Saw Palmetto
(*Serenoa repens)*

and One of Its Constituent Sterols

β-Sitosterol
[83-46-5]

Review of Toxicological Literature

Prepared for

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EXECUTIVE SUMMARY

The nomination of saw palmetto and β-sitosterol for testing is based on the potential for human exposure and the limited amount of toxicity and carcinogenicity data.

Saw palmetto (*Serenoa repens*), a member of the palm family Arecaceae, is native to the West Indies and the Atlantic Coast of North America, from South Carolina to Florida. The plant may grow to a height of 20 feet (6.10 m), with leaves up to 3 feet (0.914 m) across. The berries are fleshy, about 0.75 inch (1.9 cm) in diameter, and blue-black in color. Saw palmetto berries contain sterols and lipids, including relatively high concentrations of free and bound sitosterols. The following chemicals have been identified in the berries: anthranilic acid, capric acid, caproic acid, caprylic acid, β-carotene, ferulic acid, mannitol, β-sitosterol, β-sitosterol-*D*-glucoside, linoleic acid, myristic acid, oleic acid, palmitic acid, 1-monolaurin and 1-monomyristin. A number of other common plants (e.g., basil, corn, soybean) also contain β-sitosterol. Saw palmetto extract has become the sixth best-selling herbal dietary supplement in the United States. In Europe, several pharmaceutical companies sell saw palmetto-based over-the-counter (OTC) drugs for treating benign prostatic hyperplasia (BPH). Additional pharmaceutical preparations that contain saw palmetto extract as an ingredient have been patented as hair lotions for the treatment of seborrhea and hair loss, capsules for the treatment of hair loss, and lotions/ointments for the treatment of acne. β-Sitosterol is available as a cholesterol-lowering drug, and is an ingredient in some contraceptive drugs. Sitosterols are commercially available as raw material in 50- and 200-kg fiber drums.

An extract of saw palmetto berries can be prepared using hot water, or by supercritical extraction with CO_2. The lipophilic ingredients may be extracted with lipophilic solvents (hexane or ethanol 90% v/v). A non-standardized extract is produced by grinding the saw palmetto berries to a raw powder. Sterol fractions rich in β-sitosterol are isolated from the stillbottoms remaining after distillation of the commercially usable oils from pinewood (tall oil), corn, cottonseed, or soybeans. β-Sitosterol may also be extracted from *Anacardium occidentale*.

Plantation Medicinals (the largest U.S. producer) harvests about 5,000 tons of saw palmetto berries per year in Hendry County, Florida. The second largest producer of the berries is Wilcox Natural Products in Boone, North Carolina. The export of saw palmetto berries from Florida has become a $50 million dollar a year industry, with about 2,000 tons of the berries exported to Europe each year. No production and import volumes were found for sitosterol.

Historically, American Indians used the berries for food. Since 1994 when federal dietary supplement laws were relaxed, the most common use of the berries by Americans is as an herbal health remedy. The berries have been used for treating stomach ache, bronchitis, diabetes, cancer, and cystitis; they have also been used as a diuretic,

aphrodisiac, and for breast enlargement. Saw palmetto berries are claimed to relieve irritated throat and symptoms of the common cold. The dried berries have been used as a menstrual drug product. Saw palmetto berry extracts have been reported to be effective in the treatment of BPH. However, significant inhibition of prostate growth has not been demonstrated, and a critical analysis of data on the effects of phytotherapy (including saw palmetto berry extracts) in BPH treatment suggested that the effects were no better than placebo treatment.

β-Sitosterol is claimed to have the following pharmacological properties: androgenic, anorexic, antiadenomic, antiandrogenic, antiestrogenic, antifeedant, antifertility, antigonadotropic, antiinflammatory, antileukemic, antimutagenic, antiophidic (inhibits effect of snake bite), antiprogestational, antiprostatadenomic, antiprostatitic, antitumor, cancer preventative, candidicide, estrogenic, gonadotropic hepatoprotective, hypocholesterolemic, hypoglycemic, hypolipidemic, pesticide, spermicide, viricide, antibacterial, and antifungal. Pharmaceutical preparations claim that β-sitosterol is effective in treating diabetic male sexual dysfunction. β-Sitosterol is used in the treatment of prostatic adenoma and BPH. A rodent study suggested that β-sitosterol may be effective in the treatment of vitiligo. β-Sitosterol was not effective in the treatment of pulmonary tuberculosis, and it exhibited low potency when tested for use as an antiacne agent. In addition to its medicinal uses, sitosterol is used in German cosmetic products and was effective as a nonabsorbable indicator for cholesterol absorption.

High concentrations of β-sitosterol are found in the effluent of pulp mills. Sitosterols have also been identified in the raw effluent of municipal wastewater treatment plants, but were not detected in tap water. Sitosterols are excreted in the feces of humans, pigs, cows, horses, sheep, cats, dogs, and a number of bird species.

Human exposure to saw palmetto occurs when the extract is taken for medicinal purposes: orally as a capsule or as a tea or topically as a hair lotion or as an acne lotion/ointment. β-Sitosterol is taken orally for medicinal purposes. The largest human dietary intake of sitosterol occurs from consuming corn, bean, and plant oils. Vegetarian diets contain higher amounts of sitosterol than traditional Western meat-eating diets, and β-sitosterol is the most commonly ingested phytosterol. Common sources of β-sitosterol include a number of plant constituents or oils including wheat germ oil, corn oil, rye germ oil, cottonseed oil, soybean oil, peanut oil, olive oil, navy beans, dark red kidney beans, pinto beans, and black turtle soup beans. β-Sitosterol is also present in fats, with smaller contributions to the diet identified in nuts, cereals, bread, preserves, vegetables including potatoes, red meat products, fish, dairy products, eggs, poultry, beverages including coffee and tea, margarine, and fruits. β-Sitosterol has been identified in 8 species of shellfish marketed for consumption in the northwestern states, and in a number of edible fish. β-Sitosterol is a component of tobacco and of tobacco smoke. It has also been identified in opium, bourbon, and whiskey.

Saw palmetto extract is not recognized as safe and effective by the U.S. Food and Drug Administration (FDA) and is misbranded when labeled as an OTC drug for use as an orally administered menstrual drug product. In the U.S., saw palmetto extract may not be sold or labeled as therapeutic support for the prostate gland or reproductive organs.

In rare cases, the consumption of saw palmetto berries may cause stomach problems, while large amounts might cause diarrhea. Only minor side effects were reported in studies of BPH patients ingesting saw palmetto extract: half of the side-effect symptoms were gastrointestinal. When phytosterols (including β-sitosterol) were taken orally to lower plasma cholesterol levels, no obvious side effects were noted.

No data were found relating to chemical disposition of β-sitosterol.

In animals (including humans), sitosterol is derived exclusively from dietary intake. Sitosterol is absorbed in the intestine; humans usually absorb less than 5% of phytosterols (including sitosterol), so that about 95% of dietary phytosterols enter the colon. Absorption of phytosterols appears to be greater during infancy and childhood than during adulthood. Absorption of phytosterols in the intestine is selective and appears to decrease with increasing number of carbons in the sterol side chain. In an inhalation experiment with male rats, 78% of radiolabeled β-sitosterol administered as a component of cigarette smoke was taken up by the rats. Most of the β-sitosterol was found in the distal air spaces and parenchyma of the lung, with a smaller amount being found in the trachea. The radiolabeled β-sitosterol was slowly released by the lungs to plasma; it was immediately found in the plasma, peaked on day 2, and declined slowly (but was not totally eliminated) over the next 30 days. From the plasma, β-sitosterol was distributed to the liver, kidney, stomach, spleen, and esophagus, with a peak absorption at 5 to 8 days. Levels of β-sitosterol slowly declined in all the sampled organs except for the esophagus, in which β-sitosterol was reduced to negligible quantities after 15 days. Peak amounts of β-sitosterol were found in the liver and spleen.

When a saw palmetto extract containing ^{14}C-labeled oleic or lauric acid or β-sitosterol was fed to rats, uptake of the radioactive label was much higher in the prostate gland than in the liver or other genitourinary tissues (e.g., seminal vesicles). The amount of phytosterols in the serum is generally low even with high dietary intake, but plasma levels of sitosterol have been shown to increase up to twice the normal levels with dietary supplementation. In humans with an average diet, plasma levels ranged from 0.00166 to 0.010 mg/mL (0.000004 to 0.000024 mmol/mL).

Insects and prawns can transform phytosterols to cholesterols, which are then synthesized into steroid hormones or bile acids. However, vertebrate species lack this ability. β-Sitosterol is converted to polar compounds (di- and tri-hydroxylated C_{21}-bile acids). In rat liver mitochondria, β-sitosterol is oxidized into 26-hydroxy-β-sitosterol and 29-hydroxy-β-sitosterol metabolites. In the rat testes, β-sitosterol is directly converted by mitochondrial enzymes to the steroid hormones progesterone, pregnenolone, testosterone plus 17α-progesterone, and polar steroids. Theoretically, the presence of an

ethyl group at C_{24} should prevent or obstruct conversion of sitosterol into bile acids just as it does for the conversion of cholesterol into C_{24}-bile acids. Experiments with rats, monkeys, and humans have reported a lack of conversion of sitosterol into C_{24}-bile acids in accordance with the theory. Conflicting data, however, have been published.

