

Safety assessment of (–)-hydroxycitric acid and Super CitriMax[®], a novel calcium/potassium salt

M.G. Soni^{a,*}, G.A. Burdock^a, H.G. Preuss^b, S.J. Stohs^c, S.E. Ohia^d, D. Bagchi^c

^a Burdock Group, 780 US Highway 1, Suite 300, Vero Beach, FL 32962, USA

^b Department of Physiology and Biophysics, Georgetown University Medical Center, Washington, DC, USA

^c Department of Pharmacy Sciences, Creighton University Medical Center, Omaha, NE, USA

^d University of Houston College of Pharmacy, Houston, TX 77204, USA

Received 20 January 2004; accepted 30 April 2004

Abstract

(–)-Hydroxycitric acid (HCA) is a principle constituent (10–30%) of the dried fruit rind of *Garcinia cambogia*, a plant native to Southeastern Asia. The dried rind has been used for centuries throughout Southeast Asia as a food preservative, flavoring agent and carminative. Extensive experimental studies show that HCA inhibits fat synthesis and reduces food intake. The objective of this review is to systematically review the available safety/toxicity literature on HCA to determine its safety in-use. The primary mechanism of action of HCA appears to be related to its ability to act as a competitive inhibitor of the enzyme ATP-citrate lyase, which catalyzes the conversion of citrate and coenzyme A to oxaloacetate and acetyl coenzyme A (acetyl-CoA), primary building blocks of fatty acid and cholesterol synthesis. Super CitriMax[®], a novel calcium/potassium–HCA extract (HCA-SX), is considerably more soluble and bioavailable than calcium-based HCA ingredients. Acute oral toxicity studies in animals demonstrate that CitriMax (50% HCA as calcium salt) has a low acute oral toxicity. In a subchronic study in rats, the gavage administration of HCA-SX at doses up to 2500 mg/kg/day for a period of 90 days caused a significant decrease in body weight and reduction in feed consumption without any adverse effects. The structure, mechanism of action, long history of use of HCA and other toxicity studies indicate that HCA-SX is unlikely to cause reproductive or developmental effects. HCA-SX was not mutagenic in the presence or absence of metabolic activation in Ames genotoxicity assays in strains TA98 and TA102. HCA-SX-induced increases in number of revertants in other strains (TA100 and TA1535 in the absence of metabolic activation and in strain TA1537 in the presence of metabolic activation) but these were not considered as biologically indicative of a mutagenic effect. In several, placebo-controlled, double-blind trials employing up to 2800 mg/day HCA, no treatment-related adverse effects were reported. There is sufficient qualitative and quantitative scientific evidence, including animal and human data suggesting that intake of HCA at levels up to 2800 mg/day is safe for human consumption.

© 2004 Elsevier Ltd. All rights reserved.

Keywords: Hydroxycitric acid; Food ingredient; Super CitriMax; Toxicity; Safety

1. Introduction

Super CitriMax[®] (HCA-SX), a popular dietary supplement, is derived from the dried fruit rind of a small

pumpkin-shaped fruit (5-cm diameter) from the plant *Garcinia cambogia* (family Guttiferae), a tree that is native to Southeast Asia. HCA-SX consist of a calcium/potassium salt of (–)-hydroxycitric acid (HCA). HCA is also reportedly found in several other species of *Garcinia* genus. The dried fruit rind of *G. cambogia*, also known as *Malabar tamarind*, is commonly used in Southeast Asia (particularly southern India) as a food preservative, flavoring agent and carminative. Thus, HCA, the primary constituent of HCA-SX, has a long history of consumption through its use in foods. As stated in Boll et al. (1969), the natural occurrence of HCA was reported as early as 1883 by Lippman. Structurally, HCA is chemically nearly identical to citric acid, the

Abbreviations: HCA, (–)-Hydroxycitric acid; HCA-SX, Super CitriMax[®], a novel calcium/potassium–HCA extract; NOAEL, No observed adverse effect level; NOEL, No observed effect level; ADI, Acceptable daily intake; GC, Gas chromatography; DSHEA, Dietary Supplement Health and Education Act; ATP, Adenosine triphosphate; GCE, *Garcinia cambogia* extract

* Corresponding author. Tel.: +1-561-562-3900x104; fax: +1-561-562-3908.

E-mail address: msoni@burdockgroup.com (M.G. Soni).

agent that gives citrus fruits their characteristic tart flavor. Citric acid is present in practically all plants and in many animal tissues and fluids, and is a common food additive.

In recent years, HCA has received considerable attention because of its putative weight reduction effects in animal and human studies. Several independent animal and human studies on the safety and efficacy of HCA have appeared in the published literature. However, no systematic review on the safety of HCA based on the published reports has appeared. In the present review, the available information on the mechanism of action of HCA and a novel calcium/potassium–HCA complex (HCA-SX; Super CitriMax[®]) from pre-clinical and clinical studies, along with adverse events from the clinical trials, were critically evaluated to determine the safety of HCA-SX and HCA. No attempt was made to review or comment on findings related to the potential benefits of HCA, or any risk versus benefit considerations.

1.1. Historical perspective

1.1.1. Description, chemistry and isolation

Garcinia is a large genus of approximately 180 species of polygamous trees or shrubs, distributed in tropical Asia, Africa and Polynesia. Approximately 30 species of *Garcinia* are found in India. One of the species, *G. cambogia*, found commonly in the evergreen forests of southwest India, is a small or medium-sized tree with a rounded crown and horizontal or drooping branches. The leaves are dark green and shiny, elliptic obovate, 5–12 cm long and 2–7 cm broad. The tree flowers during the hot season, and fruits ripen during the rainy season. The fruit, which is the source of HCA, is ovoid, 5 cm in diameter, yellow, orange or red when ripe, with six to eight grooves. The fruit has six to eight seeds surrounded by a succulent aril. The *G. cambogia* fruit is included in the USDA's inventory of perennial edible fruits of the tropics (Martin et al., 1987).

The fruit contains approximately 10–30% acid calculated as citric acid on a dry weight basis (Lewis et al., 1964). In some early studies, the organic acids present in the fruits were mistakenly identified as tartaric and citric acids. In subsequent studies, the major acid in the fruit of *G. cambogia* was identified as HCA (Lewis and Nelakantan, 1965; Lewis, 1969). HCA-SX, derived from the dried fruit rind of *G. cambogia*, contains approximately 95% calcium/potassium salt of (–)-hydroxycitric acid. The calcium/potassium salt contains approximately 60% HCA, calculated as the acid. General descriptive parameters and properties of HCA-SX are summarized in Table 1.

1.1.2. Chemistry

HCA (1,2-dihydroxypropane-1,2,3-tricarboxylic acid) has two asymmetric centers; hence two pairs of distereoisomers or four different isomers are possible (Fig. 1). All four isomers, (–)-HCA, (+)-HCA, (–)-*allo*-HCA and (+)-*allo*-HCA, have been chemically synthesized starting from *trans*-aconitic acid (Martius and Maue, 1941). One of these isomers, the principal constituent of HCA-SX, occurs in *Garcinia* (Fig. 1A) and another in *Hibiscus* species (Fig. 1B) (Lewis, 1969). (–)-HCA is the principal acid in the highly acidic fruit of *G. cambogia*. The absolute configuration of (–)-HCA was determined from Hudson's lactone rule, optical rotatory dispersion curves, circular dichromism curves and calculation of partial molar rotations (Boll et al., 1969). By employing X-ray crystallography, Glusker et al. (1969, 1971) reported the structure and absolute configuration of the calcium hydroxycitrate and (–)-HCA lactone.

The acid is present at a level of 10–30% in the dried fruit rinds of *G. cambogia*. The acid can be isolated in the free form, as a mineral salt (i.e., calcium–HCA, potassium–HCA, calcium/potassium–HCA, etc. formed post extraction) or as the lactone by various methods. Lowenstein and Brunengraber (1981) have estimated the hydroxycitrate content of the fruit of *G. cambogia* by gas chromatography (GC). During concentration and

Table 1
General descriptive characteristics of HCA and Super CitriMax[®]

Botanical source	<i>Garcinia cambogia</i>
Botanical family	Guttiferae
Synonyms	(–)-Hydroxycitric acid; 1,2-dihydroxy-1,2,3-propanetricarboxylic acid; <i>Garcinia</i> acid; 1,2-dihydroxypropane-1,2,3-tricarboxylic acid
CAS No.	27750-10-3 [(–)-HCA]
Molecular formula	C ₆ H ₈ O ₈ [(–)-HCA]
Molecular weight	208 [(–)-HCA]
Physical state	Powder, non-fibrous
Color	Cream, white
Odor	Odorless
Taste	Tasteless ^a
Storage	Moisture, air and light resistant container

^a HCA in its free form has a distinctive acidic taste, but as a calcium/potassium salt it is tasteless.

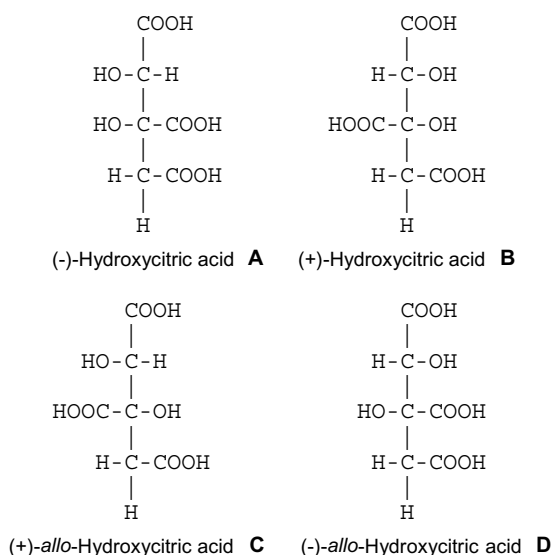


Fig. 1. Chemical structures of stereoisomers of hydroxycitric acid.

evaporation, free HCA leads to the formation of HCA lactone. Based on the information submitted to the US Patent office, several investigators have reported the preparation of HCA concentrate from *Garcinia* rinds with 23–54% HCA and 6–20% lactone (Guthrie and Kierstead, 1977; Moffett et al., 1977). Recently, Jayaprakasha and Sakariah (1998, 2000) developed high-performance liquid chromatography (HPLC) methods for the estimation of organic acids in the fruits of *G. cambogia* and commercial samples of *G. cambogia* extracts. Using these methods, dilute extracts can be quantified without concentration, drying or derivatization. An additional advantage of these methods is that the HCA and its lactone can be quantified separately. Loe et al. (2001) reported a gas-chromatography/mass spectrophotometry method for quantitative determination of blood hydroxycitrate levels.

1.1.3. Isolation

Lewis and Neelakantan (1965) reported a method on isolation of large scale HCA from the dried rinds of *G. cambogia*. In this method, the acid is extracted by heating the raw material with water under pressure. Subsequently, the extract was concentrated and pectin was removed by precipitation with alcohol. The clear filtrate was neutralized and the acid was recovered after passing through cation exchange resin. The recovered acid was concentrated, dried and recrystallized to small needle shaped crystals of lactone. In another method, Lewis (1969) reported isolation of HCA from dried rinds using acetone. The acetone extract was concentrated and the acid was extracted in water. The water extract was evaporated to yield lactone. In yet another process, aqueous extract of HCA was passed through anion exchange column for adsorption of HCA. The

adsorbed HCA was eluted with sodium/potassium hydroxide. The free acid was prepared by passing through a cation exchange column. In recent years, several manufacturers have employed different procedures (patented) to prepare salts of HCA with improved bioavailability.

