200 and 20/100 groups. Heart rate was increased in the 20/100 and 20/200 groups (Astrup *et al.*, 1991; Astrup & Toubro, 1993).

In followup to the previous study, Astrup and coworkers (1992) conducted a randomized, placebo-controlled, double blind study of 180 obese patients treated by diet, E+C (20 mg/200mg), ephedrine (20), caffeine (200), or placebo 3 times a day for 24 weeks. Weight losses were significantly greater in the E+C group than in the other groups, all of whom lost weight. Systolic and diastolic blood pressure fell in all four groups. Three E+C patients and one ephedrine patient withdrew because of adverse reactions. Approximately half of the E+C and ephedrine patients complained of transient symptoms (tremor, insomnia, dizziness).

Nasser and coworkers (1999) conducted a double-blind, placebo-controlled 8-week trial of Metabolife-356® (E, 72 mg/d; C, 240 mg/d) in healthy subjects. Of the 67 subjects, 7 dropped out before first follow-up, 5 on E+C: 1 with high blood pressure, 4 with heart palpitations. Of 60 with at least one follow-up visit, 12 dropped out before week 8, 6 on E+C: 2 with heart palpitations, 1 with irritability, and 1 with increased systolic blood pressure. Of the 48 subjects who completed the study, the 24 on E+C had greater changes in body weight, % body fat, and serum triglycerides, but they also reported increases in

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

dry mouth, heart palpitations, blood pressure, and insomnia.

Boozer and coworkers (2000) studied of the effects of E+C in obese, but otherwise healthy subjects. A total of 69 persons ingested 90 mg ephedrine alkaloids and 192 mg caffeine daily, given as three equally divided doses. An additional 68 subjects received placebo. Statistically significant increases in systolic blood pressure and heart rate were observed in the ephedra/caffeine group by week 4. A total of 46 subjects in the active group and 38 in the placebo group continued to participate in the study, extending the results through 6 months. Heart rate remained persistently increased although cardiac arrhythmias were not increased.

Huber administered the weight-loss preparations, Nutra 1 (Lean-R-Gy: Thermogenic Weight Loss and Natural Energizer), Nutra 2 (Trim-4-Life), and Nutra 3 (ProTrim) to obese volunteers for 6 months. These products contained approximately 48, 24, and 72 mg ephedrine alkaloid/day, respectively, when used as directed[3]. Nutra 3 also contained 200 mg/day of caffeine when used as directed. Symptoms were stated to be of extremely low frequency; neither systolic nor diastolic blood pressure were raised. The study was not blinded and there were no control groups, limiting its utility (American Herbal Products Association, 1999; CANTOX, 2000).

Ingerslev and coworkers (1997) conducted a 6-week study on the effects of a slimming drug on blood pressure. The 136 subjects were normotensive, hypertensives treated with adrenergic β-receptor blocking drugs, and hypertensives treated with other antihypertensive agents. Systolic blood pressure was reduced significantly (5.5 mm Hg) in the group given E+C (20mg/200mg) and treated with agents other than β-blockers. In the normotensive group given E+C, both systolic and diastolic blood pressure were reduced significantly(4.4/3.9 mmHg). In the E+C groups, 45 of 81 patients had side effects including nausea (12%), palpitations (10%), increased perspiration (6%), and

[3]Nutra 1 and Nutra 2 are derived primarily from ma huang. Nutra 3 ephedra is derived from other sources (American Herbal Products Association, 1999).

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

tremor (5%). Six were withdrawn due to such effects. In the placebo group, 21% reported undesirable effect

During the course of a 15-week study comparing dexfenfluramine with 20 mg ephedrine/200 mg caffeine given 3 times a day, 27 of 50 E+C patients complained of side effects, primarily agitation. Both systolic and diastolic blood pressures were reduced (Breum *e*t *al*., 1994).

Nørregaard and coworkers (1996) administered E+C (60 + 600 mg/day) for 1 year to heavy smokers. A total of 152 received E+C and 73 received placebo. Subjects in the treatment group reported significantly more palpitations, sweating, dizziness, and nausea during the first week but then the differences leveled off; 6 subjects withdrew because of side effects.

Six subjects administered ephedrine (75-150 mg), caffeine (150 mg), and aspirin (330 mg) (ECA) for 7-26 months lost weight without significant changes in heart rate, blood pressure, or cholesterol levels (Daly *et al.*, 1993).

Supplements of E+C (50 mg/400 mg) given daily for 10 days to 9 obese women were described as having only a weak effect on the cardiovascular system, but addition of yohimbine (10 mg/day) attenuated cardiac performance during rest and handgrip stress and increased cardiac work during dynamic exercise in a second group of 9 obese women (Waluga *et al.*, 1998).

The nervousness, psychosis, headache, dizziness, seizures, stroke, premature ventricular contraction, hypertension, myocardial infarction and death described in reports of the adverse effects of ephedra supplements are consistent with the nonselective adrenergic agonist properties of sympathomimetic amines (Boullata & Nace, 2000). Caffeine appears to potentiate these effects.

Animal Data on Ephedra Supplements:

Acute Studies. LD_{50} values for various species and routes of administration are available. Table

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

2 summarizes the information most relevant to the consumption of ephedrine alkaloid extracts used for weight control or enhanced physical ability. All observation periods were for 24 hours.

Table 2. Results of Acute Toxicity Studies Performed on Ephedra.

Species	Route of Exposure and Composition of Test Material	LD_{50} Results
Mice (ICR)	oral (gavage), ephedra extract	5.3 g/kg (extract) 24.0 g/kg (crude ephedra)
Mice (strain not specified)	oral (gavage), ephedra herb extract	4.35 g/kg (males) 5.00 g/kg (females)
Rats (strain not specified)	oral (gavage), ephedra herb extract	4.00 g/kg (males) 3.5 g/kg (females)

Source: CANTOX (2000)

Records from the American Society for the Prevention of Cruelty to Animals National Animal Poison Control Center pertaining to 47 incidents of accidental ingestion of herbal supplements containing mostly guarana and ma huang in dogs were examined. Clinical signs of toxicity included tremors, seizures, vomiting, tachycardia, hyperthermia, and nonspecific behavioral changes. Doses of guarana and ma huang at which signs of intoxication were reported ranged between 4.4 and 296 mg/kg bw and 1.3 and 89 mg/kg bw, respectively. Clinical signs developed within 8 hours after ingestion in 80% of the dogs; duration ranged from 10 to 48 hours (Ooms *et al.*, 2001).