Phytosterols are excreted in the bile and their elimination appears to be faster than that for cholesterol. The pharmacokinetics of β-sitosterol administration via intravenous (i.v.) and oral routes in the dog were best described by the two-compartment model; the distribution half-life was 3 hours and the terminal distribution half-life was 129 hours. Absolute bioavailability upon oral administration was 9%.

In contrast to healthy humans, individuals with sitosterolemia (a rare inherited lipid storage disease) have a very different pattern of sitosterol metabolism. Sistosterolemic individuals have increased intestinal absorption of the compound, loss of tissue sterol structural recognition, expanded pools, and hepatic retention.

Acute toxicity data for saw palmetto extract were not found; the acute toxicity for β-sitosterol administered intraperitoneal (i.p.) to mice is >3000 mg/kg (>7.23 mmol/kg).

Short term (60 days) subcutaneous (s.c.) exposure of male and female rats to β-sitosterol did not produce gross or microscopic lesions either in the liver or the kidney. All clinical biochemical parameters were in the normal range except for serum protein and serum cholesterol; serum cholesterol was markedly depleted in both sexes in a dose-dependent manner. Male rats fed β-sitosterol in the diet for 28 weeks experienced no adverse effects.

No chronic exposure data were found.

Saw palmetto extract may exhibit an antiestrogenic effect, as well as may block progesterone and androgenic receptors. In BPH patients, treatment with saw palmetto berry extract alone did not significantly reduce prostate volume. Antiestrogenic activity of saw palmetto extract was noted in treated BPH patients. This activity, in addition to an antiandrogenic action, may occur by competitively blocking translocation of cytosolic estrogen receptors to the nucleus. Saw palmetto extracts, including β-sitosterol, exhibited estrogenic effects when injected into immature female mice. When inbred female rats were administered β-sitosterol s.c. for 30 days, the estrus cycle was disrupted; at high doses, the incidence of persistent estrus was prolonged as long as treatment continued and a marked increase in ovarian, uterine, and pituitary weights was induced. In adult male rats, treatment with β-sitosterol for up to 60 days significantly reduced fertility, decreased sperm concentrations, and decreased testicular weight. Withdrawal from treatment for 30 days did not restore sperm count or testicular weight.

In ovariectomized albino Wistar rats, β-sitosterol s.c. for 10 days caused a significant dose-dependent increase in glycogen and total lactate dehydrogenase concentrations, significant increases in glucose-6-phosphate dehydrogenase and phosphohexose isomerase, and a significant dose-dependent increase in uterine weight. β-Sitosterol exhibited an estrogenic response when injected into neonatal male and

female rats: postpubertal pituitary response to GnRH was altered in females, and basal luteinizing hormone secretion was altered in both males and females. These doses also altered basal luteinizing hormone secretion in immature male and female rats and postpubertal pituitary response to GnRH in female rats. Very high doses of β-sitosterol administered s.c. induced irregularity in spermiogenesis in immature rabbits, and ovarian weight was reduced in 25-week-old female lambs.

Fish chronically exposed to kraft pulp mill effluent exhibited a range of reproductive responses: female mosquitofish (*Gambusia affinis*) expressed male anatomical and behavioral characteristics, including a modified anal fin resembling a gonopodium and reproductive behaviors such as mating attempts. White sucker fish (*Catostomus commersoni*) and lake whitefish (*Coregonus clupeaformis*) had lower serum 17β-estradiol, testosterone, 17α,20β-dihydroprogesterone, and 11-ketotestosterone levels compared to fish from a reference site. Laboratory exposure of rainbow trout (*Oncorhynchus mykiss*) reduced plasma testosterone levels by approximately 50%. β-Sitosterol, found in high concentrations in the effluent, is believed to be responsible for the toxicological effects. In the presence of bacteria, β-sitosterol degrades into androgens thought to be responsible for the masculinizing effects on female fish.

Saw palmetto is known to exhibit an antiandrogenic action, although the compound responsible for this action has not been identified. The effects are thought to be caused by a direct action on the androgen receptor, the inhibition of the enzyme testosterone-5-α-reductase and/or competitive inhibition of dihydrotestosterone (DHT) binding to both cytosolic and nuclear receptors. However, studies found that saw palmetto berry extract did not demonstrate any inhibition of DHT binding or inhibition of 5-α-reductase activity. The extract inhibited the formation of all the testosterone metabolites studied (DHT; androst-4-ene-3,17-dione; and 5α-androstane-3,17-dione) in both epithelial and fibroblast cells from BPH and prostate cancer tissues. Saw palmetto extract markedly inhibited both isoforms of human 5-α-reductase in the baculovirus-directed insect cell expression system, but the inhibition was noncompetitive. It inhibited DHT and testosterone binding in 11 different human tissue specimens. In humans, the antiandrogenic effect is achieved without significantly influencing systemic hormone levels, including testosterone, follicle-stimulating hormone, and luteinizing hormone.

No carcinogenicity studies were located for saw palmetto extract or β-sitosterol. However, several anticarcinogenicity studies with β-sitosterol have been conducted; in none of these studies was an increased incidence of tumors due to treatment with β-sitosterol alone reported. In a two-stage skin carcinogenicity study, female mice initiated with a single topical application of 7,12-dimethylbenz[*a*]anthracene (DMBA) followed by a twice weekly treatment for 18 weeks with the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) exhibited a lower incidence of tumors and a lower tumor multiplicity in tumor-bearing animals when topically treated with β-sitosterol 30-40 minutes before each TPA treatment. In another initiation-promotion study, β-

sitosterol was an effective inhibitor of the initiation of mammary lesions induced in rats by DMBA plus TPA.

β-Sitosterol also significantly reduced the incidence of colon tumors (predominantly adenomatous polyps) in male Fischer CD rats induced by *N*-methyl-*N*-nitrosourea (MNU). However, in a study using outbred male rats, β-sitosterol supplemented in the diet did not significantly inhibit the number of azoxymethane (AOM)-induced tumors per rat.

Only limited genotoxicity data on β-sitosterol were available. β-Sitosterol was negative for the induction of strand breaks in λDNA, and was not mutagenic in *S. typhimurium* strain TA98 with metabolic activation or in TA100 without metabolic activation. Autoxidized β-sitosterol was not mutagenic when tested in the absence of metabolic activation in *S. typhimurium* strains TA98, TA100, TA1535, and TA1538. A pyrolysis product of β-sitosterol (prepared at 450°C) was not mutagenic when tested in the presence or absence of metabolic activation in *S. typhimurium* strains TA98 and TA100. However, in another study, a pyrolysate of β-sitosterol (formed at 700°C) was mutagenic when tested in *S. typhimurium* strains TA97, TA98, and TA100 in the presence and absence of metabolic activation.

Several studies have been conducted to evaluate the antigenotoxicity of β-sitosterol. β-Sitosterol did not inhibit the ability of ascorbic acid to induce strand breaks in λDNA. In *S. typhimurium*, β-sitosterol inhibited in a dose-dependent manner the mutagenic activity of MNU in TA100 in the absence of metabolic activation, and of 2-aminoanthracene (2-AA) in TA98 in the presence of metabolic activation. In contrast, β-sitosterol did not suppress the mutagenicity of Trp-P-2 in *S. typhimurium* strain TA98 in the presence of metabolic activation. In a V79 mammalian mutagenicity assay, β-sitosterol completely inhibited the induction of oubain-resistance mutants by 2-AA in the presence of hamster hepatocytes but was inactive against MNU-induced mutations. β-sitosterol did not inhibit the binding of benzo[*a*]pyrene (B[*a*]P) to DNA in human bronchial epithelial cells. However, β-Sitosterol did inhibit the induction of transformed Class II and III foci in cultured rat tracheal epithelial cells by B[*a*]P. β-Sitosterol was reported also to inhibit the ability of DMBA to induce micronucleated polychromatic erythrocytes in $B6C3F_1$ mice using the *in vivo* bone marrow micronucleus assay.

β-Sitosterol enhanced the *in vitro* proliferative response of T-cells stimulated by suboptimal concentrations of phytohemagglutinin (PHA). Higher stimulating activity was noted when a ratio (by mass) of 100 β-sitosterol to 1 β-sitosterol glucoside was administered at the same dosage. The mixture also significantly enhanced the expression of CD25 and HLA-Dr activation antigens on T-cells *in vitro*, increased the secretion of IL-2 and γ interferon into the medium, and increased NK-cell activity. When the same mixture was ingested by volunteers for 4 weeks, proliferation of PHA-stimulated T-cells was enhanced. β-Sitosterol demonstrated antiinflammatory and antipyretic effects in rats, but not in mice. In another study using female mice, sitosterol

had a slight, but significant, inhibitory effect on TPA-induced inflammation when applied to the ear 30 minutes before topical application of TPA to the same area.