1.1.4. Specifications

Physical characteristics and specifications of HCA-SX are presented in Table 2. Analysis reveals a highly water-soluble, calcium/potassium salt of HCA extracted from *G. cambogia*, commercially known as Super CitriMax[®] (HCA-SX). Quantitative estimations show that HCA-SX consists of 95% calcium/potassium-HCA providing 60% hydroxycitric acid. HCA-SX was characterized by HPLC, UV-VIS and IR, ¹H-NMR, ¹³C-NMR spectral data and mass spectra. The stability data suggest that HCA-SX is stable over three years at temperature of 30° C and relative humidity of 65%. HCA-SX is reported to provide approximately 150 calories *per* 100 g.

1.2. Approved and historical uses

The fruits of *G. cambogia* are valued for their dried rinds, which are used extensively in Southern India for culinary purposes and particularly as a condiment, in place of tamarind or lemon, for flavoring curries, meat and seafood dishes. The fruit extract also serves as a unique flavor enhancer for beverages, gourmet spice and

Table 2
Specifications of HCA-SX (Super CitriMax[®])

Characteristic	
Loss on drying	8%
Solubility in water	>99%
PH (1 g/100 ml water)	6.0–9.0
(-)-Hydroxycitric acid	600 ± 50 mg/g
Calcium	110 ± 30 mg/g
Potassium	160 ± 40 mg/g
Sodium	<10 mg/g
Phytosterols	0.5 mg/g
Total protein	3 mg/g
Soluble dietary fiber (by difference)	8.5%
Moisture	4.5%
Microbial standards	
Total plate count	<3000 CFU/g
Yeast and mold	<10 CFU/g
<i>Escherichia coli</i>	Negative
<i>Salmonella</i>	Negative
<i>Staphylococcus aureus</i>	Negative
Heavy metals	
Lead	<10 ppm
Cadmium	<0.25 ppm
Arsenic	<10 ppm
Mercury	<0.25 ppm

as a post-prandial carminative. The fruit has also been used for centuries to make meals more ‘filling’ (Sergio, 1988; Mattes and Bormann, 2000). Dried rind of *G. cambogia*, which contains HCA, is also used in pickling fish (Sreenivasan and Venkataraman, 1959; Clouatre and Rosenbaum, 1994); the commercial pickling of fish is called “Colombo curing” (Sreenivasan and Venkataraman, 1959; Lewis et al., 1964). The organic acids present in the fruit are responsible for the bacteriostatic effect of the pickling medium by a simple lowering of the pH. In addition to flavoring and preservative effects of the fruit extract, in the traditional system of herbal medicine in India (Ayurveda), *Garcinia* is considered to be one of the prime herbs that are beneficial for health. A decoction of the fruit rind is given for rheumatism and bowel complaints. In veterinary medicine, the extract is employed as a rinse for some diseases of the mouth in cattle (Jena et al., 2002). HCA, the organoleptically characterizing ingredient of *G. cambogia*, is a popular component of several dietary supplements marketed under various trade names. It has been reported that calcium salts of HCA, which are typically less than 50% soluble, are less bioavailable compared to readily soluble calcium/potassium salts of HCA. As a dietary supplement in the US, HCA is regulated under the Dietary Supplement Health and Education Act (DSHEA) of 1994 (DSHEA, 1994).

2. Biological data

In the published literature, several studies have appeared on the biological and toxicological effects of HCA. In a number of these studies, the investigators have not identified the specific stereoisomer of hydroxycitric acid employed. Although not identified as such, in a majority of these studies, the stereoisomer used was most likely HCA for the following reasons: first, the HCA or extract used in majority of the studies was isolated from *G. cambogia*; secondly, in several mechanistic studies of biochemical reactions, the (–)-isomer is the most active in inhibiting the enzyme ATP-citrate lyase; thirdly, based on its mechanism of action in weight reduction studies, use of this isomer is expected. Whenever the investigators have specifically mentioned the isomer, it is identified in the descriptions below.

2.1. Absorption, metabolism and excretion

Loe et al. (2001) investigated the bioavailability of HCA-SX in four adult healthy human volunteers (three males, one female). Fasting subjects were given 2 g HCA-SX. Blood samples were collected every 30 min after the ingestion of HCA-SX for a period of 3.5–4 h. The HCA-SX concentration was found to range from

0.8 µg/ml 30 min after HCA ingestion to 8.4 µg/ml after 2 h. Peak plasma HCA-SX concentration ranged from 4.7 to 8.4 µg/ml. Although plasma HCA-SX levels began to fall after 2.5 h, they remained considerably higher than baseline values even after 4 h of ingestion. The rates of absorption and clearance of HCA-SX appeared to be variable, with one subject having peak concentrations at 3 h and a slower return to baseline values. These studies demonstrate that, when consumed acutely, HCA-SX is readily absorbed in humans.

2.2. Biochemical/pharmacological effects

The reported weight reduction effects of HCA are based on its action as a potent and specific inhibitor of the enzyme ATP-citrate lyase (also known as citrate cleavage enzyme), which is required for the synthesis of fatty acids. The overall biochemical changes related to the action of HCA are summarized in Fig. 2. Inhibition of ATP-citrate lyase by HCA decreases fatty acid synthesis dramatically in a variety of tissues. By inhibiting ATP-citrate lyase at the regulatory juncture of fatty acid metabolism, HCA mimics some of the regulatory activities of citrate. Thus, HCA may decrease the production of fatty acids and cholesterol; slow the glycolytic pathways and consequently increase glycogen production. The decrease in fatty acid synthesis and increase in production of glycogen has a number of biochemical effects.

Compared to citrate, HCA has a much greater affinity for the enzyme citrate lyase (Watson et al., 1969; Lowenstein, 1970; Cheema-Dhadli et al., 1973; Szutowicz et al., 1976; Hoffman et al., 1980; Jena et al., 2002). Of the four isomers of hydroxycitric acid (Fig. 1), HCA was the only potent inhibitor of ATP-citrate lyase (Sullivan et al., 1977; Stallings et al., 1979). By inhibiting ATP-citrate lyase, HCA increases the extra-mitochondrial pool of citrate (Fig. 2A and C). As a result of this action, glycolysis is inhibited and the carbon units are redirected toward glycogen production. HCA thus initiates a form of nutrient partitioning as calories are redirected away from fat production and toward glycogen production and storage. This production and storage of glycogen, in turn, influences glucoreceptors located in the liver, which may induce satiation *via* the vagus nerve (Sullivan et al., 1974a, 1984; Sullivan and Triscari, 1976). In addition to its inhibitory action on ATP-citrate lyase, HCA also affects other biochemical processes directly or indirectly. HCA may increase energy expenditure in part by increasing glycogen deposition through the indirect pathway, i.e., through extrahepatic glycolysis followed by hepatic gluconeogenesis.

The appetite suppression in HCA-fed rats appears to be a specific effect of HCA ingestion and not due to alterations of taste. This has been supported by experi-

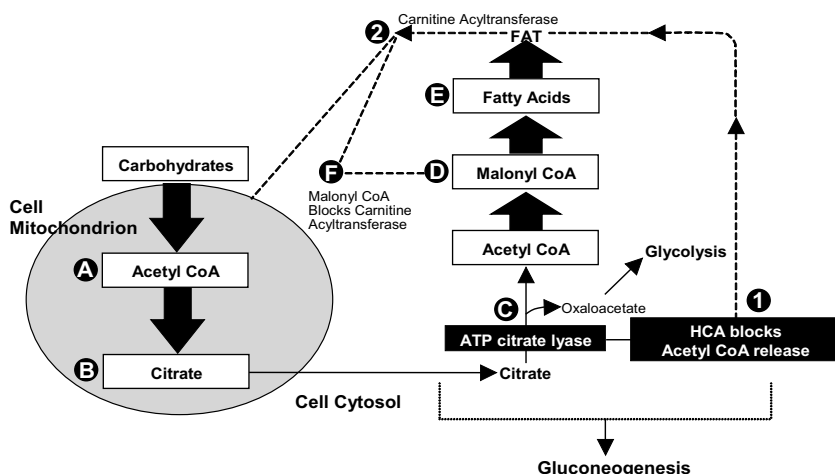


Fig. 2. Proposed biochemical action of HCA. (A) Acetyl-CoA the product of carbohydrate metabolism; (B) As acetyl-CoA cannot cross mitochondrial membrane it is converted to citrate (oxaloacetate reacts with CoA to form citrate); (C) Citrate can cross the mitochondrial membrane and can be cleaved by the enzyme ATP-citrate lyase; (D) Acetyl-CoA is converted to malonyl-CoA; (E) Malonyl-CoA forms the fatty acids; (F) Malonyl-CoA blocks the activity of carnitine acyltransferase. (1) HCA may block ATP-citrate lyase; (2) Reduction in malonyl-CoA means that carnitine acyltransferase can transport existing fat into the mitochondrion where fats can be more easily burned. See the text for additional details. Adapted from the article "Fat Burning Capabilities", AIM International, 1999.

ments in which control group of rat was fed citrate in the diet at a level equivalent to that of HCA in the experimental diet (Rao and Sakariah, 1988). As HCA and citrate are nearly identical in structure and possess a similar "sour" taste, it is reasonable to believe that HCA-mediated reduced feed intake is not related to the taste of the feed. This hypothesis is also supported by the fact that HCA-reduced feed intake occurs only during the first hour following administration, indicating that the reduced feed intake was not merely a consequence of conditioned aversive effects of HCA (Panksepp et al., 1977).

It has been suggested that because HCA inhibits the formation of acetyl-CoA in the cytoplasm, it may also inhibit the formation of the next compound (malonyl-CoA) in the pathway of fatty acid synthesis (Fig. 2). Malonyl-CoA is the "base" from which fatty acids (and thus fat) and cholesterol are formed from carbohydrates. Malonyl-CoA, in turn, inhibits the enzyme, carnitine acyltransferase, which is required for the oxidation of fat. Hence, reduction in the formation of malonyl-CoA with HCA might stimulate fat metabolism leading to a decrease in body weight. Decreased levels of malonyl-CoA suggest that carnitine acyltransferase can transport existing fat into the mitochondria where fats can be more easily burned (Fig. 2).