Subacute Studies of Ephedra Supplements. The safety of a ma huang herbal preparation containing 9% *l*-ephedrine was addressed in a repeated-dose study in BALB/c mice. Male and female mice (5/sex/dose) were dosed twice a day at 20-4,000 mg/kg for 2 weeks. The highest dose (8,000 mg/kg/day) was lethal to 50% of the animals with gastrointestinal bleeding observed at necropsy. The same toxicity findings to a lesser degree were observed in the 4,000 mg/kg groups; liver weights were not increased. From these data, the LD_{50} for *l*-ephedrine in this herbal preparation was determined to be 360 mg/kg twice daily (720 mg/kg/day). Based on body surface area corrections, this corresponds to 2.1 grams twice daily of *l*-ephedrine and 23.3 grams twice daily of ma huang in a 70 kg human (Law *et al*., 1996).

Subchronic and Chronic Studies of Ephedra Supplements. No subchronic or chronic studies of the effects of ephedrine alkaloid extracts in animals were identified in the available literature.

Genotoxicity Studies of Ephedra Supplements. Zenedrine RFA-1, a herbal tea containing ephedrine (8.5 mg/ml), pseudoephedrine (3.4 mg/ml), norpseudoephedrine (0.2 mg/ml), and norephedrine (0.2 mg/ml) was tested in the National Cancer Institute Short-Term Test Program (STTP). This material was negative in all five strains of *Salmonella* in the Ames test. Results from the mouse lymphoma assay are not yet available (Seifried, 2001).

Xue-jun and coworkers (1991) reported that *Ephedrae sinica* Staph extract was not mutagenic in *Salmonella typhimurium* TA98 or TA100 either in the absence or presence of S-9 at doses up to 40 mg/plate. The ephedra extract was also administered intraperitoneally to mice at doses described as equivalent to 1-40 times that used in traditional medicine. No changes in the ratio of polychromatic erythrocytes to total erythrocytes or in the frequency of micronucleated polychromatic erythrocytes in the bone marrow micronucleus assay were observed.

Ephedra herba was screened for mutagenic activity by the rec-assay with *Bacillus subtilis* as well as the Ames reversion assay with *S. typhimurium* TA98 and TA100 with and without metabolic activation. The ephedra extract was not mutagenic at concentrations up to 100 mg/ml (Morimoto *et al.*, 1982).

E. sinica extract inhibited the mutagenicity of benzo[a]pyrene in *S. typhimurium* TA98 with S-9. However, the extract was an ineffective antitumor agent when administered for 50 weeks in the drinking water of F344/DuCrj male rats that had received benzo[a]pyrene by subcutaneous injection. *E. sinica* extract also inhibited the mutagenicity of 3,9-dinitrofluoranthene and 1,6-dinitropyrene (Horikawa *et al.*, 1994).

Decoctions of *Ephedra foliata* extracts produced nitrosamines when reacted with excess sodium nitrite at conditions found in gastric juice (pH 2, 37EC) (Alwan *et al.*, 1986)

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

The cytoxicities of various ma huang extracts on a battery of cell lines could not be totally accounted for by their ephedrine contents, suggesting the presence of other toxins in the extracts (Lee *et al.*, 2000).

Animal Data on Ephedrine

Acute Studies. NTP (1986) reported an LD_{50} of 812 mg/kg in male mice and 1,072 mg/kg in female mice of the $B6C3F_1$ strain for ephedrine sulfate administered via gavage. An LD_{50} was not be determined in F344 rats similarly dosed at 75-1,200 mg/kg because deaths occurred in all dose groups.

Subchronic and Chronic Studies. Thirteen-week studies of ephedrine sulfate via feed were conducted by NTP (1986) in F344/N rats and $B6C3F_1$ mice. Doses were 125-2,000 ppm in rats and 310-5,000 ppm in mice. Hyperactivity and excitability, the most frequent clinical observations, were observed with the greatest incidence in animals receiving ephedrine sulfate at 1,000 ppm or higher. No compound-related histopathological effects were observed. Compound-related reduced weight gain was observed in each sex of both species even though feed consumption by dosed and control animals was comparable. These effects at concentrations of ephedrine sulfate of 500 ppm or higher were considered to be potentially life threatening over the course of a 2-year study. For this reason, the ephedrine sulfate doses selected for the 2-year study were 125 and 250 ppm in feed.

Ephedrine sulfate was tested for carcinogenic activity in a 103-week feed study in rats and mice. The compound was administered at 0, 125, or 250 ppm to 50 animals/group/sex. Throughout most of the 2-year study, mean body weights of males and females of both species were lower than those of controls and survival of the chemically exposed female rats (39/50) was greater than survival of controls (27/50). No evidence of a tumorigenic effect was observed. However, a significant decrease in the incidence of mammary gland fibroadenomas observed in ephedrine sulfate-treated female rats was thought to be associated with reduced weight gain (NTP, 1986, pp. 55-56).

Heart-related nonneoplastic lesions showed no evidence of a toxic effect related to the administration of ephedrine sulfate. These lesions are described in Table 3.

Table 3. Heart-Related Non-neoplastic Lesions in 2-Year Ephedrine Sulfate Study (NTP, 1986).