Cultured PC3 and LNCaP human prostatic cells exposed to saw palmetto extract exhibited a dose-dependent increase in cell mortality. β-Sitosterol was more effective than cholesterol in inhibiting the growth of human prostate cancer cells. *In vitro* exposure of human umbilical vein endothelial cells to sitosterol caused contraction of the endothelial cells and increased the release of intracellular lactate dehydrogenase. β-Sitosterol was highly effective in inhibiting TPA-induced tyrosine kinase activity in HL-60 cells, TPA-induced ornithine decarboxylase (ODC) activity in rat tracheal epithelial cells, and poly(ADP-ribose) polymerase activity in propane sultone-treated primary human fibroblasts. In contrast, β-sitosterol did not induce a reduction of glutathione in Buffalo rat liver cells, or TPA-induced free radical formation in primary human fibroblasts or HL-60 cells.

The ability of sitosterol to lower cholesterol levels was noted in the early 1950s, when sitosterol addition to the diet of cholesterol-fed chickens or rabbits lowered cholesterol levels in both species. Addition of sitosterol to the diet also inhibited atherogenesis in rabbits. β-Sitosterol inhibited cholesterol absorption, decreased liver cholesterol concentration, and decreased the synthesis of bile acids when administered in the diet of mice. Additionally, in a study of rats dosed with cholesterol in the diet, β-sitosterol was effective in lowering liver cholesterol, triglyceride, and fatty acid levels. Human studies have also found sitosterols to be effective in lowering cholesterol levels. In hypercholesterolemia treatment, phytosterol was able to alter lipid metabolism by reducing liver acetyl-CoA carboxylase and malic enzyme activities.

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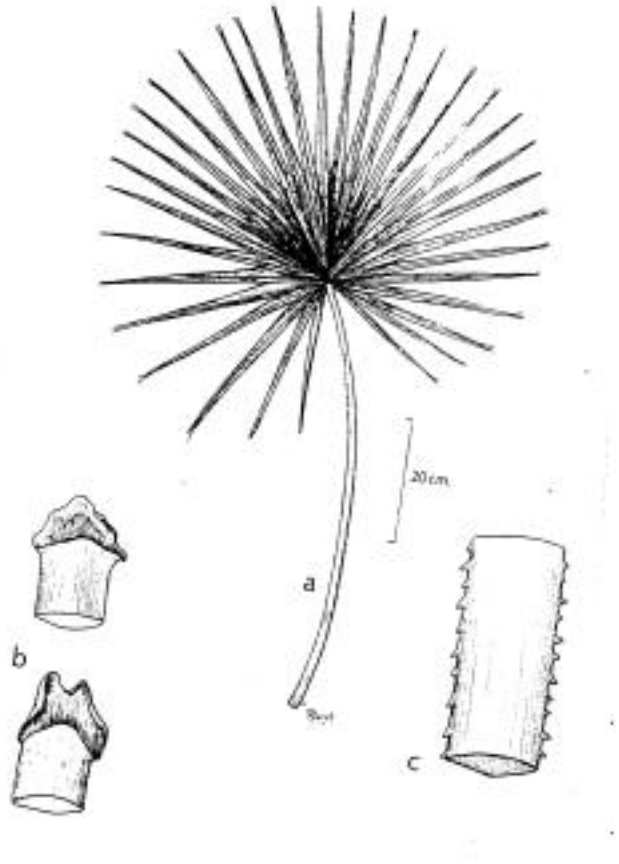
1.0 BASIS FOR NOMINATION

The nomination of saw palmetto and β-sitosterol for testing is based on the potential for human exposure and the limited amount of toxicity and carcinogenicity data.

2.0 INTRODUCTION

Saw Palmetto: a. leaf; b. ligule (adaxial and abaxial views); c. small portion of petiole. Source: Godfrey (1988)



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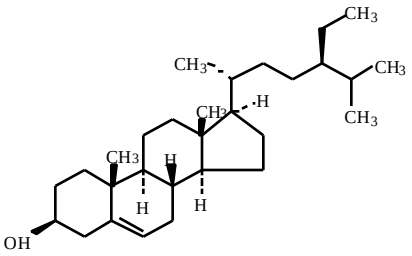

2.1 Chemical Identification

β-Sitosterol ($C_{29}H_{50}O$; mol. wt. = 414.72) is also called:

Stigmast-5-en-3-ol, (3β-) (9CI)
Cinchol
Cupreol
α-Dihydrofucosterol
22:23-Dihydrostigmasterol
22,23-Dihydrostigmasterol
(24*R*)-Ethylcholest-5-en-3β-ol
24β-Ethyl-Δ^5-cholesten-3β-ol
24α-Ethylcholesterol
Harzol
Nimbosterol
α-Phytosterol
Prostasal
Quebrachol
Rhamnol
Sito-Lande
SKF 14463
β-Sitosterin
Sitosterin
Stigmast-5-ene-3-β-ol (French)
(3β)-Stigmast-5-en-3-ol
24*R*-Stigmast-5-en-3β-ol
Δ^5-Stigmasten-3β-ol
Stigmasterol, 22,23-dihydro-

2.2 Physical-Chemical Properties

2.2.1 Saw Palmetto

Saw palmetto (*Serenoa repens*) is a member of the palm family Arecaceae (Godfrey, 1988). It is often creeping and thicket-forming with underground stems. The leaves are up to 3 feet (0.914 m) across, with segments radiating from the ends of the leafstalks (Petrides, 1988). The plant may grow to a height of 20 feet (6.10 m). The berries are fleshy, about 0.75 inch (1.9 cm) in diameter, and blue-black in color.

Saw palmetto berries contain sterols and lipids (Mendosa, 1997), including relatively high concentrations of free and bound sitosterols (Tyler, 1993; cited by

Mendosa, 1997). The following chemicals have been identified in the berries of saw palmetto: anthranilic acid, capric acid, caproic acid, caprylic acid, β-carotene, ferulic acid, mannitol, β-sitosterol, β-sitosterol-*D*-glucoside (Beckstrom-Sternberg and Duke, 1997), linoleic acid, myristic acid, oleic acid, palmitic acid (Wajda-Dubos et al., 1996), 1-monolaurin and 1-monomyristin (Shimada et al., 1997).

2.2.2 Sitosterol

Property	Information	Reference
Sitosterols (mixture of β-sitosterol and other saturated sterols)		
Physical State	White, essentially odorless, tasteless powder	Martin and Cook (1961)
Solubility	Soluble in: chloroform, carbon disulfide Slightly soluble in: alcohol Insoluble in: water	Martin and Cook (1961)
β-Sitosterol		
Solubility	alcohol, ether, acetic acid	HODOC (1997)
Melting Point (^{o}C)	140	HODOC (1997)

2.3 Commercial Availability

Saw palmetto has become the sixth best-selling herbal dietary supplement in the United States (Associated Press, 1997); the standardized extract sold in health food stores comes in 160-mg capsules (Mendosa, 1997). Saw palmetto berry powder is available from the Indiana Botanical Garden, Inc. (Shimada et al., 1997). In Europe, several pharmaceutical companies sell saw palmetto-based over-the-counter (OTC) drugs for the treatment of benign prostatic hyperplasia (BPH). Pierre Fabre, a French pharmaceutical company, markets a BPH drug called Permixon® (Associated Press, 1997) while Therabel Pharma in Belgium markets a similar drug called Prostaserene® (Braeckman, 1994). The following other BPH medications contain saw palmetto extracts as one of their principal ingredients: PA109, Curbicin, Prostagalen, Prostaselect, Prostavigol, and Strogen forte (Dreikorn and Richter, 1989; cited by Lowe and Ku, 1996). Additional pharmaceutical

preparations that contain saw palmetto extract as an ingredient have been patented as hair lotions for the treatment of seborrhea (excessive secretion of the sebaceous glands) (Jeanjean and Navarro, 1995) and hair loss, capsules for the treatment of hair loss (Crandall, 1996), and lotions and ointments for the treatment of acne (Fauran et al., 1996).

β-Sitosterol is available as a cholesterol-lowering drug under the name Cytellin®, manufactured by Eli Lilly and Company (Cohen and Raicht, 1981). β-Sitosterol is the main component of Harzol, an OTC drug for the treatment of BPH (Lowe and Ku, 1996) and is available for purchase on the Internet in Prostate Support Formula, which also contains zinc, copper, pygeum, vitamin B6, pumpkin seed powder, and nettles (Anonymous, 1997d). It is also an ingredient in some contraceptive drugs. Sitosterols (as a group) are available as raw material in 50- and 200-kg fiber drums from Henkel Corporation (Strum, 1997).

3.0 PRODUCTION PROCESSES AND ANALYSES

3.1 Saw Palmetto

An extract of saw palmetto berries can be prepared using hot water (Anonymous, 1931) or the extract may be prepared by supercritical extraction with CO_2 (Braeckman, 1994; Shimada et al., 1997). The lipophilic ingredients may be extracted with lipophilic solvents (hexane or ethanol 90% v/v) (Commission E., 1991). A non-standardized extract is produced by grinding the saw palmetto berries to a raw powder (Shimada et al., 1997).

3.2 β-Sitosterol

Sterol fractions rich in β-sitosterol are isolated from the stillbottoms remaining after distillation of the commercially usable oils from pinewood (tall oil), corn, cottonseed, or soybeans. After saponification, the sterols are enriched by 40-60% by counter-current liquid-liquid extractions using immiscible solvent pairs. β-Sitosterol is brought to a final purity of 85-100% by carbon decolorization and fractional crystallization in organic solvents. β-Sitosterol extracted from soy beans must undergo

further counter-current purification to separate the β-sitosterol from stigmasterol prior to final crystallization (Martin and Cook, 1961).