It is also suggested that other metabolic effects of HCA could contribute to the wasting of ATP and therefore promote the increased consumption of calories in the presence of hydroxycitrate. Gluconeogenesis consumes ATP; if molecules are converted to glucose and glycogen, instead of fat, this may consume ATP. Cloutre and Rosenbaum (1994) also suggested that

inhibition of ATP-citrate lyase by HCA consumes a significant amount of energy. In a related hypothesis, it has been suggested that hydroxycitrate increases thermogenesis (metabolism of fat or other compounds to generate heat rather than metabolic energy in the form of ATP). It is known that in brown adipose tissue (brown fat), there is a type of fat consumption that converts calories to heat without producing ATP. The support for this hypothesis is based on experimental animal models comparing reduced weight gain to reduced feed intake, and disposal of extra calories (McCarty, 1994). In a recent study, Roy et al. (in press) reported that HCA-SX supplementation selectively influenced ~1% of 9960 genes screened in the adipose tissue without affecting the vital genes transcribing for mitochondrial/nuclear proteins. Functional characterization of HCA-SX sensitive genes revealed up-regulation of genes encoding serotonin receptors and thus influencing the appetite.

In summary, several mechanisms of action of HCA are proposed in the published literature. The primary mechanism appears to be related to the action of HCA as a competitive inhibitor of the enzyme ATP-citrate lyase. In the cytosol, ATP-citrate lyase catalyzes the conversion of citrate and coenzyme A to oxaloacetate and acetyl coenzyme A (acetyl-CoA). Acetyl-CoA is used in the synthesis of fatty acids, cholesterol and triglycerides and also in the synthesis of acetylcholine in the central nervous system. Oxaloacetate may enter the gluconeogenic pathway, which can lead to the production of glucose and glycogen. The putative weight reduction effect of HCA is due to suppression of fatty acid and fat synthesis. In addition, HCA is thought to

suppress feed intake *via* loss of appetite by stimulation of liver gluconeogenesis.

2.3. Toxicological studies

2.3.1. Acute studies

Acute toxicity studies of CitriMax[®] [50% HCA as calcium salt] demonstrate that it is practically non-toxic, as the oral LD₅₀ was reported to be greater than 5000 mg/kg. In recent studies, acute oral and dermal toxic effects of CitriMax[®] were investigated in laboratory animals, using standard protocols and Good Laboratory Practices. In the acute oral toxicity study, the effects of CitriMax[®] were evaluated in CrI:CD[®] albino male and female rats (Kiplinger, 1994b; Ohia et al., 2002). The test material was administered once orally *via* gastric intubation to a single group of five male and five female rats at a dose level of 5000 mg/kg. Mortality, clinical observations, body weights and gross necropsy findings were evaluated. There were no deaths, remarkable body weight changes or gross necropsy findings. Clinical findings were limited to soft stool and rales for one male and two female rats, respectively. Thus, the oral LD₅₀ of CitriMax[®] in rats was found to be greater than 5000 mg/kg.

In another study, the acute dermal toxicity of CitriMax[®] was evaluated in rabbits (Kiplinger, 1994a; Ohia et al., 2002). The test material was administered once dermally at a dose of 2000 mg/kg to the shaved intact skin of five male and five female New Zealand albino rabbits for a period of 24-h, under semi-occlusive dressing. The same parameters as those mentioned above were evaluated in the acute oral toxicity study. There were no deaths, test material-related clinical findings, other than local reaction to treatment, or remarkable body weight changes. The local reaction to treatment included yellow staining and slight erythema at the application site. Desquamation was reported on eight sites. No edema or other dermal findings were noted. All dermal irritation completely subsided by day 12 or earlier. The dermal LD₅₀ of CitriMax[®] was found to be greater than 2000 mg/kg in rabbits.

Oral administration of a single dose of sodium salt of hydroxycitric acid (trisodium hydroxycitrate) at a level of 0.17 mmol/kg (~3 mg/kg) and above to Charles River CD strain female rats showed a decrease (generally dose-related) in the rate of fatty acid cholesterol synthesis in the liver, adipose tissue and small intestine (Sullivan et al., 1974c). The report did not provide a detailed examination of the animals nor mention any overt signs of toxicity.

2.3.2. Short-term studies

Sullivan et al. (1974b) also investigated the effects of daily exposure of hydroxycitrate (sodium salt) for 30 days on lipid metabolism in Charles River CD strain

female rats. At the lowest dose administered (~3 mg/kg/day) for 30 days, decreases in the synthesis of cholesterol in the liver, adipose tissue and small intestine were observed. At about 11 mg/kg/day for 30 days, body weight gain was reduced, although liver size and liver lipid content were unaffected. Higher doses, up to 1350 mg/kg/day for 15–30 days, also reduced body weight gain and feed efficiency ratio (Sullivan et al., 1974b; Chee et al., 1977; Rao and Sakariah, 1988). Reductions were also noted in serum triglyceride levels and fat levels in the epididymis at doses of approximately 1000 mg/kg/day and higher. In a brief abstract, Vasselli et al. (1998) reported that male rats given 1000 mg/kg/day hydroxycitric acid for 28 days showed a reduction in 24-h energy expenditure during the first five days of the treatment.

Chee et al. (1977) investigated the influence of HCA on *in vivo* and *in vitro* rates of fatty acid synthesis in chicken and rat liver and in rat adipose tissue. Addition of 1 mM HCA to liver slices from chickens or rats in buffer containing 10 mM glucose resulted in a depressed rate of ³H₂O incorporation into fatty acids. *In vivo* rates of fatty acid synthesis in rat adipose tissue were not influenced by consumption of a diet containing 52.6 nmol HCA/kg diet. HCA feeding for 2–3 weeks resulted in a 2-fold increase in plasma triglyceride levels of chickens, although the levels remained unchanged in rats.

Leonhardt and Langhans (2002) examined the effect of HCA on feed intake, meal patterns, body weight regain and energy conversion ratio, as well as on different blood and liver variables in Sprague–Dawley male rats after substantial body weight loss. In two separate experiments, rats (6/group) were fed a diet containing 1% or 12% fat. Supplementation of both diets with 3% HCA (~1500 mg/kg/day) after 10 days of restrictive feeding (10 g powdered standard rodent diet/day), reduced body weight regain over the whole subsequent period of *ad libitum* consumption (22 days) and decreased the energy conversion ratio at the end of the experiment. In rats fed the 12% fat diet, HCA had a long-term suppression of feed intake. HCA did not affect any metabolic variable examined in rats given 1% fat diet, while in rats fed the 12% fat diet, HCA reduced plasma triacylglycerol and increased liver fat concentration without affecting plasma *beta*-hydroxybutyrate levels. The investigators concluded that because HCA did not affect plasma *beta*-hydroxybutyrate concentration, it did not support a role for increased hepatic fatty acid oxidation in the anorectic effect of HCA.

2.3.3. Subchronic studies

The results of a 90-day oral toxicity study in rats demonstrate that HCA-SX [60% HCA as calcium/potassium salt] at levels up to 5% of the feed intake did not induce any toxicologically significant effects. Shara

et al. (2003a,b, 2004) investigated the safety of HCA-SX following administration to Sprague–Dawley male and female rats. The study meets the FDA core standards described in “Red Book” (FDA, 1982) and is adequate for evaluation of toxicity. In this study, rats (17/group/sex) weighing 294 ± 20 g (male) and 210 ± 26 g (female) were administered (*via* gavage) a daily dose of HCA-SX (dissolved in water) at an amount equivalent to what would be ingested *via* feed at a dietary level of 0% (vehicle, water), 0.2%, 2% and 5% in the diet for 90 days. The gavage dose volume was 5 ml/kg body weight. The feed consumption was measured twice weekly and the gavage dose was adjusted accordingly. The selection of gavage route rather than through feed was based on the fact that (1) gavage administration most simulates the method of intake in humans, consumed over a relatively short period of time; and (2) high doses of HCA-SX are known to suppress appetite. The approximate daily dose of HCA-SX was 0, 100, 1000 and 2500 mg/kg/day. During the course of the study, the rats were examined daily for mortality/morbidity and clinical signs. Body weights and feed consumption were monitored twice weekly. Blood for clinical pathology evaluation was collected on days 30, 60 and 90 of treatment. Hematology, serum chemistry and serum iron analysis was performed on all animals. Urine samples (24 h) from five rats *per sex per group* were collected during days 2–4 and one 24-h period during days 83–90. At necropsy all major tissue were retained. Organ weights were taken for adrenal glands (pair), brain, heart, kidneys (pair), liver, prostate and seminal vesicles, spleen, testes (pair) and thymus. Organs (brain, pituitary gland, adrenal

gland, eyes, trachea, esophagus, thyroid, heart, lung, salivary gland, liver, kidneys, spleen, pancreas, stomach, intestine mesenteric lymph nodes, ovaries, mammary gland, uterus, urinary bladder, testis and skin) were fixed in formalin for histopathological examination.

Morbidity and mortality observations did not reveal any unusual findings. Compared to the respective control group, HCA-SX caused a significant reduction in feed consumption in both male and female rats at the end of 90 days, but not at the end of 30 or 60 days (Table 3). At the end of 90 days, the feed intake in the 100, 1000, 2500 mg/kg/day groups was decreased by 13%, 27% and 25% in male rats and by 16%, 20% and 23% in female rats, respectively. In both male and female rats, HCA-SX treatment resulted in a significant reduction in body weight at the end of 60 and 90 days (Table 4). The feed intake to body weight ratio was not significantly altered during the study. Male animals consumed more feed than the females throughout the study. Ophthalmologic observations did not reveal any treatment-related lesions. Compared to controls, no statistically significant or biologically relevant differences in organ weights in any of the HCA-SX treated groups were noted. Hematological analysis of multiple indices, including WBC, RBC, hemoglobin, hematocrit and platelet count, and total serum protein and albumin did not reveal any significant differences between the groups at the end of 30, 60 or 90 days. Similarly, clinical chemistry parameters (alkaline phosphatase, blood urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase, cholesterol, total bilirubin, glucose, calcium, chloride, phosphorus, sodium, potassium, iron,

Table 3
Effect of HCA-SX (Super CitriMax®) on feed intake in rats

Feed intake days	Male rats HCA-SX (mg/kg/day)/feed intake (g)				Female rats HCA-SX (mg/kg/day)/feed intake(g)			
	0	100	1000	2500	0	100	1000	2500
1–4	33 ± 6	41 ± 9	33 ± 7	39 ± 14	30 ± 9	38 ± 16	38 ± 12	28 ± 8
29–32	98 ± 12	72 ± 7	75 ± 15	76 ± 20	75 ± 13	78 ± 24	84 ± 18	73 ± 7
57–60	102 ± 5	102 ± 13	84 ± 17	77 ± 4	95 ± 11	85 ± 18	86 ± 9	81 ± 7
88–90	112 ± 8	97 ± 14*	82 ± 7*	84 ± 6*	92 ± 15	77 ± 7*	74 ± 7*	71 ± 8*

Feed intake was determined twice or three times *per week*. Mean feed intake during days 1–4, 29–32, 57–60 and 88–90 are presented in the table. Values are means ± SD.

*Significantly different from the control group at $p < 0.05$.