MALE MICE	Control (50)	Low Dose (50)	High Dose (50)
Heart			
Inflammation, chronic focal		1 (2%)	1 (2%)
Perivasculitis	1 (2%)	1 (2%)	
Myocardium			
Inflammation, chronic			1 (2%)
Inflammation, chronic focal			1 (2%)
Degeneration, NOS		1 (2%)	
Pulmonary Vein			
Mineralization		1 (2%)	
FEMALE MICE	**Control (50)**	**Low Dose (50)**	**High Dose (49)**
Heart			
Thrombosis, NOS			1 (2%)
Myocardium			
Mineralization		1 (2%)	
Degeneration, NOS		1 (2%)	
Cytomegaly			1 (2%)
MALE RATS	**Control (50)**	**Low Dose (50)**	**High Dose (50)**
Myocardium			
Mineralization	12 (24%)	6 (12%)	4 (8%)
Inflammation, chronic	11 (22%)	9 (18%)	3 (6%)
Fibrosis	44 (88%)	45 (90%)	46 (92%)
Degeneration, NOS	11 (22%)	3 (6%)	7 (14%)
Mitral Valve			
Inflammation, chronic			1 (2%)
Artery			
Inflammation, active chronic			1 (2%)
Pulmonary Artery			
Mineralization	4 (8%)	1 (2%)	3 (6%)
Splenic Artery			
Hypertrophy, NOS		1 (2%)	
Testicular Artery			
Inflammation, fibrinoid		1 (2%)	
FEMALE RATS	**Control (49)**	**Low Dose (50)**	**High Dose (50)**
Myocardium			
Inflammation, chronic	8 (16%)		1 (2%)
Fibrosis	29 (59%)	32 (64%)	36 (72%)
Degeneration, NOS	6 (12%)	4 (8%)	3 (6%)
Pulmonary Artery			
Mineralization			2 (4%)

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

Genotoxicity: Ephedrine sulfate at 100-10,000 µg/plate was negative in *S. typhimurium* TA97, TA98, TA100, TA1535 with or without metabolic activation (NTP, 1986).

Ephedrine sulfate was also negative in Chinese hamster ovary (CHO) cells without S-9. With metabolic activation, the test was equivocal for both sister chromatid exchanges (SCE) and chromosomal aberrations (CA) (NTP, 1986).

Ephedrine hydrochloride was tested in the National Cancer Institute Short-Term Test Program (STTP) and was negative in all five strains of *Salmonella* in the Ames test. Results from the mouse lymphoma assay are not yet available (Seifried, 2001).

Metabolism of Ephedrine and Ephedra: Ephedrine is rapidly absorbed and extensively distributed throughout the body after oral administration, with distribution to the liver, lungs, kidneys, spleen, and brain. Wide interindividual variation in plasma levels has been observed after oral dosing (CANTOX, 2000).

In a study by Gurley and coworkers (1998), the absorption and distribution of ephedrine was similar whether it was supplied as ephedra extract in combination with other botanicals or as a single ingredient ephedrine capsule. In contrast, White and coworkers (1997) reported that ephedrine ingested in the form of ma huang had a time to maximum plasma concentration (T_{max}) of almost 4 hours, compared with the T_{max} for pure ephedrine of 2 hours.

The major route of elimination for ephedrine in humans is urinary excretion. Ephedrine is eliminated in the urine largely as unchanged drug, with a half-life of about 3 to 6 hours (McEvoy, 2000). Up to 95% of an oral dose may be excreted in the urine within 24 hours. The urinary excretion of ephedrine is pH-dependent due to the presence of an ionizable group in the ephedrine molecule and is increased in acidic urine. In alkaline urine, excretion is reduced to 20 to 35% of the dose (CANTOX, 2000).

The metabolism of ephedrine in humans, dogs and several species of rodents proceeds

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

primarily by three reactions; aromatic hydroxylation, *N*-demethylation, and oxidative deamination. The extent to which ephedrine is metabolized and the major metabolites vary quantitatively between species. The extent of aromatic hydroxylation is greatest in rats, followed by rabbits, guinea pigs, and dogs, with no aromatic hydroxylation observed in humans. *N*-demethylation of ephedrine is greatest in rabbits followed by dogs, guinea pigs, rats, and humans. Deamination is greatest in rabbits, followed by humans and rats (CANTOX, 2000).

Ephedrine, 8-20%, is metabolized in humans by *N*-demethylation to PPA. A total of 4-13% of an oral dose of ephedrine undergoes oxidative deamination yielding 1-phenylpropan-1,2-diol and further side-chain oxidation to benzoic acid and hippuric acid (CANTOX, 2000).

The duration and magnitude of ephedrine activity are known to be affected by antacids and agents which alter the pH of urine thus affecting absorption and excretion, respectively. Ephedrine was not found to affect theophylline clearance (CANTOX, 2000; Upton, 1991).

Other Biological Effects

Teratogenicity. No information on the teratogenicity of ma huang extracts was found in the available literature.

Kanai and coworkers (1986a,1986b) investigated the cardiovascular teratogenicity of ephedrine in pregnant Wistar-Imamichi rats injected ip on days 9,10, or 11 of gestation with single doses of 0.1, 1, 10, or 50 mg/kg of ephedrine. The frequency of cardiovascular abnormalities was dose dependent, ranging from 8.1 to 26.9%, although no significant differences were seen based on day of gestation dosed. All cardiovascular malformations were ventricular septal defects; no extracardiac malformations were observed.

Evidence of cardiovascular teratogenicity has also been observed in chick embryos

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

(Nishikawa *et al.*, 1985).

Placental transfer of ephedrine occurs at 70% of the maternal blood levels. Ephedrine is also excreted in breast milk. No clinical studies have been conducted with ephedrine in pregnant or nursing mothers (CANTOX, 2000).

Effects Contributed by Other Ingredients in Ephedrine Alkaloid Supplements:

The possibility of effects contributed by other agents should be considered since they are often a major component of herbal preparations at far greater concentrations than the ephedrine alkaloids. These agents include stimulants, diuretics and cathartics, and heart protectors.

Stimulants.

Guarana: Guarana seed has been used by Rainforest Indian tribes as a stimulant, astringent, and in treating diarrhea and for curing fevers, headaches, and cramps. Guarana seeds contain up to 5% (25,000-75,000 ppm) of guaranine as well as trace amounts of theophylline (500-750 ppm) and theobromine (300-500 ppm). Guarana seeds also contain large quantities of tannins. Guaranine would affect the body in the same manner as caffeine (Blumenthal, 1998; Guarana.com, 2001a, 2001b, 2001c; Raintree.com, 2001; Vitacost.com, 2001).