β-Sitosterol may be extracted from *Anacardium occidentale* by shade drying the tender leaves, coarsely powdering them, and extracting with hexane by cold percolation. Extracts may be concentrated under reduced pressure (Malini and Vanithakumari, 1990).

4.0 PRODUCTION AND IMPORT VOLUMES

Plantation Medicinals (the largest producer in the U.S.) harvests about 5,000 tons of saw palmetto berries per year in Hendry County, Florida (Mendosa, 1997). The second largest producer of the berries is Wilcox Natural Products in Boone, North Carolina. The export of saw palmetto berries from Florida has become a $50 million dollar per year industry, with about 2,000 tons of the berries exported to Europe each year (total production volumes in Florida were not provided) (Associated Press, 1997). Information on import volumes was not found.

Production and import volumes were not found for sitosterol.

5.0 USES

5.1 Saw Palmetto

Historically, American Indians used the berries for food. Since 1994 when federal dietary supplement laws were relaxed, the most common use of the berries by Americans is as an herbal health remedy (Associated Press, 1997). Estrogenic, antiestrogenic, and antiandrogenic pharmacological properties are discussed in **Section 9.2** of this report.

The berries have been used for treating stomach ache, bronchitis, diabetes, cancer, and cystitis; they have also been used as a diuretic, aphrodisiac, and for breast enlargement (Croom and Walker, 1995).

Saw palmetto berries are claimed to relieve irritated throat and symptoms of the common cold. The recommended preparation involves steeping a teaspoon of berries in a cup of boiling water and cooling. Drinking one or two cups a day is recommended

(Anonymous, 1931).

The dried berry has been used as a menstrual drug product (Novitch and Schweiker, 1982). Preparations including saw palmetto extract are claimed to be effective in treating seborrhea (excessive secretion of the sebaceous glands) (Jeanjean and Navarro, 1995), acne (Fauran et al., 1983), and hair loss (Crandall, 1996).

Saw palmetto extracts have been reported to be effective in the treatment of mild to moderate BPH (Braeckman, 1994; Bracher, 1997), producing an effective response in 30 to 45 days compared to the 6 to 12 months required for most other BPH drugs (Braeckman, 1994). However, significant inhibition of prostate growth has not been demonstrated (Bracher, 1997), and the German Federal Health Agency requires saw palmetto labels to state that "This medication relieves only the difficulties [pain and frequent urination] associated with an enlarged prostate without reducing the enlargement" (Mendosa, 1997). A recent critical analysis of data on the effects of phytotherapy (including saw palmetto extracts) in BPH treatment suggested that the effects were no better than placebo treatment (Dreikorn and Schonhofer, 1995).

5.2 β-Sitosterol

β-Sitosterol is claimed to have the following pharmacological properties: androgenic, anorexic, antiadenomic, antiandrogenic, antiestrogenic, antifeedant, antifertility, antigonadotropic, antiinflammatory, antileukemic, antimutagenic, antiophidic (inhibits effect of snake bite), antiprogestational, antiprostatadenomic, antiprostatitic, antitumor, cancer preventative, candidicide, estrogenic, gonadotropic hepatoprotective, hypocholesterolemic, hypoglycemic, hypolipidemic, pesticide, spermicide, viricide (Beckstrom-Sternberg and Duke, 1997), antibacterial, and antifungal (Padmaja et al., 1993; cited by Ling and Jones, 1995). Pharmaceutical preparations claim that β-sitosterol is effective in treating diabetic male sexual dysfunction (Shlyankevich, 1995). Estrogenic, antiestrogenic, and antiandrogenic effects of β-sitosterol are discussed in **Section 9.2** of

this report. Studies on the hypocholesterolemic action are presented in **Section 9.8.2**, and the analgesic and antiinflammatory properties are presented in **Sections 9.8.4** and **9.8.5**, respectively.

β-Sitosterol is used in the treatment of prostatic adenoma (Budavari, 1996) and BPH (Berges et al., 1995). Advertisements for β-sitosterol-containing products claim that treatment with β-sitosterol (60 mg daily) for 6 months results in a 53% increase in urine flow rate (Anonymous, 1997d).

A study using mice indicated that β-sitosterol may be effective in the treatment of vitiligo (an autoimmune condition characterized by destruction of melanocytes, also called leukoderma) (Lee et al., 1994).

β-Sitosterol was not effective in the treatment of pulmonary tuberculosis (Donald et al., 1996), and it exhibited low potency when tested for use as an antiacne agent (Kubo et al., 1994).

In addition to its medicinal uses, sitosterol is used in German cosmetic products (Schrader, 1983) and was effective as a nonabsorbable indicator for cholesterol absorption (Terry et al., 1995).

6.0 ENVIRONMENTAL OCCURRENCE AND PERSISTENCE

Saw palmetto is a scrubby palm tree native to the West Indies and the Atlantic Coast of North America, from South Carolina to Florida (Murray, 1994; cited by Mendosa, 1997).

Table 1 shows common plants containing β-sitosterol and the respective concentrations found in each (Beckstrom-Sternberg and Duke, 1997).

Table 1. Plants containing β-sitosterol

Common Name	Scientific Name	Plant Part	Concentration	Reference
Cherimoya	*Annona cherimola*	Seed	10,000-14,000 ppm	Beckstrom-Sternberg & Duke (1997)
Hawthorn	*Crataegus laevigata*	Flower	6,500-7,800 ppm	
		Leaf	5,100-6,200 ppm	
Black Cumin	*Nigella sativa*	Seed	3,218 ppm	
Evening-Primrose	*Oenothera biennis*	Seed	1,186-2,528 ppm	
Sage	*Salvia officinalis*	Leaf	5-2,450 ppm	
		Stem	1,214 ppm	
Sang-Pai-Pi	*Morus alba*	Leaf	2,000 ppm	
Sicklepod	*Senna obtusifolia*	Seed	1,000-2,000 ppm	
Buckwheat	*Fagopyrum esculentum*	Seed	1,880 ppm	
Basil	*Ocimum basilicum*	Leaf	896-1,705 ppm	
		Flower	1,051 ppm	
		Root	408 ppm	
		Sprout Seedling	230 ppm	
		Stem	230 ppm	
Corn	*Zea mays*	Kernels	1,300 ppm	
Sallow Thorn	*Hippophae rhamnoides*	Seed	550-970 ppm	
Soybean	*Glycine max*	Seed	900 ppm	Beckstrom-Sternberg and Duke (1997, cont.)
Licorice	*Glycyrrhiza glabra*	Root	500 ppm	
Common Violet	*Viola odorata*	Plant	330 ppm	
Ashwagandha	*Withania somniferum*	Root	200 ppm	
Saw Palmetto	*Serenoa repens*	Fruit	189 ppm	
Giant Cordgrass	*Spartina cynosuroides*	Tops	110 mg	Miles et al. (1983)
Tobacco	*Nicotiana* sp.	Leaves	n.p.	Holden et al. (1988)
Cashew	*Anacardium occidentale*	Leaves	n.p.	Malini and Vanithakumari (1990)
Opium Poppy	*Papaver somniferum*	n.p.	n.p.	Malaveille et al. (1982)
Cotton	*Gossypium* sp.	Bracts	n.p.	Gilbert et al. (1979)
n.p.	*Phyllanthus corcovadensis*	Leaves	n.p.	Santos et al. (1995)
		Stems		
		Roots		
Prickly Lettuce	*Lactuca sativa*	Seed Oil	n.p.	Said et al. (1996)
Savoy Chieftain Cabbage	*Brassica oleracea*	Leaves	n.p.	Lawson et al. (1989)
Chick Pea Plant	*Cicer arietinum*	Pea	n.p.	Gattuso et al. (1988)

Abbreviations: n.p. = not provided

High concentrations of β-sitosterol are found in the effluent of pulp mills. These effluents are released into streams and lakes (Anonymous, 1995; Cooper and Kavlock, 1997). In a characterization of plant sterols released from U.S. pulp and paper mills, the discharge rate of β-sitosterol was generally the highest of the investigated sterols — campesterol, stigmasterol, β-sitosterol, and stigmastanol (Cook et al., 1997). The lowest β-sitosterol discharge rate was found in a plant using recycled fibers (100 mg β-sitosterol/ton or 0.24 mmol/ton of pulp produced) and the highest discharge rate was found in a plant using the kraft/thermo-mechanical/groundwood pulping process (20,300 mg β-sitosterol/ton or 48.9 mmol/ton of pulp produced).

Sitosterols have also been identified in the raw effluent of municipal wastewater treatment plants (Nguyen et al., 1994; Quéméneur and Marty, 1994; Garric et al., 1996; Marty et al., 1996; Stumpf et al, 1996), but were not detected in tap water (Stumpf et al., 1996). Sitosterols are excreted in the feces of humans, pigs, cows, horses, sheep, possums, cats, dogs, hens, seagulls, ducks, magpies, rosellas, and swans (Leeming et al., 1996).

β-Sitosterol was identified as a component of soybean dust originating during harbor activities in Barcelona, Spain (Aceves et al., 1991).

7.0 HUMAN EXPOSURE

Human exposure to saw palmetto occurs when the extract is taken for medicinal purposes: orally as a capsule (Mendosa, 1997) or as a tea (Anonymous, 1931) or topically as a hair lotion (Jeanjean and Navarro,1995) or an acne lotion/ointment (Fauran et al.,1983).