Table 4
Effect of HCA-SX (Super CitriMax®) on body weight in rats at the end of 30, 60 and 90 day

Day of feeding	Male rats HCA-SX (mg/kg/day)/body weight (g)				Female rats HCA-SX (mg/kg/day)/body weight (g)			
	0	100	1000	2500	0	100	1000	2500
1	300 ± 21	291 ± 18	294 ± 23	291 ± 16	209 ± 29	217 ± 24	206 ± 24	209 ± 27
30	368 ± 24	367 ± 30	363 ± 21	359 ± 36	264 ± 30	253 ± 21	234 ± 21	247 ± 29
60	436 ± 30	407 ± 18*	394 ± 31*	388 ± 38*	291 ± 29	267 ± 17*	247 ± 17*	272 ± 29*
90	484 ± 25	430 ± 22*	424 ± 34*	408 ± 38*	311 ± 23	274 ± 18*	254 ± 16*	270 ± 29*

Body weights were recorded weekly, but values at the end of 30, 60 and 90 days are presented in the table. Values are means ± SD.

*Significantly different from the control group at $p < 0.05$.

total iron binding capacity and iron/total iron bonding capacity) did not show any significant differences among the groups at the end of 30, 60 or 90 days of treatment.

The histological investigations of brain, pituitary, trachea, thyroid, heart, lung, liver, pancreas, stomach, ovaries, uterus, testis and skin at the end of 30, 60 and 90 days of treatment revealed lesions in a number of organs, the majority of which were incidental findings and were not related to treatment. The histological findings in the liver revealed hepatocyte vacuolation, which was either minimal or mild. In the 30 day group, this change was only noted in the high dose animals. In the 60 day group, the change was noted in animals in all three groups administered the test material; however, it was seen in three of the four animals in the high dose group, but in only one of the four animals from the other two groups. The 90 day group, however, did not fit the pattern; there was minimal vacuolation in one negative control animal and minimal vacuolation in one animal in each of the low and medium dose groups, while none of the high dose group had these lesions. It appears that the lesion regressed with time. Alternatively, the vacuolation may be the result of the time duration between the last feeding and euthanasia (according to study pathologist). Morphologically, the change was quite minimal. Another histological finding noted was in the glandular stomach of a number of animals. Scattered minimal or mild foci of gastric gland dilation with accumulation of what appeared to be proteinaceous material in the lumen was noted in all groups. The study pathologist indicated that the change may be associated with the gavage procedure. The change was minimal and may represent an exaggeration of a normal variation in gland morphology. No lesions were noted in any of the other organs examined including the testes and ovaries.

The 90-day study reveals that the administration of HCA-SX at dose levels of 0, 100, 1000 and 2500 mg/kg/day to rats did not induce any toxicologically significant changes. The minimal histological changes noted at 30 and 60 days were not found in the animals treated for 90 days. Based on the data from this study, a no observed adverse effect level (NOAEL) of HCA-SX for male and female rats was observed at 2500 mg/kg/day.

2.3.4. Reproduction studies

Based on pragmatic maternal observations, reduced weight gain is one end point of maternal toxicity. Because HCA is known to reduce weight in animals, the possibility of weight reduction-associated effects on reproductive and developmental endpoints, including the fetal size and ossification, cannot be ignored. However, as described in the following subsections, based on (1) mechanism of action of HCA and (2) available empirical observation, this compound would not be expected to cause reproductive or developmental toxicity.

2.3.4.1. Mechanism of action studies. During fetal development, rapid growth necessitates the production of large quantities of lipid. Lipid is supplied both from the mother by transfer across the placenta and through rapid rates of synthesis by the fetal tissues. The proposed mechanism of action of HCA suggests changes in fat synthesis and storage (utilization and deposition), which may be critical to late gestational development. Jones and Ashton (1976) reported that despite changes in fat synthesis and storage, lipid synthesis in slices of fetal liver from guinea pigs was not inhibited by HCA. Additional evidence also supports the notion that fatty acid synthesis pathways in fetal rat liver differ from those in adult rat liver (Watson et al., 1969; Watson and Lowenstein, 1970; Jones and Ashton, 1976). Another study in explants of 18-day fetal rat lung tissue cultured for 48 h has shown that HCA did not affect fatty acid synthesis stimulated by the hormone dexamethasone (known to stimulate the synthesis of fatty acids) (Xu et al., 1990). Together, these studies suggest that HCA does not affect fatty acid synthesis in the fetus.

Feeding of Zucker obese (*fa/fa*) female rats with HCA has been shown to decrease feed intake and body weight (Greenwood et al., 1981). Interestingly, the percent of body fat in these rats remained unchanged and they maintained the fat cell size equivalent to that of controls. The results of this study suggest that despite a substantial reduction in body weight of the obese rats by HCA, these animals still maintained fat cell size. As the body composition and fat cell size is maintained during HCA feeding, this study supports the above-described premise that HCA will not affect fatty acid synthesis in either the maternal animals or their offspring.

2.3.4.2. Empirical observations. The fact that *Garcinia cambogia* extract has been in use for perhaps several thousand years and has not been associated with a birth defect or reproductive problem, suggests that HCA is unlikely to cause reproductive or developmental toxicity. Also, during the last 10 years, approximately 5 billion doses of HCA-SX (including CitriMax[®]) have been sold as a dietary supplement, yet there have been no reports of significant adverse, reproductive or teratologic effects. Mild gastric upset is the most common, yet infrequent, complaint.

Dietary restriction resulting in a decrease in body weight of about 20% has been shown to adversely affect fertility and reproduction in rats and mice (Henry, 1991). However, decrease in body weight in the range of 10–15% did not affect fertility or reproduction. In the 90-day study, HCA-SX at the dose of 5% in rats decreased body weight by 12–15%. Human observations also suggest a ~10% weight loss upon consumption of HCA for a period of 4–12 weeks. Hence, it is expected

that HCA will not affect fertility and reproduction on the basis of weight loss.

Several studies in rabbits indicate that reduced feed consumption and associated weight loss are associated with resorptions, abortions and umbilical hernia. However, *per* OECD (IPCS/OECD, 2003) guidelines, body weight changes in rabbits may not be a useful indicator of maternal toxicity because normal fluctuations in body weight occur during pregnancy.

In summary, the above-described mechanistic and empirical observations, support the likelihood that HCA-SX under normal conditions of use would not cause reproductive or developmental toxicity.

2.3.5. Genotoxicity

In an *in vitro* bacterial reverse mutation test (plate incorporation and preincubation methods), mutagenic potentials of HCA-SX were investigated (Aujoulat, 2003). Five strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA1535 and TA1537) were used to investigate the effects of HCA-SX, both in the presence and absence of metabolic activation (\pm S9). In the plate incorporation method, the dose levels used were 52, 164, 512, 1600 and 5000 μ g/plate. No statistically or biologically significant increases in the number of revertants were noted in any strain, either with or without metabolic activation. In the preincubation method, HCA-SX was used at dose levels of 492, 878, 1568, 2800 and 5000 μ g/plate. No statistically or biologically significant increases in the number of revertants were noted in the strains TA98 and TA102, either with or without metabolic activation. Statistically significant increases were observed in the strain TA100 and TA1535 without metabolic activation and in the strain TA1537 with metabolic activation. The tests with these strains were repeated several times, and it was concluded that the increases observed in the TA100 and TA1535 strains without metabolic activation and in the TA1537 strain with metabolic activation were not biologically indicative of a mutagenic effect. The statistical significance was considered to be questionable, because it was obtained at the lowest or the alternate dose levels with no dose relationship. Secondly, in strain TA100 without metabolic activation and in the strain TA102 with metabolic activation, precipitation was noted. Under the experimental conditions and according to the criteria of the test study plan, it was concluded by the investigator (study director) that HCA-SX did not induce mutagenic effects in the bacterial reverse mutation test, either with or without metabolic activation.

Similar to HCA-SX studies, *in vitro* bacterial genotoxicity studies were negative for citric acid and its sodium and tripotassium salts. An *in vivo* cytogenetics study with citric acid also indicated no genetic toxicity (EPA, 2001).

2.3.6. Skin irritation studies

In a primary dermal irritation study, original CitriMax[®] was classified as non-irritating. It should be noted that in some of the toxicity studies, CitriMax[®] (50% HCA as calcium salt) was used instead of Super CitriMax[®] (60% HCA as calcium/potassium salt). Irritation potentials of CitriMax[®] were investigated using standard protocols and Good Laboratory Practices in rabbits (Kiplinger, 1994c; Ohia et al., 2002). Single 500 mg doses of the test material were applied for a four-hour exposure period to the shaved intact skin of six (two males and four females) New Zealand white rabbits, under semi-occlusive dressing. At the end of the exposure, the bandages were removed and sites were washed. Application sites were evaluated in accordance with the method of Draize at approximately 30–60 min and 24, 48 and 72 h after patch removal. At the initial observation, performed after completion of exposure, the test material induced very slight erythema on a single animal. All dose sites were stained yellow. No edema or other dermal findings were noted. Irritation was reversible and completely subsided by study day 1. The primary irritation for CitriMax[®] was calculated as 0.0, and the compound was classified as non-irritating. As described earlier in acute dermal toxicity studies, at high doses (2000 mg/kg), CitriMax[®] shows minimal irritation. These observations from the acute dermal toxicity study further support the minimal irritation level of CitriMax[®]. As both CitriMax[®] and Super CitriMax[®] are salts of HCA, the studies of CitriMax[®] are relevant for the evaluation of Super CitriMax[®].

In a study to detect sensitization (in 24/48 h covered patch tests), a 5% concentration of 'hydroxycitr' (hydroxycitric acid) was used. The results of this study indicate that the compound would be unlikely to irritate the skin of most healthy humans (Ginanneschi et al., 1989). Additional details of the study were not available.

2.3.7. Eye irritation studies

In an eye irritation study, the primary ocular irritation potential of CitriMax[®] [50% HCA as calcium salt] was tested by instilling single 54 mg doses of the test material into the lower conjunctival sac of the right eye of six albino New Zealand rabbits (Kiplinger, 1994d; Ohia et al., 2002). The dose of 54 mg was based on the determination that it occupied a volume of 0.1 ml. The left eye of each animal served as control. The eyes were examined for ocular reactions in accordance with the method of Draize at approximately 1, 24, 48 and 72 h post-dosing and on days 4, 7, 14 and 21 post-dosing. Sodium fluorescein was used to aid in revealing possible corneal damage at 72 h and on days 7, 14 and 21 post-dosing. On day 7 post-dosing, three of six rabbits had small areas of inflammatory exudates with enlarged blood vessels present at the apex of the lower conjunctival sac. One of these three rabbits also had

inflammatory exudates on the nictitating membrane. In two of the three affected rabbits the inflammatory exudates completely subsided by day 21 post-dosing. The Maximum Average Score for the test material was 15.0 (average) at 1 h. The test material induced positive iridal and conjunctival reactions for all rabbits, although no corneal reactions were noted. Within 48 h post-dosing, iridal irritation completely subsided. With the exception of inflammatory exudates, conjunctival irritation completely subsided by study termination (day 21) or earlier in all animals.

The results of the eye irritation study indicate that CitriMax[®] causes ocular irritation with production of inflammatory exudates in some animals. Positive iridal and conjunctival reactions were present in all animals, which subsided within 48 h. A total maximum Draize score of 110 is possible. An average Draize score of 15.0 was obtained in the study, indicating mild irritation. As both CitriMax[®] and Super CitriMax[®] are salts of HCA, the studies of CitriMax[®] may be relevant for Super CitriMax[®].