Limited testing of guarana has been conducted. No significant toxicological effects were observed in rats and mice given guarana at 1000-2000 mg/kg (ip and po) and motor activity was not affected. Progress in weight gain was similar to controls in rats administered 0.3 or 3 mg/ml guarana in water/Tween as the only source of liquid for 12 months. Histopathological examination found no alterations in heart, lungs, stomach, small and large intestine, liver, pancreas, kidneys, bladder, or spleen (Mattei *et al.*, 1998).

Mice that ingested a guarana suspension at a dose of 0.3 mg/ml had the same average lifespan as controls even after 23 months of treatment (Espinola *et al.*, 1997).

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

Aqueous extracts of guarana did not affect the blood glucose in mice with epinephrine-induced glycogenolysis (Miura *et al.*, 1998).

In contrast, aqueous extracts of guarana were genotoxic as assessed by lysogenic induction in *Escherichia coli,* and this extract also induced frameshift mutations in *Salmonella typhimurium* TA97 (da Fonseca *et al.*, 1994). Aqueous guarana extract induced cytotoxicity in Chinese hamster ovary cells and bacterial cells (*Photobacterium phosphoreum*) causing the authors to conclude that high doses could be harmful to human health (Santa Maria *et al.*, 1998).

Caffeine [58-08-3]. Caffeine is pharmacologically similar to the other xanthine drugs, theobromine and theophylline; however, these three agents differ in the intensity of their actions on various structures (McEvoy, 2000).

Caffeine causes transient increases in heart rate, force of contraction, cardiac output, and heart work. Caffeine constricts cerebral vasculature but dilates peripheral blood vessels, decreasing peripheral vascular resistance. The effect on blood pressure is offset by increased cardiac output. The overall effect of caffeine on heart rate and blood pressure depends on whether CNS or peripheral effects predominate. Adverse CNS effects include insomnia, restlessness, nervousness, and mild delirium. Adverse GI effects include nausea, vomiting, and gastric irritation. It is not known whether caffeine is teratogenic in humans; however, it is teratogenic in rats (McEvoy, 2000).

Caffeine has been tested for carcinogenicity in two separate studies in rats: (1) males and female of the Wistar strain, at doses of 0.1 & 0.2% in drinking water for 78 weeks; and (2) males and females of the Sprague-Dawley strain, at doses of 200, 430, 930, 2000 mg/L in drinking water for 2 years. Both studies were negative (CCRIS, 2001).

As seen in Table 4, below, clastogenicity and mutagenicity have been demonstrated in some *in vitro* and animal studies although many other studies are negative.

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

Table 4. Results of Tests for Genotoxicity of Caffeine.

S. typhimurium TA98, TA100, TA1535, TA1537, TA1538 w/wo S-9: negative (CCRIS)
E. coli wp2 w/wo S-9: negative (CCRIS)
E. coli polA (W3119 vs P3478), rec-assay, DNA effects: positive (Genetox)
Mammalian polychromatic erythrocytes; micronucleus test , CA: positive (Genetox)
Nonhuman cells and human lymphocytes, SCE, *in vitro*: positive (Genetox)
Nonhuman bone marrow, CA, *in vivo*: no conclusion (Genetox)
Human lymphocytes or leukocytes, CA, *in vitro*: positive (Genetox)
Mammalian germ cells (male), CA, *in vivo*: no conclusion (Genetox)
Human lymphocytes, SCE, *in vitro*,: no conclusion (Genetox)
Rodents, male, dominant lethal test: negative (Genetox)
Mouse, male, heritable translocation test for CA: no conclusion (Genetox)
Mammalian polychromatic erythrocytes, micronucleus test (CA): no conclusion (Genetox)
Mouse, female, spot test (gene mutation): no conclusion (Genetox)
Neurospora crassa, aneuploidy, CA: positive (Genetox)
Hordeum vulgare, Vicia faba, Allium cepa, CA: positive (Genetox)
Syrian hamster embryo (SHE) cells, cell transformation (clonal assay): negative (Genetox)
Saccharomyces cerevisiae, aneuploidy, CA: positive (Genetox)
Bacillus subtilis (H17 vs M45T), Rec-assay, spot test, DNA effects: no conclusion (Genetox)
Mouse, male, specific locus test, gene mutation: negative (Genetox)
Mouse, male, sperm morphology: negative (Genetox)
Drosophila melanogaster, SLRL; aneuploidy, sex chromosome gain & loss, CA: positive (Genetox)
Mouse, male, germ cells, UDS *in vivo*: negative (Genetox)
Chinese hamster lung (V79) cells, forward gene mutation at the HPRT or ouabain locus: negative (Genetox)

Source: The National Library of Medicine TOXNET databases, Chemical Carcinogenesis Research Information System (CCRIS) and Genetic Toxicology (Genetox), searched in October 2001.
CA = chromosome aberrations; SCE = sister chromatid exchange; SLRL = sex-linked recessive lethal; UDS = unscheduled DNA synthesis; w/wo = with and without

Diuretics/Cathartics.

Fumitory: Fumitory contains isoquinolines as the main ingredient. A light, antispasmodic effect on the upper digestive tract is sufficiently documented. Average daily dosage is 6 g of cut herb or equivalent. No side effects, contraindications, or interactions with other drugs are known (Blumenthal, 1998).

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

Cascara sagrada [8015-89-2]: Cascara sagrada consists of the dried bark of *Rhamnus purshiana* D.C. as well as its preparations. The bark contains anthranoids, mainly of the aloe-emodin type, in addition to those of the chrysophanol and physcion type. 1,8-Dihydroxyanthracene derivatives have a laxative effect, and cascara sagrada bark is used to treat constipation. German Commission E warns against long-time use/abuse, which may cause disturbances of electrolyte balance, especially potassium deficiency, which can lead to disorders of heart function and muscular weakness (Blumenthal, 1998).