β-Sitosterol is taken orally for medicinal purposes (Anonymous, 1997d). The largest human dietary intake of sitosterol occurs from consuming corn, bean, and plant oils. Vegetarian diets contain higher amounts of sitosterol than traditional Western meat-eating diets (Ling and Jones, 1995), and the most commonly ingested phytosterol is β-sitosterol (Jones et al., 1997). Common sources of β-sitosterol are in the following plant

constituents or oils: wheat germ oil, corn oil, rye germ oil, cottonseed oil, soy and calabar beans, rice embryos (Budavari, 1996), soybean oil, peanut oil (Thorpe, 1972), olive oil (Huang et al., 1991), navy beans, dark red kidney beans, pinto beans, and black turtle soup beans (Drumm et al., 1990). β-Sitosterol is also present in fats, with smaller contributions to the diet identified in nuts, cereals, bread, preserves, vegetables including potatoes, red meat products, fish, dairy products, eggs, poultry, margarine (Pyle et al., 1976), fruits (Oka et al., 1973), and beverages (Morton et al., 1995) including coffee (Turchetto et al., 1993) and tea (Oka et al., 1973).

β-Sitosterol has been identified in eight species of shellfish marketed for consumption in the northwestern states: Manila clam, blue mussel, Pacific oyster, sea and bay scallops, California squid, Pandalus pink shrimp, and Dungeness crab (King et al., 1990). β-Sitosterol has also been identified in mackerel, rainbow trout, smelt, sardines, and chimaeras (Takagi et al., 1979).

β-Sitosterol is a component of tobacco and has been confirmed as a component of tobacco smoke (Holden et al., 1988; Eatough et al., 1989). It has also been identified in opium (Malaveille et al., 1982), bourbon (Gaveler et al., 1987; Rosenblum et al., 1991, 1993), and whiskey (type not specified) (Grayson, 1985). Its presence in whiskey (type not specified) could be caused by extraction from the wood barrels during aging (Grayson, 1985).

8.0 REGULATORY STATUS

Under 21 CFR Part 310 (Federal Register, 1993), the Food and Drug Administration (FDA) issued a final rule under the Federal Food, Drug, and Cosmetic Act (the Act), effective November 10, 1993, that certain active ingredients in OTC products are not generally recognized as safe and effective or are misbranded. Among these, saw palmetto is not recognized as safe and effective and is misbranded when labeled as an OTC drug for use as an orally administered menstrual drug product. A previous version of the Act stated that saw palmetto extract may not be sold or labeled as therapeutic

support for the prostate gland or reproductive organs (FDA, 1991).

Saw palmetto for use as an herbal health remedy is also regulated under the Dietary Supplement Health and Education Act of 1994 (DSHEA) (Anonymous, 1997b). Under this Act, a dietary supplement is defined as a "product intended to supplement the diet that contains one or more of the following ingredients—a vitamin, mineral, herb or other botanical, an amino acid, or a concentrate, metabolite, constituent, extract, or combination of any of these ingredients." Supplements must be provided in dosage form and may not be regulated as food additives.

9.0 TOXICOLOGICAL DATA

Summary: In rare cases, the consumption of saw palmetto berries may cause stomach problems, while large amounts might cause diarrhea. Only minor side effects were reported in studies of BPH patients taking an oral dose of 160 mg saw palmetto extract twice daily for three months: half of the side-effect symptoms were gastrointestinal. When phytosterols (including β-sitosterol) were taken orally to lower plasma cholesterol levels, no obvious side effects were noted.

No data were found relating to chemical disposition of β-sitosterol. In animals (including humans), sitosterol is derived exclusively from dietary intake. Sitosterol is absorbed in the intestine; humans usually absorb less than 5% of phytosterols (including sitosterol), so that about 95% of dietary phytosterols enter the colon. Absorption of phytosterols appears to be greater during infancy and childhood than during adulthood. β-Sitosterol absorption in the rat involves the sitosterol partitioning between an oil and a micellular phase within the intestine, followed by uptake of sitosterol by mucous membranes, and then esterification within the mucosal cells. Absorption of phytosterols in the intestine is selective and appears to decrease with increasing number of carbons in the sterol side chain. In an inhalation experiment with male Sprague-Dawley rats, 78% of radiolabeled β-sitosterol administered as a component of cigarette smoke was taken up. Most of the β-sitosterol was found in the distal air spaces and parenchyma of the lung, with a smaller amount being found in the trachea.

When a saw palmetto extract containing ^{14}C-labeled oleic or lauric acid or β-sitosterol was fed to rats, uptake of the radioactive label was much higher in the prostate gland than in the liver or other genitourinary tissues (e.g., seminal vesicles). The amount of phytosterols in the serum is generally low even with high dietary intake, but plasma levels of sitosterol have been shown to increase up to twice the normal levels with dietary supplementation. In humans with an average diet, plasma levels ranged from 0.00166 to 0.010 mg/mL (0.000004 to

0.000024 mmol/mL). Following inhalation of tobacco smoke by rats, radiolabeled β-sitosterol was slowly released by the lungs to plasma; it was immediately found in the plasma, peaked on day 2, and declined slowly (but was not totally eliminated) over the next 30 days. From the plasma, β-sitosterol was distributed to the liver, kidney, stomach, spleen, and esophagus, with a peak absorption at 5 to 8 days. Levels of β-sitosterol slowly declined in all the sampled organs except for the esophagus, in which β-sitosterol was reduced to negligible quantities after 15 days. Peak amounts of β-sitosterol were found in the liver and spleen.

Insects and prawns can transform phytosterols to cholesterols, which are then synthesized into steroid hormones or bile acids. However, vertebrate species lack this ability. In rat bile, β-sitosterol is converted to polar compounds (di- and tri-hydroxylated C_{21}-bile acids). In rat liver mitochondria, β-sitosterol is oxidized into 26-hydroxy-β-sitosterol and 29-hydroxy-β-sitosterol metabolites. In the rat testes, β-sitosterol is directly converted by mitochondrial enzymes to the steroid hormones progesterone, pregnenolone, testosterone plus 17α-progesterone, and polar steroids. Theoretically, the presence of an ethyl group at C_{24} should prevent or obstruct conversion of sitosterol into bile acids just as it does for the conversion of cholesterol into C_{24}-bile acids. Experiments with rats, monkeys, and humans have found an apparent lack of conversion of sitosterol into C_{24}-bile acids in accordance with the theory. Conflicting data, however, have been published.

Phytosterols are excreted in the bile and their elimination appears to be faster than that for cholesterol. The pharmacokinetics of β-sitosterol administration via i.v. and oral routes in the beagle dog were best described by the two-compartment model; distribution half-life was 3 hours and the terminal distribution half-life was 129 hours. Absolute bioavailability upon oral administration was 9%.

In contrast to healthy humans, individuals with sitosterolemia (a rare inherited lipid storage disease) have a very different pattern of sitosterol metabolism. Sistosterolemic individuals have increased intestinal absorption of the compound, loss of tissue sterol structural recognition, expanded pools, and hepatic retention.

Acute toxicity data for saw palmetto extract were not found; the acute toxicity for β-sitosterol administered i.p. to mice is >3000 mg/kg (>7.23 mmol/kg).

Short term (60 days) s.c. exposure of male and female albino Wistar rats to β-sitosterol at 2 mL/kg/day (0.0048 mmol/kg/day) did not produce gross or microscopic lesions either in the liver or the kidney. All clinical biochemical parameters were in the normal range except for serum protein and serum cholesterol; serum cholesterol was markedly depleted in both sexes in a dose-dependent manner. Male Fischer CD rats fed 0.2% β-sitosterol in the diet for 28 weeks experienced no adverse effects.

No chronic exposure data were found.

Saw palmetto berry extract may exhibit an antiestrogenic effect, as well as it may block progesterone and androgenic receptors. In BPH patients, using saw

palmetto extract plus cyproterone acetate (CPA) as treatment, a significant reduction of prostate volume was identified with use of the combination treatment as compared with treatment using each of the drugs alone. Antiestrogenic activity of saw palmetto extract was noted in treated BPH patients. This activity, in addition to an antiandrogenic action, may occur by competitively blocking translocation of cytosolic estrogen receptors to the nucleus. Saw palmetto extracts, including β-sitosterol, exhibited estrogenic effects when injected into immature female mice. When inbred female albino rats were administered 1.5 mg/kg/day (0.00362 mmol/kg/day) β-sitosterol s.c. for 30 days, the estrus cycle was disrupted in 60% of the animals At a dose of 2.5 mg/100 g/day (0.00603 mmol/kg/day), the incidence of persistent estrus was prolonged as long as treatment continued (30 days), and a marked increase in ovarian, uterine, and pituitary weights was induced. In adult male albino Wistar rats, a low dose (0.5 mg/kg/day; 0.00121 mmol/kg/day) β-sitosterol significantly decreased sperm concentrations after 48 days of treatment and decreased testicular weight after 32 and 48 days of treatment, respectively. At the high dose (5 mg/kg/day; 0.0121 mmol/kg/day), fertility was reduced after 42 and 48 days of exposure, sperm concentrations were reduced after 16, 32, and 48 days of exposure, and testicular weight was significantly decreased in a time-dependent manner. Withdrawal from treatment for 30 days did not restore sperm concentration or testicular weight.