2.3.8. Sensitization studies

In a briefly reported study investigating nine patients who were allergic to propolis, one individual also developed a patch-test reaction to 'hydroxycitr. 5%' (presumed to be hydroxycitric acid), after 24/48-h covered contact. Additional details of the study were not provided (Ginanneschi et al., 1989).

2.4. Observations in humans

It is generally recognized that well-designed, quality human clinical studies are preferable to data derived from animals, particularly when assessing the potential risks to humans from exposure to substances. Clinical testing of food additives for safety is acceptable by the FDA, when it cannot be addressed adequately by non-human studies. Humans may be exposed to large quantities of some food additives. Testing of these substances in animals at doses that greatly exaggerate their anticipated human exposure may not be possible. Human clinical studies for these substances may provide additional confidence in the safety of the food additive (FDA, 1993). The clinical database on HCA intake includes two sets of data, observational case reports and clinical investigations. Table 5 provides an index of all clinical studies reviewed.

A total of 15 clinical studies involving approximately 914 subjects, examining the effects of HCA (Table 5) have appeared in the literature. Except for two studies (Girola et al., 1996; Kovacs et al., 2001), the dosages of *Garcinia* extract in these studies ranged from 1500 to 4667 mg/day (25–78 mg/kg/day). The equivalent dosage of HCA in these studies ranged from 900 to 2800 mg/day (15–47 mg/kg/day). Of the 15 clinical studies re-

ported (Table 5), 14 were placebo-controlled, double-blind trials (with 816 participants) and one was a single arm placebo-controlled trial. Double-blind, placebo-controlled studies are considered the least likely to result in bias. The clinical studies provide an opportunity to assess the safety and 'tolerability' of HCA and HCA-SX intake in fairly diverse populations. Collectively, these studies are of sufficient quality and consistency to draw certain conclusions regarding the safety of HCA-SX.

The placebo-controlled double-blind trials (Table 5) lasted for periods of up to 12 weeks (most studies i.e., 7 of 14, were for eight weeks), and the daily dosage of HCA in these studies ranged from 110 to 2800 mg. In these studies, clinical tolerance was evaluated by recording side effects appearing during the trial. In seven, double-blind, placebo-controlled trials, no side effects were noted or reported. In the other studies, although side effects were reported, they were not significantly different from the control group. These studies demonstrate that HCA did not cause adverse effects and was well-tolerated. Some of these studies are further described below.

In a 12 week, randomized, double-blind, placebo-controlled trial, Heymsfield et al. (1998) investigated the efficacy of *G. cambogia* extract (50% HCA) for body weight and fat mass loss in overweight humans. Subjects were randomized to receive either 1500 mg of HCA *per* day or placebo. Both groups were prescribed a high-fiber, low-energy diet. Body weight was evaluated every other week, and fat mass was measured at weeks 0 and 12. A total of 135 subjects were randomized to either active HCA ($n = 66$) or placebo ($n = 69$). No overt signs of toxicity were observed among volunteers consuming 1500 mg/day HCA (~15–18 mg/kg/day) for 12 weeks. Forty-two subjects in the active treatment group and 42 in the placebo group completed the study. The number of reported adverse reactions was not significantly different between the placebo group and hydroxycitric acid group. Patients in both groups lost a significant amount of weight; however, between-group weight loss differences were not significant. There were no significant differences in estimated body fat mass loss between treatment groups, and the fraction of subject weight loss as fat was not influenced by treatment. The investigators concluded that *G. cambogia* extract failed to produce significant weight loss and fat mass loss beyond that observed with placebo (Heymsfield et al., 1998; Heymsfield, 1999). In this study, adverse events were also recorded. The reported adverse events are summarized in Table 6. The number of reported adverse events was not significantly different between the placebo and treatment groups.

Several investigators criticized the design of the Heymsfield et al. (1998) clinical trial, including a lack of bioavailability of HCA used in the trial as well as the dosage of HCA used, which in both cases was low

Table 5
Summary of clinical studies of HCA-SX (Super CitriMax®) and HCA in humans and safety

Study design	Subject diagnosis	Number subjects	Dosage in mg/day (duration)	Clinical observations	Adverse events	Reference
Randomized, placebo-controlled, double-blind	Overweight	40 enrolled, with 35 completing trial (18; 5 male; 13 female)	1500 mg as CitriMax® GCE* (eight weeks)	Reduction in body weight, cholesterol, triglycerides	Two reported headache and nausea; One placebo reported similar symptoms	Ramos et al. (1995)
Single arm, open label	Obese adults	77 enrolled, with 55 completing trail	1500 mg GCE* and 300 µg chromium picolinate/day; healthy diet/exercise (eight weeks)	Weight loss		Badmaev and Majeed (1995)
Randomized, placebo-controlled, double-blind	Obese subjects	54 enrolled, with 39 completing trial	1500 mg GCE* and 300 µg chromium nicotinate/day; low fat substitution diet (eight weeks)	Weight loss (no statistical analysis)	One reported itching around mouth	Conte (1993)
Randomized, placebo-controlled, double-blind		200 enrolled, with 186 completing trail	1500 mg GCE, 600 µg chromium picolinate, 1200 mg L-carnitine/day; low fat high-fiber diet (four weeks)	Weight loss; fat mass loss		Cited in Heymsfield et al. (1998)
Randomized, placebo-controlled, double-blind	Obese subjects	60 enrolled, number of subjects completing not reported	1320 mg HCA/day; low fat diet (eight weeks)	Weight loss	No adverse events reported	Thom (1996)
Randomized, placebo-controlled, double-blind	Obese subjects	50, with 48 completing the trial	2400 mg GCE, 150 mg caffeine and 120 µg chromium/day; low fat diet (six weeks)	Weight loss; Reduction in blood pressure, total cholesterol and hip and waist circumference		Rothacker and Waitman (1997)
Randomized, placebo-controlled, double-blind		150 enrolled, number of subjects completing not reported	110 mg GCE, 38 mg chrome and 480 mg chitosan/day (or half all ingredients); hypocaloric diet (four weeks)	Weight loss		Girola et al. (1996)
Randomized, placebo-controlled, double-blind	Overweight	135 enrolled, with 84 completing the trial	3000 mg GCE (containing 1500 mg HCA/day); high-fiber, low-energy diet (12 week)	No significant changes in weight (see discussion)	Not significantly different adverse effects (see Table 6)	Heymsfield et al. (1998)
Randomized, placebo-controlled, double-blind	Mildly over weight subjects	89 female (42 on HCA)	2400 mg <i>G. cambogia</i> ; 1200 mg HCA (12 weeks)	Weight loss	No adverse events reported	Mattes and Bormann (2000)
Randomized, placebo-controlled, single-blind		24 enrolled (all subjects completed study)	1500 mg HCA-SX containing 900 mg HCA/day (two weeks)	Decreased energy intake	No adverse events reported	Westerterp-Plantenga and Kovacs (2002)

Table 5 (continued)

Study design	Subject diagnosis	Number subjects	Dosage in mg/day (duration)	Clinical observations	Adverse events	Reference
Randomized, placebo-controlled, single-blind		21 enrolled (7 male, 14 female) (two weeks)	500 mg HCA (alone or with 3 g medium chain triglycerides)	No change in energy intake	No adverse events reported	Kovacs et al. (2001)
Randomized, placebo-controlled, single-blind	Obese subjects	48 enrolled (number of subjects completing not reported)	4667 mg HCA-SX containing 2800 mg HCA/day (eight weeks)	Weight loss	No adverse events reported	Preuss et al. (2002)
Randomized, placebo-controlled, single-blind	Obese subjects	60 enrolled (53 subjects completed the study)	4667 mg HCA-SX containing 2800 mg HCA/day (eight weeks)	Decrease in body weight and BMI	No significantly different adverse events noted	Preuss et al. (2004a)
Randomized, placebo-controlled, single-blind	Obese subjects	30 enrolled (29 subjects completed the study)	4667 mg HCA-SX containing 2800 mg HCA/day (eight weeks)	Decrease in body weight and BMI	No significantly different adverse events noted	Preuss et al. (2004b)
Randomized, placebo-controlled, double-blind	Preobese or obese class I (BMI 25–35 kg/m ²)	40 enrolled (all subjects completed the study)	1000 mg HCA/day (eight weeks)	No significant changes in body weight; decrease in visceral fat area	No adverse effects were noted	Hayamizu et al. (2001)

*GCE = *G. cambogia* extract.

compared to successful animal trials (Badmaev et al., 1999; Firenzuoli and Gori, 1999; Schaller, 1999). Additionally, the use of a low-calorie diet (1200 kcal) may have negated any effect HCA might have had on inhibiting appetite and reducing food intake. These investigators (Badmaev et al., 1999; Firenzuoli and Gori, 1999; Schaller, 1999) cited several other studies assessing the weight loss effects of *G. cambogia* extract (Conte, 1993, 1994; Hobbs, 1994; Badmaev and Majeed, 1995; Girola et al., 1996; Rothacker and Waitman, 1997). While these studies were designed and conducted to determine efficacy and not safety, the authors were, nonetheless, obligated to and did report adverse effects, of which, there were none of any significance bearing on the safety of HCA.

In a randomized clinical trial, Ramos et al. (1995) studied the effects of a lyophilized extract of *G. cambogia* in healthy overweight subjects. Subjects were randomly divided into two groups, each consisting of 20 subjects, and were given placebo or *G. cambogia* extract (500 mg capsule) before each meal for eight weeks. Compared to the placebo group, administration of the extract resulted in a significant decrease in body weight, cholesterol and triglycerides. No changes in blood chemistry parameters, including glutamic oxaloacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT) and glucose were noted. Of the 20 subjects in the treatment group, two experienced headaches and nausea. In the placebo group, one subject reported symptoms of headache and nausea. The investigators stated that no symptoms of adverse reactions common to anorexigenic drugs derived from adrenergic sources were observed. In another study by Rothacker and Waitman (1997), it is stated that the administration of a proprietary formulation of *G. cambogia* caused 'no serious adverse events' in 25 overweight subjects. This was also a randomized, double-blind, placebo-controlled trial, with a slightly higher number of subjects (50 obese subjects, of which 48 completed the study) and for a shorter duration of treatment (six weeks). The formulation was given prior to every meal and provided a total daily dose of HCA slightly lower than 1500 mg and with additives (15 mg caffeine and 120 µg chromium).

In a randomized, double-blind, placebo-controlled study for eight weeks, Thom (1996) investigated the

Table 6
Number of subjects reporting side effects during HCA and placebo intake

Reported effects	HCA group	Placebo group
GI discomfort	13	6
Headache	9	12
Upper respiratory tract symptoms	16	13

efficacy and tolerability of HCA in 60 obese subjects (44 females and 16 males; subjects completing the study were not reported but two subjects stopped the treatment). Each subject received HCA (1320 mg/day) or an identical placebo capsule three times *per day*, 30 min before breakfast, lunch and dinner. All subjects were on a low fat diet of 1200 kcal/day and were instructed to exercise three times *per week*. Compared to the placebo group, a significant decrease in weight was noted in HCA-treated subjects. Significant reductions in blood pressure, total cholesterol and hip and waist circumferences were noted in both groups and between groups. Appetite scores during the study, using visual analog scales, were significantly decreased in the HCA-treated group, but not in the placebo group. The investigator reported that the tolerability of the treatment was excellent. Two patients stopped the treatment due to stomach pain, one in the HCA group and one in placebo group.