Experiments pertaining to the genotoxicity of cascara sagrada and its preparations are not available. Data obtained for aloe-emodin (481-72-1) are outlined in Table 5 below.

Table 5. Results of Tests for Genotoxicity for Aloe-emodin.

S. typhimurium TA97A,TA1537, TA1538 w/wo S-9; TA 102 w S-9; TA1978 wo S-9: positive (CCRIS)
S. typhimurium TA100 wo S-9; TA102 wo S-9; TA1535 w/wo S-9; TA1978 w S-9: negative (CCRIS)
S. typhimurium TA98, TA100 w S-9: mixed results (CCRIS)
Chinese hamster V-79/8-azaguanine wo S-9: positive (CCRIS)
Chinese hamster V-79(HPRT)/6-thioguanine w/wo S-9: negative (CCRIS*)*
UDS Rodent hepatocytes, thymidine incorporation: mixed results (CCRIS)

Source: The National Library of Medicine TOXNET database, Chemical Carcinogenesis Research Information System)were searched in October 2001.
w/wo = with/without

Heart Tonics.

Hawthorn: Preparations of hawthorn berry have been applied to the treatment of coronary circulation, coronary complications, and weak heart. Preparations of hawthorn flowers have been applied to enhance the activity of myocardium, prevent stress-related heart disease, strengthen the heart and circulatory system, and for coronary insufficiency. Hawthorn leaf preparations have been applied prophylactically to improve the perfusion and nutrition of the myocardium, for simple circulatory disorders of the coronary artery, for diminished cardiac output due to hypertension and pulmonary disease, and for hypotension. According to German Commission E, the effectiveness of hawthorn for the claimed applications has not been documented and there are no scientific data on which

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

to base the pharmacology and toxicology of the herb (Blumenthal,1998; Springboard4health.com, 2001).

Willow: White willow bark is considered a natural aspirin. Its salicin derivatives are oxidized in the liver and blood to produce salicylic acid. Average daily dosage corresponds to 60-120 mg total salicin. It has pain-relieving effects like aspirin: typical dosages, up to six 400 mg capsules a day, may be too low to achieve the effect of aspirin (American Herbal Products Association, 2000; Blumenthal, 1998).

Both willow bark and aspirin are salicylates, a class of compounds that work by virtue of their salicylic acid content. All salicylates share substantially the same side effects. The major adverse effects include irritation of the gastric mucosa, adverse effects when used during pregnancy (stillbirth, bleeding, prolonged gestation and labor, low birth weigh infants), stroke, and adverse effects in children with fever and dehydration. Approximately 5% of the population is hypersensitive to salicylates (FDA, 1993).

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)

References

Alwan, S.M., Al-Hindawi, M.K., Abdul-Rahman, S.K. & Al-Sarraj, S. (1986) Production of nitrosamines from ephedrine, pseudoephedrine and extracts of *Ephedra foliata* under physiological conditions. *Cancer Lett*., **31**(2), 221-226

AMBI Inc. (2001) *Lite Bites*®. [http://www.ambiinc.com]. Searched November 8, 2001

American Herbal Products Association (1999) 1999 AHPA Ephedra International Symposium, Arlington, VA, December 9-10. Speaker presentations available from AHPA, Silver Spring, MD

American Herbal Products Association (2000) Willow salix spp. *Another Healthy Herb!* [http://www.ahpa.org/ahh/willow.htm]. Searched October 2, 2001

Anon. (1999) Herbal dietary supplements. Reprinted from *Health & Fitness Magazine* (October 1, 1999). *Metabolife - News and Media*. [http://www.metabolife.com/news/100199.htm]. Searched October 24, 2001

Astrup, A., Breum, L., Toubro, S., Hein, P. & Quaade, F. (1992) The effect and safety of an ephedrine/caffeine compound compared to ephedrine, caffeine and placebo in obese subjects on an energy restricted diet. A double blind trial. *Int. J. Obesity*, **16**(4), 269-277

Astrup, A. & Toubro, S. (1993) Thermogenic, metabolic, and cardiovascular responses to ephedrine and caffeine in man. *Int. J. Obesity*, **17**(Suppl. 1), S41-S43

Astrup, A., Toubro, S., Cannon, S., Hein, P. & Madsen, J. (1991) Thermogenic synergism between ephedrine and caffeine in healthy volunteers: A double-blind, placebo-controlled study. *Metab. Clin. Exper*., **40**(3), 323-329

Blau, J.J. (1998) Ephedrine nephrolithiasis associated with chronic ephedrine abuse. *J. Urol*., **160**(3, Pt.1), 825

Blumenthal, M. (1998) Cascara Sagrada bark, Fumitory, Hawthorn leaf with flower, Hay flower, White Willow bark. *The German Commission E Monographs.* Austin, TX, American Botanical Council, pp. 104-105, 133, 142-144, 230

Blumenthal, M. & King, P. (1996) The agony of the ecstasy: herbal high products get media attention. *Herbalgram*. [http://www.herbalgram.org.browse.php.130]. Searched October 24, 2001

Botanical Dermatology Database (2001) *Ephedraceae (Joint-Pine Family)*. [http://bodd.cf.ac.uk/BotDermFolder/BotDermE/EPHE.html]. Searched October 24, 2001

Boozer, C.N., Daly, P.A., Blanchard., D., Nasser, J.A., Solomon, J.L. & Homel, P. (2000) Herbal ephedra/caffeine for weight loss: A 6-month safety and efficacy trial. Abstract presented at NAASO - North American Association for the Study of Obesity Annual Meeting, October 29-November 2,

2000 [cited in CANTOX, 2000]

Boullata, J.I. & Nace, A.M. (2000) Safety issues with herbal medicine. *Pharmacotherapy*, **20**(3), 257-269

Breum, L., Pedersen, J.K., Ahlstrøm, F. & Frimodt-Møller, J. (1994) Comparison of an ephedrine/caffeine combination and dexfenfluramine in the treatment of obesity: A double-blind, multi-centre trial in general practice. *Int. J. Obesity*, **18**, 99-103

Bruno, A., Nolte, K.B. & Chapin, J. (1993) Stroke associated with ephedrine use. *Neurology*, **43**(7), 1313-1316

Bucci, L.R. (2000) Selected herbals and human exercise performance. *Am. J. Clin. Nutr.*, **72**(Suppl. 2), 624S-636S

CANTOX (2000) *Safety Assessment and Determination of a Tolerable Upper Limit for Ephedra*. Report prepared by CANTOX Health Sciences International, Ontario, for the Council for Responsible Nutrition, Washington, DC, 194 pp.