In ovariectomized albino Wistar rats, β-sitosterol (0.5, 2.5, or 5.0 mg/kg/d; 0.00121, 0.00603, or 0.0121 mmol/kg/d) s.c. for 10 days caused a significant dose-dependent increase in glycogen and total lactate dehydrogenase concentrations, significant increases in glucose-6-phosphate dehydrogenase and phosphohexose isomerase, and a significant dose-dependent increase in uterine weight. β-Sitosterol at doses of 0.003 and 0.030 mg (0.00000723 and 0.0000723 mmol) exhibited an estrogenic response when injected into neonatal male and female rats: postpubertal pituitary response to GnRH was altered in females, and basal luteinizing hormone secretion was altered in both males and females. These doses also altered basal luteinizing hormone secretion in immature male and female rats and postpubertal pituitary response to GnRH in female rats. Very high doses of β-sitosterol administered s.c. induced irregularity in spermiogenesis in immature rabbits. Ovarian weight was reduced when β-sitosterol was administered s.c. to 25-week-old female lambs at doses of 0.5 to 20.0 mg/d (0.00121 to 0.0482 mmol/d) for 2, 4, or 8 weeks. With increasing doses, β-sitosterol inhibited follicular growth and distribution did not extend past the 6 and 7 granulosal layers.

Fish chronically exposed to kraft pulp mill effluent exhibited a range of reproductive responses: female mosquitofish (*Gambusia affinis*) expressed male anatomical and behavioral characteristics, including a modified anal fin resembling a gonopodium and reproductive behaviors such as mating attempts. White sucker fish (*Catostomus commersoni*) and lake whitefish (*Coregonus clupeaformis*) had lower serum 17β-estradiol, testosterone, 17α,20β-

dihydroprogesterone, and 11-ketotestosterone levels compared to fish from a reference site. Laboratory exposure of rainbow trout (*Oncorhynchus mykiss*) reduced plasma testosterone levels by approximately 50%. β-Sitosterol, found in high concentrations in the effluent, is believed to be responsible for the toxicological effects. In the presence of bacteria, β-sitosterol degrades into androgens thought to be responsible for the masculinizing effects on female fish. Furthermore, the fish downstream of the mills reach maturation several years later than expected. In a laboratory experiment on goldfish (presumably *Carassius auratus*) injected with β-sitosterol, the same reduction in gonadal weight and hormone levels was observed.

Saw palmetto is known to exhibit an antiandrogenic action, although the compound responsible for this action has not been identified. The effects are thought to be caused by a direct action on the androgen receptor, the inhibition of the enzyme testosterone-5-α-reductase and/or competitive inhibition of dihydrotestosterone (DHT) binding to both cytosolic and nuclear receptors. However, studies found that saw palmetto berry extract did not demonstrate any inhibition of DHT binding or inhibition of 5-α-reductase activity. The extract inhibited the formation of all the testosterone metabolites studied (DHT; androst-4-ene-3,17-dione; and 5α-androstane-3,17-dione) in both epithelial and fibroblast cells from BPH and prostate cancer tissues. Saw palmetto extract markedly inhibited both isoforms of human 5-α-reductase in the baculovirus-directed insect cell expression system, but the inhibition was noncompetitive. It inhibited DHT and testosterone binding in 11 different human tissue specimens. In humans, the antiandrogenic effect is achieved without significantly influencing systemic hormone levels, including testosterone, follicle-stimulating hormone, and luteinizing hormone.

Bourbon concentrate (containing β-sitosterol) induced an estrogenic response (decreased luteinizing hormone (LH) levels and increased sex hormone binding of globulin and HDL cholesterol) in normal post-menopausal women.

No carcinogenicity studies were located for saw palmetto extract or β-sitosterol. However, several anticarcinogenicity studies with β-sitosterol were conducted; in none of these studies was an increased incidence of tumors due to treatment with β-sitosterol reported. In a two-stage skin carcinogenesis study, female ICR mice initiated with a single topical application of DMBA followed by a twice weekly treatment for 18 weeks with the tumor promoter TPA exhibited a lower incidence of tumors and a lower tumor multiplicity when topically treated with β-sitosterol (0.005 mmol) 30-40 minutes before each TPA treatment. In another initiation-promotion study, β-sitosterol was an effective inhibitor of the initiation of mammary lesions induced in rats by DMBA plus TPA.

β-Sitosterol also significantly reduced the incidence of colon tumors (predominantly adenomatous polyps) induced in male Fischer CD rats by MNU. The anticarcinogenicity of β-sitosterol was related to its ability to decrease MNU-induced colonic epithelial cell proliferation. However, in a study using outbred

male Sprague-Dawley rats, β-sitosterol supplemented in the diet (2000 mg/kg; 4.82 mmol/kg) did not significantly inhibit the number of AOM-induced tumors per rat. When β-sitosterol (at the same dose) was given in combination with 13-*cis*-retinoic acid and selenous acid, the number of AOM-induced tumors per animal were significantly decreased.

Only limited genotoxicity data on β-sitosterol were available. β-Sitosterol, at 1000 μM, was negative for the induction of strand breaks in λDNA, and was not mutagenic at concentrations up to 600 μL/plate (1.4 μmol/plate) in *Salmonella typhimurium* strain TA98 with metabolic activation or in TA100 without metabolic activation. Autoxidized β-sitosterol was not mutagenic when tested at doses up to 5000 μg/plate in the absence of metabolic activation only in *S. typhimurium* strains TA98, TA100, TA1535, and TA1538. A pyrolysis product of β-sitosterol (prepared at 450°C) was not mutagenic when tested up to 1000 μg/plate in the presence or absence of metabolic activation in *S. typhimurium* strains TA98 and TA100. However, in another study, a pyrolysate of β-sitosterol (formed at 700°C) was mutagenic when tested at 400 μg/plate to *S. typhimurium* strains TA97, TA98, and TA100 in the presence and absence of metabolic activation. The pyrolyzate product was more mutagenic in strain TA97 than strains TA98 or TA100.

Several studies have been conducted to evaluate the antigenotoxicity of β-sitosterol. β-Sitosterol at 1000 μM did not inhibit the ability of ascorbic acid (250 μM) to induce strand breaks in λDNA. In *S. typhimurium*, β-sitosterol at concentrations up to 600 μL/plate (1.4 μmol/plate) inhibited in a dose-dependent manner the mutagenic activity of N-methyl-N-nitrosourea (MNU) in TA100 in the absence of metabolic activation, and of 2-aminoanthracene (2-AA) in TA98 in the presence of metabolic activation. In contrast, β-sitosterol (0.01 to 1000 μg/plate; 0.000024-2.4 μmol/plate) did not suppress the mutagenicity of 0.1 nM Trp-P-2 in *S. typhimurium* strain TA98 in the presence of metabolic activity. In a V79 mammalian mutagenicity assay, β-sitosterol at 50 and 250 μg/mL (0.12 and 0.60 μM) completely inhibited the induction of oubain-resistance mutants by 2-AA at 25 mg/mL in the presence of hamster hepatocytes but was inactive against MNU (50 μg/mL)-induced mutations. β-Sitosterol did not inhibit the binding of B[*a*]P to DNA in human bronchial epithelial cells. However, β-sitosterol at 2.41 μM inhibited by 43% the induction of transformed Class II and III foci in cultured rat tracheal epithelial cells by B[*a*]P. β-Sitosterol was reported also to inhibit (by 60%) the ability of DMBA to induce micronucleated polychromatic erythrocytes in $B6C3F_1$ mice using the *in vivo* bone marrow micronucleus assay.

Soybean dust (which contained β-sitosterol) originating from harbor activities in Barcelona, Spain, was concluded to have contributed to asthma outbreaks in the city. β-Sitosterol enhanced the *in vitro* proliferative response of T-cells stimulated by suboptimal concentration of PHA. Higher stimulating activity was noted when a ratio (by mass) of 100 β-sitosterol to 1 β-sitosterol glucoside was administered at the same dosage. One microgram per milliliter of 100:1 β-sitosterol/β-sitosterol glucoside also significantly enhanced the expression

of CD25 and HLA-Dr activation antigens on T-cells *in vitro*, increased the secretion of IL-2 and γ interferon into the medium, and increased NK-cell activity. When the same 100:1 ratio was ingested by volunteers for 4 weeks, proliferation of PHA-stimulated T-cells was enhanced. β-Sitosterol demonstrated antiinflammatory and antipyretic effects in rats, but not in mice. In another study using female ICR mice, sitosterol had a slight, but significant, inhibitory effect on TPA-induced inflammation when applied to the ear 30 minutes before topical application of TPA to the same area.

Cultured PC3 and LNCaP human prostatic cells exposed to saw palmetto extract exhibited an increase in cell mortality. *In vitro* exposure of human umbilical vein endothelial cells to 700 μM sitosterol for 72 hours caused contraction of the endothelial cells and increased the release of intracellular lactate dehydrogenase. β-Sitosterol was highly effective in inhibiting TPA-induced tyrosine kinase activity in HL-60 cells, TPA-induced ornithine decarboxylase (ODC) activity in rat tracheal epithelial cells, and poly(ADP-ribose) polymerase activity in propane sultone-treated primary human fibroblasts. In contrast, β-sitosterol did not induce a reduction of glutathione in Buffalo rat liver cells, or TPA-induced free radical formation in primary human fibroblasts or HL-60 cells.