In a double-blind, placebo-controlled, parallel group trial, 89 mildly overweight females were prescribed 5020-kJ (~1180 kcal) diets for 12 weeks (Mattes and Bormann, 2000). Of the 89 participants, 42 ingested 400-mg caplets of *Garcinia cambogia* 30–60 min prior to meals for a total dose of 2.4 g/day (1.2 g/day HCA), the remaining subjects ingested matched placebo. At the end of 12 weeks, both groups lost body weight, with the HCA group achieving a significantly greater reduction. No effects of the HCA were observed on appetitive variables.

In a randomized, double-blind, placebo-controlled trial, Girola et al. (1996) studied lipid lowering effects of a formulation containing *G. cambogia* given to 150 subjects (the number completing the trial was not reported) for four weeks. Three groups of subjects, each with 50 volunteers, received 0, 1 or 2 capsules (containing 240 mg chitosan, 55 mg *G. cambogia* extract and 19 mg chrome) *per day* for four weeks. All subjects in this study were on a low-calorie diet. Data were expressed as “overweight reduction”: 12.5% for two doses/day; 7.9% for one dose/day; and 4.3% for placebo. The incidence of ‘adverse events’ (nausea and/or headache) in both of the treated groups (receiving 27.5 and 55 mg hydroxycitric acid/day) was no higher than in the placebo group, and there were no clinically significant effects on the blood (biochemistry or cellular composition). No pathologic or clinically significant change in blood chemistry or hematological assay was observed. Dose-dependent decreases were noted in the levels of LDL cholesterol, total cholesterol and triglycerides, while HDL cholesterol levels were increased.

Lim et al. (2002) investigated the short-term effects of HCA administration on the exercise performance of athletes. Subjects were administered 250 mg of HCA or placebo as a control for five days, after the performance of each cycle ergometer exercise (60% VO_2 max for

60 min followed by 80% VO_2 max until exhaustion). Compared to control subjects, the respiratory exchange ratio was significantly decreased in the HCA-treated subjects. Administration of HCA resulted in a significant increase in fat oxidation, and carbohydrate oxidation was significantly decreased during exercise, presumably resulting in the increase in the cycle ergometer exercise time to exhaustion after 1 h of 60% VO_2 max exercise. HCA administration did not affect the blood parameters such as glucose, lactate, free fatty acids or glycerol concentration. The investigators concluded that short-term administration of HCA enhances endurance performance by increasing fat oxidation, which spares glycogen utilization during moderate intensity exercise in athletes.

In a randomized, placebo-controlled single-blinded, cross-over trial, Westerterp-Plantenga and Kovacs (2002) investigated the effects of two weeks of daily administration of HCA-SX on energy intake and satiety in overweight men ($n = 12$) and women ($n = 12$). Subjects consumed 100 ml tomato juice (placebo) three times daily for two weeks and then 100 ml tomato juice with 300 mg HCA, after a two-week washout period. Compared to placebo, HCA treatment resulted in a significant decrease (15–30%) in 24-h energy intake, without changes in the appetite profile, dietary restraint, mood, taste perception or hedonics, while body weight tended to decrease. In another similar type of study, Kovacs et al. (2001) investigated the effects of HCA and HCA combined with medium chain triglycerides on satiety and food intake in seven male and 14 female normal to moderately obese subjects. Subjects consumed three self-selected meals and four isoenergetic snacks daily with either no supplementation, with 500 mg HCA, or 500 mg HCA and 3 g medium chain triglycerides. Subjects in all three groups showed body weight loss, but this reduction was not different between treatments. Two weeks of supplementation with HCA and HCA combined with medium chain triglycerides did not result in increased satiety or decreased energy intake compared to placebo.

Hayamizu et al. (2001) conducted a placebo-controlled, double-blind trial to examine the effects of HCA on body fat accumulation in 40 human subjects with body mass index (BMI) of 25–35 kg/m². Subjects were randomized to either HCA (1000 mg/day) or a placebo group for a period of eight weeks. Compared to the placebo group, a significant reduction in the visceral fat area and visceral fat area/subcutaneous fat area, as measured by computed tomography scan, was noted. However, no significant decreases in body weight loss and the waist–hip ratio were noted. No adverse effects were observed during the period of the study. At the end of the 4- and 8-week time points of the treatment, hematological (white blood cells, red blood cells, hemoglobin, hematocrit and platelets) and clinical

chemistry (SGPT, SGOT, γ -glutamyl transpeptidase, lactate dehydrogenase, blood urea nitrogen, creatinine, glucose, insulin, acetoacetic acid, 3-hydroxybutyric acid and total ketone bodies) parameters were studied. Hematological and clinical chemistry parameters did not show any significant alterations between the groups, although significant decreases in hemoglobin and number of red blood cells were noted; these decreases were within the treated and placebo group, not between them.

In another study, Hayamizu et al. (2002) studied the effects of *G. cambogia* in human volunteers to determine a no observed adverse effect level (NOAEL) of HCA in humans. NOAEL was determined by examining the adverse effects of high doses of HCA (4000 mg) in 44 healthy subjects. No adverse effects were noted in any subjects. No treatment related changes in hematology and clinical chemistry parameters were noted. The investigators determined the NOAEL of *G. cambogia* extract to be at least as high as 4000 mg HCA/day. These investigators also studied effects of sequential increasing doses of HCA from 1000 mg to 4000 mg/day. HCA was given daily for 10 days to 18 subjects (7 male and 11 female). No changes in hematological (WBC, RBC, hemoglobin, hematocrit, platelets) and clinical chemistry (SGPT, SGOT, γ -glutamyl transpeptidase, lactate dehydrogenase, blood urea nitrogen, creatinine, glucose, insulin, free fatty acid, LDL-cholesterol, triacylglycerol, total cholesterol, acetoacetic acid, 3-hydroxybutyric acid and total ketone bodies) parameters were noted at the end of the treatment period. This study demonstrates that *G. cambogia* extract at 4000 mg/day HCA is safe for healthy humans.

Recently, in a series of double-blind, placebo-controlled, randomized, human clinical trials, Preuss and colleagues investigated the efficacy and safety of HCA-SX. In three of these studies, a total of 138 moderately obese subjects participated. Subjects received HCA-SX at a daily dose of 4667 mg (2800 mg HCA) alone or in combination with niacin-bound chromium and standardized *Gymnema sylvestre* extract (Bagchi et al., 2002a,b; Preuss et al., 2002, 2004a,b) for eight weeks. Food was prepared and delivered to the subjects, and all subjects received a daily diet of 2000 kcal. Subjects also underwent a 30-min walking exercise program, 5 days a week. The subjects received the treatment 30–60 min before breakfast, lunch and dinner for eight weeks. Changes in body weight, lipid profile, serum leptin levels, body mass index (BMI), urinary excretion of fat metabolites (malondialdehyde, formaldehyde, acetaldehyde and acetone) and appetite control were assessed. In addition to these parameters, hematology, clinical chemistry parameters and urinalysis were conducted. HCA-SX, and to a greater extent the combination of the three ingredients, resulted in a significant weight loss, reduction in BMI, increased fat oxidation, favorable lipid profile (decreased LDL and triglyceride levels and,

increased HDL levels), reduction in circulating plasma leptin levels, increase in serum serotonin levels, and decreased appetite as determined by reductions in food intake. Results of hematology, blood chemistry and urinalysis did not reveal any treatment related changes. In all of these studies, no significant differences in adverse effects were noted in the treatment group compared to placebo.

3. Summary and conclusion

Super CitriMax[®] (HCA-SX) is a novel extract from the dried fruit rind of the plant *Garcinia cambogia*, a tree native to Southeast Asia and grown widely in India. The primary constituent of HCA-SX is (–)-hydroxycitric acid (HCA), which is structurally similar to citric acid, a common food additive. The dried fruit rind of *G. cambogia*, is commonly used in Southeast Asia as a food preservative, flavoring agent and carminative. In recent years, HCA-SX and other products containing HCA have been commonly consumed worldwide as a dietary supplement. HCA has been shown to be a potent inhibitor of ATP-citrate lyase and thus limits the availability of acetyl-CoA units required for fatty acid synthesis and lipogenesis during a lipogenic diet (carbohydrate-rich diet). The claimed weight reducing effects of HCA are attributed not only to reduced food intake and increased energy expenditure, but also to a suppression of fatty acid synthesis and an enhancement of glycogen synthesis in liver.

In a bioavailability study in human subjects, oral HCA-SX supplementation was found to be bioavailable in human plasma. Acute oral toxicity studies demonstrate that CitriMax[®] has a low acute oral toxicity. The oral LD₅₀ of CitriMax[®] in rats was found to be greater than 5000 mg/kg. In the dermal toxicity study in rabbits, CitriMax[®] induced slight and transient erythema with no edema. The dermal LD₅₀ of CitriMax[®] was found to be greater than 2000 mg/kg in rabbits. In the eye irritation study, CitriMax[®] caused ocular irritation with production of inflammatory exudate in some animals. The results of the skin and eye studies indicate that CitriMax[®] can cause mild irritation.

In a subchronic study in rats, the gavage administration of HCA-SX at doses up to 2500 mg/kg/day for a period of 90 days caused a significant decrease in body weight and reduction in feed consumption. Organ weights, hematology and clinical chemistry analysis did not reveal any signs of toxicity. Histological investigations of the major tissues did not reveal any treatment-related pathological changes except for minimal or mild hepatocyte vacuolation at 30 and 60 days, but not at 90 days. The study investigators suggested that the vacuolation may be related to the time duration between last feeding and euthanasia. Based on the data from this

study, the no observed adverse effect level (NOAEL) of HCA-SX for male and female rats was determined as 2500 mg/kg body weight/day.

No developmental or embryotoxic studies of HCA were found, but the structure, mechanism of action, long history of use and other toxicity studies indicate that HCA is unlikely to cause reproductive or developmental effects. HCA-SX was not mutagenic in the presence or absence of metabolic activation in Ames genotoxicity assays in strains TA98 and TA102. The positive results noted in the TA100 and TA153 strains without metabolic activation and in the TA1537 strain with metabolic activation were not considered biologically relevant to mutagenic effects.

In 14 placebo-controlled, double-blind trials and one single arm, open trial, employing up to 2800 mg/day HCA or 4667 mg/day (78 mg/kg/day) HCA-SX for up to 90 days, no treatment-related adverse effects were reported. In some of these studies, side effects were reported, but the incidence did not significantly differ from those of the placebo group. The majority of the side effects were minor and transient. The frequency of side effects was not related to the duration of intake of HCA. In some of these studies, hematology and clinical chemistry parameters were studied, and no treatment related changes in these parameters were noted. Because double-blind, placebo-controlled studies are considered the least likely to result in bias; the clinical studies of CitriMax[®], HCA-SX and HCA provide an opportunity to assess the safety and ‘tolerability’ in fairly diverse populations. Collectively, these studies are of sufficient quality and consistency and demonstrate that HCA did not cause adverse effects and was well-tolerated.