CCRIS (2001) Caffeine, aspirin, aloe-emodin, salicylic acid. *Chemical Carcinogenesis Research Information System*. National Library of Medicine Toxnet database. [Record Nos. 1314, 3243, 3526, 6714]. [http://toxnet.nlm.nih.gov]. Searched October & November, 2001

CDC (2001) *Obesity and Overweight - A Public Health Epidemic*. [http://www.cdc.gov/nccdphp/dnpa/obesity/epidemic.htm]. Searched October 26, 2001

Coates, P.M. (2001) Personal communication [fax] from Paul Coates, Ph.D., Director, Office of Dietary Supplements, National Institutes of Health, to Imogene Sevin, Ph.D., Technical Resources International., Inc., November 19, 2001

Consumers Union (2001) Sports-supplement dangers. Some products supposedly increase muscle or energy. But they could cause serious harm. *Consumer Reports Online*, June 2001. [http://www.consumerreports.org/]. Searched October 26, 2001

Council for Responsible Nutrition (2001) Blue Cross/Blue Shield estimates one million kids use sports-enhancing supplements, drugs. *Natural Products Industry Insider* [http://www.naturalproductsinsider.com/hotnews/]. Posted August 29, 2001, searched October 26, 2001

da Fonseca, C.A., Leal, J., Costa, S.S. & Leitão, A.C. (1994) Genotoxic and mutagenic effects of guarana (*Paullinia cupana*) in prokaryotic organisms. *Mutat. Res.*, **321**(3), 165-173

Daly, P.A., Krieger, D.R., Dulloo, A.G., Young, J.B. & Landsberg, L. (1993) Ephedrine, caffeine and aspirin: Safety and efficacy for the treatment of human obesity. *Int. J. Obesity*, **17**(Suppl. 1), S73-S78

Duke, J.A. & Beckstrom-Sternberg, S.M. (2001) Chemicals in: *Ephedra sinica* STAPF

(Ephedraceae) – Chinese ephedra, ma huang. *Dr. Duke's Phytochemical and Ethnobotanical Databases.* [http://www.ars-grin.gov/cgi-bin/duke]. Searched October 24, 2001

Ephedra Education Panel (2000) *Comments of the Expert Panel of the Ephedra Education Council on the Safety of Dietary Supplements Containing Ephedrine Alkaloids and on the Adverse Event Reports (AERs) and Health Assessments Released by the Food and Drug Administration.* September 29, Ephedra Education Council, Washington, DC

Espinola, E.B., Dias, R.F., Mattei, R., & Carlini, E.A. (1997) Pharmacological activity of Guarana (*Paullinia cupana* Mart.)in laboratory animals. *J. Ethnopharmacol*., **55**(3), 223-229

FDA (1993) *Illnesses and Injuries Associated with the Use of Selected Dietary Supplements*. [http://vm.cfsan/fda.gov/]. Searched October 1, 2001

FDA (1994) Adverse Events with Ephedra and other Botanical Dietary Supplements. *FDA Medical Bulletin*. [http://vm.cfsan.fda.gov/]. Searched October 1, 2001

FDA (1995) FDA warns consumers against Nature's Nutrition Formula One. *FDA Press Release, February 28, 1995*. [http://vm/cfsan.fda.gov/]. Searched October 1, 2001

FDA (1997) Dietary supplements containing ephedrine alkaloids; Proposed rule. *Federal Register*, June 4, 1997, pp. 30677-30724

FDA (2000) *Guidance for Industry. Street Drug Alternatives*. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Rockville, MD

GAO (1999) *Report to the Chairman and Ranking Minority Member, Committee on Science, House of Representatives - Dietary Supplements, Uncertainties in Analyses Underlying FDA's Proposed Rule on Ephedrine Alkaloids.* United States General Accounting Office, Washington, DC, GAO/HEHS/GGD-99-90, 79 pp.

Garriott, J.C., Simmons, L.M., Poklis, AA. & MacKell, M.S. (1985) Five cases of fatal overdose from caffeine-containing "look-alike" drugs. *J. Anal. Toxicol*., **9**(3), 141-143

Gene-Tox (2001) Aspirin, Caffeine, Aloe-emodin. *Genetic Toxicology (*Gene-Tox*)*. National Library of Medicine Toxnet database. Records 41, 175, 1491. [http://toxnet.nlm.nih.gov.cgi-bin/sis/search]. Searched October and November 2001

Greenway, F. (2001) The safety and efficacy of pharmaceutical and herbal caffeine and ephedrine use as a weight loss agent. *Obesity Rev.*, **2**, 199-211

Gualtieri, J. & Harris, C. (1996) Dilated cardiomyopathy in a heavy ephedrine abuser. *J. Toxicol (Clin. Toxicol.)*, **34**(5), 581-582

Guarana.com (2001a) Facts and fiction. *Guarana*. [http://www.guarana.com/facts.html]. Searched October 18, 2001

Guarana.com (2001b) Research. *Guarana*. [http://www.guarana.com/research.html]. Searched October 18, 2001

Guarana.com (2001c) Guarana and weight loss? Think twice. *Guarana*. [http://www.guarana.com/weightloss.html]. Searched October 18, 2001

Gurley, B.J., Gardner, S.F. & Hubbard, M.A. (2000) Content versus label claims in ephedra-containing dietary supplements. *Am. J. Health Syst. Pharm*., **57**(10), 963-969