The ability of sitosterol to lower cholesterol levels was noted in the early 1950s, when sitosterol addition to the diet of cholesterol-fed chickens or rabbits lowered cholesterol levels in both species. Addition of sitosterol to the diet also inhibited atherogenesis in rabbits. In a study of laying hens, a diet including 4% plant sterols reduced cholesterol absorption by 40%. β-Sitosterol inhibited cholesterol absorption, decreased liver cholesterol concentration, and decreased the synthesis of bile acids when administered at 1% in the diet of mice. Additionally, in a study of rats dosed with 3% cholesterol in the diet, β-sitosterol was effective in lowering liver cholesterol, triglyceride, and fatty acid levels. Human studies have also found sitosterols to be effective in lowering cholesterol levels: a dose of 6000 mg (14.5 mmol) β-sitosterol per day (route not specified) decreased cholesterol levels by 9% and 722 mg/day (route not specified) decreased cholesterol levels by 11%. Children treated with 6000 mg/day (14.5 mmol/day) β-sitosterol for 3 months experienced a 17% reduction in total cholesterol, a 19.5% reduction in low-density lipoprotein (LDL) cholesterol, and no change in high-density lipoprotein (HDL) cholesterol levels. Men with myocardial infarction were pretreated with varying amounts of fat and cholesterol for 6 to 12 weeks and were then given sitosterol at doses of 12,000 to 18,000 mg/day for 12 to 24 weeks. A 17% reduction in total cholesterol levels was noted. Also, 2000 mg sitosterol per day effectively reduced LDL cholesterol by 20% when used as treatment for familial hypercholesterolemia. Familial-type hypercholesterolemic children had a 6% reduction in total cholesterol, a 7% LDL cholesterol reduction, a 15% HDL cholesterol reduction, and a 23% increase in triglycerides when treated with 12,000 mg/day (28.9 mmol/day) β-sitosterol for 3 months. In hypercholesterolemia

treatment, phytosterol was able to alter lipid metabolism by reducing liver acetyl-CoA carboxylase and malic enzyme activities. Treatment of hypercholesterolemia with a combination of β-sitosterol and lovastatin was found to be significantly more effective in decreasing LDL cholesterol than treatment with lovastatin alone. The mode of action is thought to involve inhibition of cholesterol absorption, even though plant sterols are very poorly absorbed. Ingestion of 1000 mg of β-sitosterol reduced absorption of a 500 mg cholesterol-containing meal by 42%. The mechanism is thought to involve crystallization and co-precipitation of cholesterol.

β-Sitosterol (3-100 mg/kg; 0.00723-0.241 mmol/kg) administered i.p. to mice caused a dose-dependent inhibition of acetic acid-induced abdominal constriction; the ID_{50} was 9 mg/kg (0.0217 mmol/kg). β-Sitosterol was equipotent with aspirin in its analgesic effects.

β-Sitosterol was more effective than cholesterol in inhibiting the growth of human prostate cancer cells.

9.1 General Toxicology

9.1.1 Human Data

In rare cases, the consumption of saw palmetto berries may cause stomach problems (Commission E, 1991). Large amounts (not specified) might cause diarrhea (Spoerke, 1980; cited by Mendosa, 1997).

In a study of 305 BPH patients taking an oral dose of 160 mg saw palmetto extract twice daily for three months, 25 patients (5%) reported minor side effects: half of the side-effect symptoms were gastrointestinal (i.e., gastralgia, nausea, vomiting, constipation, and diarrhea). Other minor side effects included dizziness, insomnia, fatigue, muscular pain, tachycardia, angina pectoris, extrasystole, angiopathy, breathlessness, urinary infection, dry mouth, testicular pain, and vesicle tenesmus (Braekman, 1994).

In another study of 110 BPH patients (55 receiving saw palmetto extracts), fewer patients reported side effects when treated orally with saw palmetto extracts (160 mg twice daily) than from the placebo treatment (control) (Champault et al., 1984). The reported side effects (e.g., headaches) were minor.

When phytosterols (including β-sitosterol) were taken orally to lower plasma cholesterol levels, no obvious side effects were noted either by the subject (Farquhar et

al., 1956; Heinemann et al., 1986; Miettinen et al., 1995; all cited by Jones et al., 1997) or by physician examination (Becker et al., 1992, 1993; both cited by Jones et al., 1997). Blood parameters remained within normal ranges (Becker et al., 1992, 1993; both cited by Jones et al., 1997). Subjects consuming up to 18,000 mg/day of phytosterols derived from soy oil or tall oil, include β-sitosterol, for 3 years had almost no side effects; a few subjects reported constipation (Lees et al., 1977; cited by Jones et al., 1997).

9.1.2 Chemical Disposition, Metabolism, and Toxicokinetics

9.1.2.1 Chemical Disposition

No data were found relating to chemical disposition of β-sitosterol.

9.1.2.2 Absorption

In animals (including humans), sitosterol is derived exclusively from dietary intake. Sitosterol is absorbed in the intestine, although cholesterol is preferentially absorbed over sitosterol in mammalian systems. Humans usually absorb less than 5% of phytosterols (including sitosterol) (Salen et al., 1989; cited by Ling and Jones, 1995; Cayen, 1980; Miettinen et al., 1990), so that about 95% of dietary phytosterols enter the colon (Salen et al., 1989; Miettinen et al., 1990; Salen et al., 1970; all cited by Ling and Jones, 1995).

Absorption of phytosterols appears to be greater during infancy and childhood than during adulthood, as noted by a 5- to 15-fold increase in plasma phytosterols in infants fed a phytosterol-rich infant formula compared to adults (adult diet not specified) (Mellies et al., 1976; cited by Finocchiaro and Richardson, 1983). Infants fed a vegetable oil-based diet accumulated plant sterols in aortic tissues.

A study found that β-sitosterol absorption in the rat involves the sitosterol partitioning between an oil and a micellular phase within the intestine (Borgstrom, 1976; cited by Bhattacharyya, 1981). The next step involves the uptake of sitosterol by mucous membranes, followed by esterification within the mucosal cells (Bhattacharyya,

1981). Bhattacharyya (1981) further hypothesized that the last step involves incorporation and transport by chylomicrons.

Absorption of phytosterols in the intestine is selective and appears to decrease with increasing number of carbons in the sterol side chain. Variations in side chains also exert a differential in absorption (Child and Kuksis, 1983; cited by Ling and Jones, 1995; Bhattacharyya, 1981). β-Sitosterol is moderately absorbed in animals, compared with campesterol (Ikeda et al, 1988; cited by Ling and Jones, 1995; Bhattacharyya, 1981) and stigmasterol (Sylven, 1970; cited by Ling and Jones, 1995; Bhattacharyya, 1981). The 5α saturated derivative of β-sitosterol (sitostanol) is not absorbed at all (Heinemann et al., 1986; Vanhanen and Miettinen, 1992; both cited by Ling and Jones, 1995).

In an inhalation experiment with male Sprague-Dawley rats, radiolabeled β-sitosterol was administered as a component of cigarette smoke (Holden et al., 1988). Seventy-eight percent of the β-[4-^{14}C]sitosterol dose was taken up by the rats. Most of the β-sitosterol was found in the distal air spaces and parenchyma of the lung, with a smaller amount being found in the trachea.

9.1.2.3 Distribution

To investigate the distribution of saw palmetto extract, an extract containing ^{14}C-labeled oleic or lauric acid or β-sitosterol was fed to rats (Plosker and Brogden, 1996). Uptake of the radioactive label was much higher in the prostate gland than in the liver or other genitourinary tissues (e.g., seminal vesicles).

The amount of phytosterols in the serum is generally low even with high dietary intake, but plasma levels of sitosterol have been shown to increase up to twice the normal levels with dietary supplementation (Salen et al., 1970; cited by Ling and Jones, 1995). In humans with an average diet (not specified), plasma levels ranged from 0.003 to 0.010 mg/mL (0.0000072 to 0.000024 mmol/mL) (Cayen, 1980). In another study, healthy humans were found to have plasma levels of β-sitosterol between 0.00166 and 0.00332 mg/mL (0.000004 and 0.000008 mmol/mL) (Jones et al., 1997).

In a study of rats fed a purified diet containing phytosterols (including β-sitosterol) for 3 weeks, a 5-fold increase in plasma phytosterol levels was detected (Garcia et al., 1997).

Following inhalation of tobacco smoke by rats, radiolabeled β-sitosterol was slowly released by the lungs to plasma (Holden et al., 1988). β-Sitosterol was immediately found in the plasma, peaked on day 2, and declined slowly (but was not totally eliminated) over the next 30 days. From the plasma, β-sitosterol was distributed to the liver, kidney, stomach, spleen, and esophagus, with a peak absorption at five to eight days. Other organs were not sampled. Levels of β-sitosterol slowly declined in all the sampled organs except for the esophagus, in which β-sitosterol was reduced to negligible quantities after 15 days. Peak amounts of β-sitosterol were found in the liver and spleen.