Based on the above-described information, safe use levels of HCA for human consumption can be determined. Ordinarily, acceptable daily intakes (ADIs) or safe levels for human consumption are determined following consideration of use levels required to achieve desired effect and as a factor of the no-effect level in an animal study. In the case of “macroingredients” (as is HCA or Super CitriMax[®]), additional issues come into play, such that conventional testing methodologies (e.g., testing at a dose of ± 100 -fold of the human intake level, which may result in a dose $>5\%$ of the diet or substances which produce satiety) preclude an interpretable outcome (Borzelleca, 1992). In such cases, where otherwise heroic doses would be mandated, tolerance data from human studies may provide needed corroborative information (Dybing et al., 2002).

By these criteria, HCA or HCA-SX cannot be tested by conventional means because (1) its minimum effective use level (or recommended dose) in humans is high (~ 4500 mg/day) and a 100-fold safety factor, if applied, would require administration of a level of 45% in the diet for animal studies and; (2) HCA or HCA-SX is known to have an effect on satiety, effectively preventing

consumption at high levels. Because of these restrictions, HCA-SX was studied at a dose equivalent to 5% of the diet (2500 mg/kg) for 90 days. This dose (2500 mg/kg, equivalent to 150,000 mg in a 60 kg human) was without adverse effect in the test animals. Several clinical studies have been conducted (Table 5), that corroborate these results, including three double-blind, placebo-controlled studies using 2800 mg/day HCA or 4667 mg/day HCA-SX for eight weeks, which reported no adverse effects. These studies and others cited above, taken in toto, support safety of (–)-HCA at 2800 mg/person/day.

In summary, on the basis of scientific procedures, which include human, animal, analytical, and other scientific studies, and history of exposure and use, the consumption of HCA or HCA-SX at dose level of 2800 or 4667 mg/day, respectively, is considered safe.

References

- Aujoulat, M., 2003. Super CitriMax HCA-600-SXS—Bacterial reverse mutation test (Plate incorporation and preincubation methods). MDS Pharma, MDS Study no. 293/001, pp. 1–52.
- Badmaev, V., Majeed, M., 1995. Open field, physician controlled, clinical evaluation of botanical weight loss formula Citrin. Nutraceuticals, Dietary Supplements and Functional Foods. Las Vegas, Nevada, July 11–13 (cited in Heymsfield et al., 1998).
- Badmaev, V., Majeed, M., Conte, A.A., 1999. *Garcinia cambogia* for weight loss. JAMA 282, 233–235.
- Bagchi, D., Preuss, H.G., Ohia, S.E., Rao, C.V.S., Bagchi, M., 2002a. Weight management and mechanisms of action of a novel, natural extract of (–)-hydroxycitric acid (HCA-SX) and a combination of a HCA-SX plus niacin-bound chromium (NBC) and *Gymnema sylvestre* extract (GSE). In: 9th World Congress on Clinical Nutrition, June 24–26, 2002, AB041.
- Bagchi, M., Preuss, H.G., Ohia, S.E., Rao, C.V.S., Bagchi, D., 2002b. Efficacy of a novel (–)-hydroxycitric acid extract in weight management. Journal of the American College of Nutrition 21, 481 (abstract).
- Boll, P.M., Sorensen, E., Balieu, E., 1969. Naturally occurring lactones and lactames. The absolute configuration of the hydroxycitric acid lactones: hibiscus acid and garcinia acid. Acta Chemica Scandinavica 23, 286–293.
- Borzelleca, J.F., 1992. Macronutrient substitutes: safety evaluation. Regulatory Toxicology and Pharmacology 16, 253–264.
- Chee, H., Romsos, D.R., Leveille, G.A., 1977. Influence of (–)-hydroxycitrate on lipogenesis in chickens and rats. Journal of Nutrition 107, 112–119.
- Cheema-Dhadli, S., Halperin, M.L., Leznoff, C.C., 1973. Inhibition of enzymes which interact with citrate by (–)-hydroxycitrate and 1,2,3-tricarboxybenzene. European Journal of Biochemistry 38, 98–102.
- Cloutre, D., Rosenbaum, M., 1994. The Diet and Health Benefits of HCA (Hydroxycitric Acid). Keats Publishing Co., New Canaan, CT. p. 9.
- Conte, A.A., 1993. A non-prescription alternative in weight reduction therapy. American Journal of Bariatric Medicine Summer, 17–19 (cited in Heymsfield et al., 1998).
- Conte, A.A., 1994. The effects of (–)-hydroxycitrate and chromium (GTF) on obesity. Journal of the American College of Nutrition 13, 535.
- DSHEA, 1994. Dietary Supplements Health and Education Act of 1994. US Food and Drug Administration, Center for Food Safety

- and Applied Nutrition, Washington, DC. Available from <<http://vm.cfsan.fda.gov/~dms/dietsupp.html>> (visited on October, 28, 2003).
- Dybing, E., Doe, J., Groten, J., Kleiner, J., O'Brien, J.O., Renwick, A.G., Schlatter, J., Steinberg, P., Tritscher, A., Walker, R., Younes, M., 2002. Hazard characterisation of chemicals in food and diet: dose response mechanisms and extrapolation issues. *Food Chemical Toxicology* 40, 237–282.
- EPA, 2001. U.S. High Production Volume (HPV) Chemical Challenge Program Assessment Plan For Acetic Acid and Salts Category, July 8, 2003, pp. 1–20. Available from <<http://www.epa.gov/chemrtk/acetisalt/c13102tp.pdf>>.
- FDA, 1982. Toxicological principles for the safety assessment of direct food additives and color additives used in food ('Redbook'). US Food and Drug Administration, Washington, DC.
- FDA, 1993. Toxicological principles for the safety assessment of direct food additives and color additives used in food "Red Book II" (Draft). US Food and Drug Administration, Washington, DC.
- Firenzuoli, F., Gori, L., 1999. *Garcinia cambogia* for weight loss. *JAMA* 282, 234, author reply 235.
- Ginanneschi, M., Acciai, M.C., Sertoli, A., Bracci, S., 1989. Propolis allergy: Synthesis and patch testing of *gamma,gamma*-dimethylallyl caffeic acid ester and its *o*-methyl derivatives. *Contact Dermatitis* 21, 267–269.
- Girola, M., De Bernardi, M., Contos, S., 1996. Dose effect in lipid-lowering activity of a new dietary integrator (Chitosan, *Garcinia cambogia* extract, and Chrome). *Acta Toxicologica et Therapeutica* 17, 25–40.
- Glusker, J.P., Minkin, J.A., Casciato, C.A., Soule, F.B., 1969. Absolute configuration of the naturally occurring hydroxycitric acids. *Archives of Biochemistry and Biophysics* 13, 573–577.
- Glusker, J.P., Minkin, J.A., Casciato, C.A., 1971. The structure and absolute configuration of the calcium salt of garcinia acid, the lactone of (–)-hydroxycitric acid. *Acta Crystallography B* 27, 1284–1293.
- Greenwood, M.R., Cleary, M.P., Gruen, R., Blase, D., Stern, J.S., Triscari, J., Sullivan, A.C., 1981. Effect of (–)-hydroxycitrate on development of obesity in the Zucker obese rat. *American Journal of Physiology* 240, E72–E78.
- Guthrie, R.W., Kierstead, R.W., 1977. Hydroxycitric acid derivatives. US Patent 4005086 and US Patent 4006166.
- Hayamizu, K., Ishii, Y., Kaneko, I., Shen, M., Sakaguchi, H., Okuhara, Y., Shigematsu, N., Miyazaki, S., Shimasaki, H., 2001. Effects of long-term administration of *Garcinia cambogia* extract on visceral fat accumulation in humans: a placebo-controlled double blind trial. *Journal of Oleo Science* 50, 805–812.
- Hayamizu, K., Ishii, Y., Kaneko, I., Shen, M., Okuhara, Y., Sakaguchi, H., Shigematsu, N., Shimasaki, H., 2002. No-observed-adverse-effect level (NOAEL) and sequential-high-doses administration study on *Garcinia cambogia* extract in humans. *Journal of Oleo Science* 51, 365–369.
- Henry, C.J., 1991. Impact of dietary restriction on bioassay and recommendations for future research: panel discussion. In: Fishbein, L. (Ed.), *Biological Effects of Dietary Restriction*. Springer-Verlag, New York, NY, pp. 321–336.
- Heymsfield, S.B., 1999. Author reply: *Garcinia cambogia* for weight loss. *JAMA* 282, 235.
- Heymsfield, S.B., Allison, D.B., Vasselli, J.R., Pirotbelli, A., Greenfield, D., Nunez, C., 1998. *Garcinia cambogia* (hydroxycitric acid) as a potential antiobesity agent: a randomized controlled trial. *JAMA* 280, 1596–1600.
- Hobbs, L.S., 1994. (–)-Hydroxycitrate (HCA). In: *The New Diet Pills*. Pragmatic Press, Irvine, CA, pp. 161–174.
- Hoffman, G.E., Andres, H., Weiss, L., Kreisel, C., Sander, R., 1980. Properties and organ distribution of ATP citrate (pro-3S)-lyase. *Biochimica Biophysica Acta* 620, 151–158.
- Jayaprakasha, G.K., Sakariah, K.K., 1998. Determination of organic acids in *Garcinia cambogia* (Desr.) by HPLC. *Journal of Chromatography* 806, 337–339.
- Jayaprakasha, G.K., Sakariah, K.K., 2000. Determination of (–)-hydroxycitric acid in commercial samples of *Garcinia cambogia* extracts by liquid chromatography using ultraviolet detection. *Journal of Liquid Chromatography Related Technology* 23, 915–923.
- Jena, B.S., Jayaprakasha, G.K., Singh, R.P., Sakariah, K.K., 2002. Chemistry and biochemistry of (–)-hydroxycitric acid from *Garcinia*. *Journal of Agricultural and Food Chemistry* 50, 10–22.
- Jones, C.T., Ashton, I.K., 1976. Lipid biosynthesis in liver slices of the foetal guinea pig. *Biochemistry Journal* 154, 149–158.
- Kiplinger, G.R., 1994a. Acute dermal toxicity study of CitriMax in albino rabbits. WIL Research Laboratories, Inc., Ashland, OH, October 31, 1994, WI 1-245002, pp. 1–35 (Report provided by InterHealth Nutraceutical, Inc., USA).
- Kiplinger, G.R., 1994b. Acute oral toxicity study of CitriMax in albino rats. WIL Research Laboratories, Inc., Ashland, OH, October 31, 1994, WI 1-245001, pp. 1–22 (Report provided by InterHealth Nutraceutical, Inc., USA).
- Kiplinger, G.R., 1994c. Primary dermal irritation study of CitriMax in albino rabbits. WIL Research Laboratories, Inc., Ashland, OH, October 31, 1994, WI 1-245003, pp. 1–17 (Report provided by InterHealth Nutraceutical, Inc., USA).
- Kiplinger, G.R., 1994d. Primary Eye Irritation Study of CitriMax in Albino Rabbits. WIL Research Laboratories, Inc., Ashland, OH, October 31, 1994, WI 1-245004, pp. 1–19 (Report provided by InterHealth Nutraceutical, Inc., USA).
- Kovacs, E.M., Westerterp-Plantenga, M.S., de Vries, M., Brouns, F., Saris, W.H., 2001. Effects of 2-week ingestion of (–)-hydroxycitrate and (–)-hydroxycitrate combined with medium-chain triglycerides on satiety and food intake. *Physiology and Behavior* 74, 543–549.
- Leonhardt, M., Langhans, W., 2002. Hydroxycitrate has long-term effects on feeding behavior, body weight regain and metabolism after body weight loss in male rats. *Journal of Nutrition* 132, 1977–1982.
- Lewis, Y.S., 1969. Isolation and properties of hydroxycitric acid. In: Lowenstein, J.M. (Ed.), *Methods in Enzymology*. In: *Citric Acid Cycle*, vol. 13. Academic Press, New York, NY, pp. 613–619.
- Lewis, Y.S., Neelakantan, S., 1965. (–)-Hydroxycitric acid—the principal acid in the fruits of *Garcinia cambogia* Desr. *Phytochemistry* 4, 619–625.
- Lewis, Y.S., Neelakantan, S., Murthy, C., 1964. Acids in *Garcinia cambogia*. *Current Science* 33, 82–83.
- Lim, K., Ryu, S., Ohishi, Y., Watanabe, I., Tomi, H., Suh, H., Lee, W.K., Kwon, T., 2002. (–)-hydroxycitrate ingestion increases fat oxidation during exercise in athletes short-term. *Journal of Nutrition Science and Vitaminology (Tokyo)* 48, 128–133.
- Loe, Y.C., Bergeron, N., Rodriguez, N., Schwarz, J.M., 2001. Gas chromatography/mass spectrometry method to quantify blood hydroxycitrate concentration. *Analytical Biochemistry* 291, 148–154.
- Lowenstein, J., 1970. Experiments with (–)-hydroxycitrate. In: Bartley, W., Kornberg, H., Quayle, J. (Eds.), *Essays in Cell Metabolism*. Wiley Interscience, New York, NY, pp. 153–166.
- Lowenstein, J., Brunengraber, H., 1981. Hydroxycitrate. In: Lowenstein, J. (Ed.), *Methods in Enzymology, Lipids*, vol. 72. Academic Press, New York, NY, pp. 486–497.
- Martin, F.W., Campbell, C.W., Ruberte, R.M., 1987. Perennial edible fruits of the tropics: an inventory. United States Department of Agriculture, Agricultural Research Service, Washington, DC, p. 212.
- Martius, C., Maue, R., 1941. Preparation, physiological behavior, and importance of hydroxycitric acid and its isomers. *Zeitschrift fur Physiological Chemistry* 269, 33–39.