Gurley, B.J., Gardner, S.F., White, L.M & Wang, P.L. (1998) Ephedrine pharmacokinetics after the ingestion of nutritional supplements containing *Ephedra sinica* (ma huang). *Therapeut. Drug Monit*., **20**(4), 439-445

Haller, C.A. & Benowitz, N.L. (2000) Adverse cardiovascular and central nervous system events associated with dietary supplements containing ephedra alkaloids. *N. Engl. J. Med*., **343**(25), 1833-1838

Horikawa, K., Mohri, T., Tanaka, Y. & Tokiwa, H. (1994) Moderate inhibition of mutagenicity and carcinogencity of benzo[a]pyrene, 1,6-dinitropyrene and 3,9-dinitrofluoranthene by Chinese medicinal herbs. *Mutagenesis* **9**(6), 523-526

Ingerslev, J., Svendsen, T.L. & Mørk, A. (1997) Is an ephedrine caffeine treatment contraindicated in hypertension? *Intl. J. Obesity*, **21**(8), 666-673

Kaats, G.R. & Adelman, J.A. (1994) Effects of a multiple herbal formulation on body composition, blood chemistry, vital signs, and self-reported energy levels and appetite control. *Intl. J. Obesity Related Metabol. Disord.*, **18**(Suppl. 2), 145 [Abstract]

Kalix, P.(1991) The pharmacology of psychoactive alkaloids from ephedra and catha. *J. Ethnopharmacol*., **32**(103), 201-208

Kanai, T., Nishikawa, T., Satoh, A. & Kajita, A. (1986a) Cardiovascular teratogenicity of ephedrine in rats. *Senten Ijo (Congen. Anom.)*, **26**(3), 246

Kanai, T., Nishikawa, T., Satoh, A., & Kajita, A. (1986b) Cardiovascular teratogenicity of ephedrine in rats. *Teratology*, **34**(3), 469 [Abstract]

Karch, S.B. (2000) Ma huang and the ephedra alkaloids. In: Cupp, M.J. (ed.) *Toxicology and Clinical Pharmacology of Herbal Products*. Humana Press, Inc., Totowa, NJ, pp. 11-30

Kernan, W.N., Viscoli, C.M., Brass, L.M., Broderick, J.P., Brott, T., Feldmann, E., Morgenstern, L.B., Wilterdink, J.L.; Horwitz, R.I. (2000) Phenylpropanolamine and the risk of hemorrhagic stroke. *N. Engl. J. Med*., **343,** 1826-1832

Kolecki, P. (2001) Toxicity, sympathomimetic. *Emedicine Journal*, **2**(6). [http://www.emedicine.

com/emerg.topic562.htm]. Searched June 11, 2001

Law, M.Y., Pedersen, G.H., Hennen, W.J., McCausland, C.W., & Sidwell, R.W. (1996) Sub-acute toxicity study of ma huang in mice. *Fundam. Appl. Toxicol*., **30**(1 Pt. 2), 111

Lee, M.K., Cheng, B.W.-H., Che, C.T. & Hsieh, D.P.H. (2000) Cytotoxicity assessment of ma huang (ephedra) under different conditions of preparation. *Toxicol. Sci*., **56**(2), 424-430

Leikin, J.B. & Klein, L. (2000) Ephedra causes myocarditis. *Clin. Toxicol*., **38**(3), 353-354

Lwanami, N., Ohtsnka, Y. & Kubo, H. (1985) Determination of ephedrine alkaloids in ephedra herba and oriental pharmaceutical preparations by HPLC. *Acta Pharm. Sinica (Yao Hsueh Hseuh Pao*), **20**(30), 149-153 [Chinese] [abstract] [cited in CANTOX, 2000]

Mack, R.B. (1997) "All but death, can be adjusted." Ma huang (ephedrine) adversities. *NC M. J.*, **58**(1), 68-70

Mattei, R., Dias, R.F., Espinola, E.B., Carlini, E.A. & Barros, S.B.M. (1998) Guarana (*Paullinia cupana*): toxic behavioral effects in laboratory animals and antioxidant activity *in vitro*. *J. Ethnopharmacol*., **60**(2), 111-116

Matthews, G., Smolinske, S. & White, S. (1997) Ephedrine-related stroke in a teenager. *J. Toxicol (Clin. Toxicol*.), **35**(5), 555

McEvoy, G.K., ed. (2000) *AHFS Drug Information*, Bethesda, MD, American Society of Hospital Pharmacists, Inc., pp. 1175-1185, 1209-1213, 2167-2170

Merck (2000) Ephedrine. *The Merck Index*, 12.3 ed. (CD-Rom). Boca Raton, FL, Chapman & Hall. [Monograph Number 3645]

Metabolife (2001) Metabolife 356®. [http://www.metbolife.com/shop/356.html]. Searched October 3, 2001

Miura, T., Tatara, M., Nakamura, K. & Suzuki, I. (1998) Effect of guarana on exercise in normal and epinephrine-induced glycogenolytic mice. *Biol. Pharm. Bull.*, **21**(6), 646-648

Morimoto, I., Watanabe, F., Osawa, T., Okitsu, T. & Kada, T. (1982) Mutagenicity screening of crude drugs with *Bacillus subtilis* rec-assay and *Salmonella/*microsome reversion assay. *Mutat. Res*. **97**, 81-102

Nadir, A., Agrawal, S., King, P.D. & Marshall, J.B. (1996) Acute hepatitis associated with the use of a Chinese herbal product, ma huang. *Am. J. Gastroenterol.*, **91**(7), 1436-1438

Nasser, J.A., Wang, V., Chen, G.C., Solomon, J.L., Heymsfield, S.B. & Boozer, C.N. (1999) Efficacy trial for weight loss of an herbal supplement of Ma huang and guarana. *FASEB J*., **13**(5

Pt. 2), A874

Nishikawa, T.H.J., Bruyere, H.J., (Jr.), Takagi, Y., Gilbert, E.F. & Uno, H. (1985) Cardiovascular teratogenicity of ephedrine in chick embryos. *Toxicol. Lett*., **29**(1), 59-63 [cited in CANTOX, 2000]

Nørregaard, J., Jørgensen, S., Mikkelsen, K.L., Tønnesen, P., Iversen, E., Sørensen, T., Søberg, B. & Jakobsen, H.B. (1996) The effect of ephedrine plus caffeine on smoking cessation and postcessation weight gain. *Clin. Pharmacol. Therapeut*., **60**(6), 679-686

NTP (1986) *Toxicology and Carcinogenesis Studies of Ephedrine Sulfate (CAS NO. 134-72-5) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies) (Technical Report Series No. 307;* NIH Publication No. 86-2563*).* Research Triangle Park, NC, National Toxicology Program, 186 pp

Nutritionfocus.com (1999) *Ephedra*. [http://www.nutritionfocus.com]. Searched October 24, 2001

Office of Women's Health (2000) *Transcript of Public Meeting on the Safety of Dietary Supplements Containing Ephedrine Alkaloids*, vol. II of II., August 9, 2000, Washington, DC, 541 pp

Ooms, T.G., Khan, S.A. & Means, C. (2001) Suspected caffeine and ephedrine toxicosis resulting from ingestion of an herbal supplement containing guarana and ma huang in dogs: 47 cases (1997-1999). *J. Am. Vet. Med. Assoc*., **218**(2), 225-229

Pape, S.M., Kracov, D.A. & Prochnow, J.R. (1999) [Patton Boggs LLP] Supplemental Comments of the Dietary Supplement Safety and Science Coalition, Submitted March 5, 1999, to FDA, Docket No. 98N-0148, 6 pp

Powell, T., Hsu, F.F., Turk, J. & Hruska, K. (1998) Ma huang strikes again: ephedrine nephrolithiasis. *Am. J. Kidney Dis*., **32**(1), 153-159

Public Citizen (2001) Public Citizen calls on FDA to ban dietary supplements containing ephedra. September 5, 2001. *Press Room.* [http://www.citizen.org/pressroom]. Searched November 28, 2001

Raintree.com (2001) Guarana. *Database for Guarana*. [http://www.rain-tree.com/guarana.htm]. Searched October 18, 2001

Santa Maria, A., Lopez, A., Diaz, M.M., Munoz-Mingarro, D. & Pozuelo, J.M. (1998) Evaluation of the toxicity of guarana with *in vitro* bioassays. *Ecotoxicol. Environ. Saf*., **39**(3), 164-167

Seifried, H. (2001) Personal communication [e-mail] from Harold Seifried, Ph.D., National Cancer Institute, Nutritional Sciences Research Group, to Ted Junghans, Technical Resources International, Inc., November 16, 2001

Springboard4health.com (2001) Hawthorne. *The Nutrition Notebook.* [http://www. springboard4 health.com/notebook/herbs_hawthorne.html]. Searched October 2, 2001

Theoharides, T.C. (1997) Sudden death of a healthy college student related to ephedrine toxicity from a ma huang-containing drink. *J. Clin. Psychopharmacol*., **17**(50), 437-439

Traub, S.J., Hoyek, W. & Hoffman, R.S. (2001) [Letter to the editor]. *N. Engl. J. Med.*, **34**(14), 1096

Upton, R.A. (1991) Pharmacokinetic interactions between theophylline and other medication (Part I). *Clin. Pharmacokinet*., **20**(1), 66-80

US Department of Health and Human Services (1996) FDA statement on street drugs containing botanical ephedrine. *HHS News*. April 10, 1996. [http//vm.cfsan.fda.gov/]

Vahedi, K., Domigo, V., Amarenco, P. & Bousser, M.G. (2000) Ischaemic stroke in a sportsman who consumed MaHuang extract and creatine monohydrate for body building. *J. Neurol. Neurosurg. Psychiatry*, **68**(1), 112-113

Van Mieghem, W., Stevens, E. & Cosemans, J. (1978) Ephedrine-induced cardiopathy. *Br. Med. J.*, **1**(6116), 816

Vardigan, B. (2001) *Special Report Ephedra: One Year Later*. [www.ahealthyme.com]. Searched November 7, 2001

Vitacost.com (2001) Guarana. *Encyclopedia of Health Concerns and Individual Nutrients.* [http://www.vitacost.com/science/nutrients/guarana.html]. Searched October 18, 2001

Waluga, M., Janusz, M., Karpel, E., Hartleb, M. & Nowak, A. (1998) Cardiovascular effects of ephedrine, caffeine and yohimbine measured by thoracic electrical bioimpedance in obese women. *Clin. Physiol*., **18**(1), 69-76

White, L.M., Gardner, S.F., Gurley, B.J., Marx, M.A., Wang, P.L. & Estes, M. (1997) Pharmacokinetics and cardiovascular effects of ma huang (*Ephedra sinica*) in normotensive adults. *J. Clin. Pharmacol*., **37,** 116-122

Wilner, B. (2001), *NFL Bans Drug Ephedra*. USAToday.com. [http://www.usatoday.com. nfl/stories/2001-09-08-ephedra.htm]. Searched October 26, 2001

Wilson, A.F., Novey, H.S., Cloninger, P., Davis, J. & White, D. (1976) Cardiopulmonary effects of long-term bronchodilator administration. *J. Allergy. Clin. Immunol*., **58**(1, Pt.2), 204-212

Xue-Jun, Y., De-xiang, L., Hechuan, W. & Yu, Z. (1991) A study on the mutagenicity of 102 raw pharmaceuticals used in Chinese traditional medicine. *Mutat. Res*., **260**(1), 73-82

Zaacks, S.M., Klein, L., Tan, C.D., Rodriguez, E.R. & Leikin, J.B. (1999) Hypersensitivity myocarditis associated with ephedra use. *J. Toxicol. Clin. Toxicol*., **37**(4), 485-489

Zahn, K.A., Ki, R.L. & Purssell, R.A.(1999) Cardiovascular toxicity after ingestion of “herbal ecstacy”. *J. Emerg. Med*., **17**(2), 289-291

Prepared for NCI by Technical Resources International, Inc. to support chemical nominations under contract no. N02-CB-07007 (10/01)