- Mattes, D.R., Bormann, L., 2000. Effects of (–)-hydroxycitric acid on appetitive variables. *Physiology and Behavior* 71, 87–94.
- McCarty, M.F., 1994. Promotion of hepatic lipid oxidation and gluconeogenesis as a strategy for appetite control. *Medical Hypotheses* 42, 215–225.
- Moffett, S.A., Bhandari, A.K., Ravindranath, B., Balasubra-Manvam, K., 1977. Hydroxycitric acid concentrate and food products prepared therefrom. US Patent 5656314.
- Ohia, S.E., Opere, C.A., LeDay, A.M., Bagchi, M., Bagchi, D., Stohs, S.J., 2002. Safety and mechanism of appetite suppression by a novel hydroxycitric acid extract (HCA-SX). *Molecular and Cellular Biochemistry* 238, 89–103.
- Panksepp, J., Pollack, A., Meeker, R.B., Sullivan, A.C., 1977. (–)-Hydroxycitrate and conditioned aversions. *Pharmacology Biochemistry Behavior* 6, 683–687.
- Preuss, H.G., Bagchi, D., Rao, C.V.S., Echard, B.W., Satyanarayana, S., Bagchi, M., 2002. Effect of hydroxycitric acid on weight loss, body mass index and plasma leptin levels in human subjects. *FASEB Journal* 16, A1020.
- Preuss, H.G., Bagchi, D., Bagchi, M., Rao, C.V.S., Dey, D.K., Satyanarayana, S., 2004a. Effects of natural extract of (–)-hydroxycitric acid (HCA-SX) and a combination of HCA-SX plus niacin-bound chromium and *Gymnema sylvestre* extract in weight loss. *Diabetes, Obesity and Metabolism* 24, 45–58.
- Preuss, H.G., Bagchi, D., Bagchi, M., Rao, C.V.S., Satyanarayana, S., 2004b. Efficacy of a novel, natural extract of (–)-hydroxycitric acid (HCA-SX) and a combination of HCA-SX, niacin-bound chromium and *Gymnema sylvestre* extract in weight management in human volunteers. *Nutrition Research*, in press.
- Ramos, R.R., Saenz, J.L., Aguilar, C.F., 1995. Extract of *Garcinia cambogia* in the control of obesity. *Investigacion Medica Internacional* 22, 97–100.
- Rao, R., Sakariah, K., 1988. Lipid lowering and antiobesity effect of (–)-hydroxycitric acid. *Nutrition Research* 8, 209–212.
- Rothacker, D.Q., Waitman, B.E., 1997. Effectiveness of a *Garcinia cambogia* and natural caffeine combination in weight loss: a double-blind placebo-controlled pilot study. *International Journal of Obesity* 21 (Suppl. 2), 53.
- Roy, S., Rink, C., Khanna, S., Phillips, C., Bagchi, D., Bagchi, M., Sen, C., in press. Body weight and abdominal fat gene expression profile in response to a novel hydroxycitric acid based dietary supplement. *Gene Expression*.
- Schaller, J.L., 1999. *Garcinia cambogia* for weight loss. *JAMA* 282, 234, author reply 235.
- Sergio, W., 1988. A natural food, the Malabar Tamarind, may be effective in the treatment of obesity. *Medical Hypotheses* 27, 39–40.
- Shara, M., Ohia, S.E., Schmidt, R.E., Yasmin, T., Zardetto-Smith, A., Kincaid, A., Bagchi, M., Chatterjee, A., Bagchi, D., Stohs, S.J., 2003a. Dose- and time-dependent effects of a novel (–)-hydroxycitric acid extract on body weight, hepatic and testicular lipid peroxidation, DNA fragmentation and histopathological data over a period of 90-days. *Molecular and Cellular Biochemistry* 254, 339–346.
- Shara, M., Ohia, S.E., Stohs, S.J., 2003b. 90-Day oral toxicity of super CitriMax in male and female Sprague–Dawley rats. *Toxicology Research Center, Creighton University Medical Center, Omaha, NE, February 21, 2003, ARC#0598*, pp. 1–134.
- Shara, M., Ohia, S.E., Schmidt, R.E., Yasmin, T., Zardetto-Smith, A., Kincaid, A., Bagchi, M., Chatterjee, A., Bagchi, D., Stohs, S.J., 2004. Physico-chemical properties of a novel (–)-hydroxycitric acid extract and its dose- and time-dependent effects on body weight, selected organ weights, hepatic lipid peroxidation and DNA fragmentation, hematology and clinical chemistry, and histopathological changes over a period of 90-days. *Molecular and Cellular Biochemistry* 260, 171–186.
- Sreenivasan, A., Venkataraman, R., 1959. Chromatographic detection of the organic constituents of *Gorikapuli* (*Garcinia cambogia* Desr.) used in pickling fish. *Current Science* 28, 151–152.
- Stallings, W., Blount, T.F., Srere, P.A., Glusker, J.P., 1979. Structural studies of hydroxycitrate and their relevance to certain enzymatic mechanisms. *Archives of Biochemistry and Biophysics* 193, 431–448.
- Sullivan, A., Triscari, J., 1976. Possible interrelationship between metabolite flux and appetite. In: Novin, D., Wyriwicka, W., Bray, G. (Eds.), *Hunger: Basic Mechanisms and Clinical Implications*. Raven Press, New York, NY, pp. 115–125.
- Sullivan, A., Triscari, J., Miller, O., 1974a. The influence of (–)-hydroxycitrate on in vivo rates of hepatic lipogenesis, glycogenesis and cholesterolgenesis. *Federation Proceedings* 33, 656.
- Sullivan, A.C., Triscari, J., Hamilton, J.G., Miller, O.N., 1974b. Effect of (–)-hydroxycitrate upon the accumulation of lipid in the rat. II. Appetite. *Lipids* 9, 129–134.
- Sullivan, A.C., Triscari, J., Hamilton, J.G., Miller, O.N., Wheatley, V.R., 1974c. Effect of (–)-hydroxycitrate upon the accumulation of lipid in the rat. I. Lipogenesis. *Lipids* 9, 121–128.
- Sullivan, A.C., Singh, M., Sere, P.A., Glusker, J.P., 1977. Reactivity and inhibitor potential of hydroxycitrate isomers with citrate synthase, citrate lyases and ATP citrate lyases. *Journal of Biological Chemistry* 252, 7583–7590.
- Sullivan, A., Triscari, J., Comai, K., 1984. Pharmacological modulation of lipid metabolism for the treatment of obesity. *International Journal of Obesity* 8 (Suppl. 1), 241–248.
- Szutowicz, A., Stepien, M., Lysiak, W., Angielski, S., 1976. Effect of (–)-hydroxycitrate on the activities of ATP citrate lyase and the enzymes of acetyl-CoA metabolism in rat brain. *Acta Biochim Poland* 23, 227–234.
- Thom, E., 1996. Hydroxycitrate (HCA) in the treatment of obesity. *International Journal of Obesity and Related Metabolic Disorders* 20 (Suppl. 4), 75.
- Vasselli, J.R., Shane, E., Boozer, C.N., Heymsfield, S.B., 1998. *Garcinia cambogia* extract inhibits body weight gain via increased energy expenditure (EE) in rats. *FASEB Journal* 12, A505.
- Watson, J.A., Lowenstein, J.M., 1970. Citrate and the conversion of carbohydrate into fat. Fatty acid synthesis by a combination of cytoplasm and mitochondria. *Journal of Biological Chemistry* 245, 5993–6002.
- Watson, J., Fang, M., Lowenstein, J., 1969. Tricarballoylate and hydroxycitrate: substrate and inhibitor of ATP: citrate oxaloacetate lyase. *Archives of Biochemistry and Biophysics* 135, 209–217.
- Westerterp-Plantenga, M.S., Kovacs, E.M., 2002. The effect of (–)-hydroxycitrate on energy intake and satiety in overweight humans. *International Journal of Obesity and Related Metabolic Disorders* 26, 870–872.
- Xu, Z.X., Smart, D.A., Rooney, S.A., 1990. Glucocorticoid induction of fatty acid synthase mediates the stimulatory effect of the hormone on cholinephosphate cytidyltransferase activity on fetal rat lung. *Biochimica Biophysica Acta* 1044, 70–76.