9.1.2.4 Metabolism

Insects and prawns can transform phytosterols to cholesterols, which are then synthesized into steroid hormones or bile acids (Pollak and Kritchevsky, 1981; Svoboda et al., 1967; Douglass et al, 1981; all cited by Ling and Jones, 1995). However, an ability to transform phytosterols to cholesterols has not been shown in vertebrate species (Ling and Jones, 1995). β-Sitosterol is converted to polar compounds (di- and tri-hydroxylated C_{21}-bile acids) in the bile acid fraction of rat bile (Subbiah and Kuksis, 1973; Skrede et al., 1985; Muri-Boberg et al., 1991; Lund et al., 1991; all cited by Ling and Jones, 1995; Boberg et al., 1990a). In rat liver mitochondria, β-sitosterol is oxidized into 26-hydroxy-β-sitosterol and 29-hydroxy-β-sitosterol metabolites (Aringer et al., 1976). In the rat testes, β-sitosterol is directly converted by mitochondrial enzymes to the steroid hormones progesterone, pregnenolone, testosterone plus 17α-progesterone, and polar steroids (Subbiah and Kuksis, 1975).

Theoretically, the presence of an ethyl group at C_{24} should prevent or obstruct conversion of sitosterol into bile acids just as it does for the conversion of cholesterol into

C_{24}-bile acids (Boberg et al, 1990b). Experiments with rats (Subbiah and Kuksis, 1973; cited by Boberg et al., 1990b), monkeys (Kritchevsky et al, 1981; cited by Boberg et al., 1990b), and humans have found an apparent lack of conversion of sitosterol into C_{24}-bile acids in accordance with the theory (Boberg et al., 1990b). An earlier study by Salen et al. (1970; cited by Boberg et al., 1990b), however, found that humans did convert β-sitosterol into C_{24} bile acids.

9.1.2.5 Excretion

Phytosterols are excreted in the bile and the elimination appears to be faster than that for cholesterol (Lin et al., 1984; cited by Ling and Jones, 1995). In a study of Fischer CD rats, administration of 0.2% β-sitosterol in the diet for 28 weeks led to a 7- to 8-fold higher concentration of β-sitosterol in the feces compared to that normally excreted in the feces of rats fed a control diet (Raicht et al., 1980).

9.1.2.6 Pharmacokinetics

The pharmacokinetics of β-sitosterol administration via different routes was investigated in the beagle dog (Ritschel et al., 1990). The concentration-time profiles for intravenous (i.v.) and oral routes were best described by the two-compartment model; distribution half-life was 3 hours and the terminal distribution half-life was 129 hours. Absolute bioavailability upon oral administration was 9%. β-Sitosterol administration in a polyethylene glycol melt, did not increase the extent of absorption, but the rate of absorption was significantly increased.

9.1.2.7 Sitosterolemia

In contrast to healthy humans, individuals with sitosterolemia (a rare inherited lipid storage disease) have a very different pattern of sitosterol metabolism (Bhattacharyya and Connor, 1974; cited by Ling and Jones, 1995; Salen et al., 1989). Sistosterolemic individuals have increased intestinal absorption of the compound, loss of

tissue sterol structural recognition, expanded pools, and hepatic retention. Salen et al. (1989) postulated that these changes are a response to reduced cholesterol synthesis in these subjects.

9.1.3 Acute Exposure

Acute toxicity data for saw palmetto extract was not found; acute toxicity values for β-sitosterol are presented in **Table 2**. Acute exposure studies discussed in this section are presented in **Table 3**.

Table 2. Acute Toxicity Values for β-sitosterol

Route	Species (sex and strain)	LD_{50}	Reference
i.p.	mice (sex and strain n.p.)	>3000 mg/kg (>7.23 mmol/kg)	Gupta et al. (1980)

Abbreviations: i.p. = intraperitoneal; n.p. = not provided

In experimental animals, very high doses of phytosterols caused diarrhea (Pollak, 1985; cited by Ling and Jones, 1995). In rats, the induction of P-450 by phenobarbital in rats fed a purified diet containing 20% casein and 5% olive oil only occurred when 0.1% oxidized β-sitosterol was added to the diet; pure crystal β-sitosterol had no effect (Marshal and McLean, 1971; cited by Finocchiaro and Richardson, 1983).

9.1.4 Short-Term and Subchronic Exposure

The studies outlined in this section are also presented in **Table 4**. No data were available for saw palmetto extract.

Short term (60 days) s.c. exposure of albino Wistar rats to β-sitosterol at 2 mL/kg/day (0.0048 mmol/kg/day) did not produce gross or microscopic lesions either in the liver or the kidney. All clinical biochemical parameters (including hemoglobin, blood glucose, serum bilirubin, serum GPT and GOT) were in the normal range except for serum

protein and serum cholesterol; serum cholesterol was markedly depleted in both sexes in a dose-dependent manner (Malini and Vanithakumari, 1990).

Table 3. Acute Exposure to Sitosterol

Species, Strain, Age	Number and Sex of Animals	Chemical Form	Dose	Exposure/ Observation Period	Results/Comments	Reference
Experimental animals, (species, strain, and age n.p.)	n.p.	Phytosterols, purity n.p.	very high dose (actual dose n.p.)	n.p.	Caused diarrhea	Pollack (1985; cited by Ling and Jones, 1995)
Rats (strain and age n.p.)	n.p.	oxidized β-sitosterol or pure crystalline β-sitosterol	**Control**: phenobarbital, route n.p.; 20% casein and 5% olive oil in the diet **Treatment**: phenobarbital, casein and olive oil as specified for controls plus 0.1% oxidized sitosterol	n.p.	Cytochrome P-450 not induced by phenobarbital unless diet contained oxidized β-sitosterol. Pure crystalline β-sitosterol in the diet had no effect.	Marshal and McLean (1971; cited by Finocchario and Richardson, 1983)

Abbreviations: n.p. = not provided; s.c. = subcutaneous

Table 4. Short-Term and Subchronic Exposure to β-Sitosterol

Species, Strain, Age	Number and Sex of Animals	Chemical Form	Dose	Exposure/ Observation Period	Results/Comments	Reference
Rats (albino, age n.p.)	10 M, 10 F per group	β-sitosterol, purity n.p.	2.5, 5.0, or 10.0 mg/kg/day (0.006, 0.012, or 0.024 mmol/kg/day), s.c. in 0.2 mL sterile olive oil/100 g body weight/day	60 day exposure	No clear-cut evidence of gross or microscopic liver or kidney lesions were found. The following blood/serum parameters were in the normal range: hemoglobin, blood glucose, serum biliruben, serum GPT, and serum GOT. Serum protein and serum cholesterol were not in the normal range; serum cholesterol was markedly depleted in both sexes in a dose-dependent manner.	Malini and Vanithakumari (1990)
Rats (Fischer CD, 6 wk-old)	10 M per group	β-sitosterol, 95% pure	0.2% β-sitosterol in the diet	28 wk exposure	No adverse effects and no deaths occurred.	Raicht et al. (1980)

Abbreviations: s.c. = subcutaneous; F = female; M = male; n.p. = not provided

Male Fischer CD rats fed 0.2% β-sitosterol in the diet for 28 weeks experienced no adverse effects; no deaths occurred and colon tumors were not induced (Raicht et al., 1980).

9.1.5 Chronic Exposure

Chronic exposure data were not found.

9.2 Reproductive and Teratological Effects

Reproductive and teratological effects discussed in this section are summarized in **Table 5**. Studies on the estrogenic, antiestrogenic, and antiandrogenic effects of saw palmetto extract and β-sitosterol are included in this section.

9.2.1 Humans

Saw palmetto extract may exhibit an antiestrogenic effect, as well as blocking progesterone and androgenic receptors (Lavalle, 1997). Di Silverio et al. (1992) noted the antiestrogenic activity of saw palmetto extract in treating BPH. Among 18 men receiving active therapy (480 mg Permixon® orally/day for 3 months), only 1 was positive for estrogen receptors in the nucleus fraction of prostatic cells, compared with 14 out of 17 for controls. Twelve men in both groups were deemed positive for estrogen receptors in the cytosolic fraction. The author noted that the antiestrogenic activity of saw palmetto extract, in addition to an antiandrogenic action, may be competitively blocking translocation of cytosolic estrogen receptors to the nucleus. In a later paper, Di Silverio et al. (1993) performed a multicenter double blind study on BPH patients, using cyproterone acetate (CPA) plus saw palmetto berry extract as treatment. BPH was hypothesized to involve both interaction of stromal and epithelial compartments in prostate mass growth. Thus, a combination of an antiandrogen (active on the epithelial component) and an antiestrogen (active on the stromal component) was sought. A

statistically significant reduction of prostate volume was identified with use of the combination treatment as compared with treatment using each of the drugs (CPA and saw palmetto) alone.

9.2.2 Mice

Saw palmetto extracts, including β-sitosterol, exhibited estrogenic effects when injected (dose not provided) into immature female mice (strain not provided) (Tyler, 1993; cited by Mendosa, 1997). The activity was found to be relatively low when compared to the female sex hormones themselves.

Table 5. Reproductive Effects of Saw Palmetto and β-Sitosterol

Species, Strain, Age	Number and Sex of Animals	Chemical Form, Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
9.2.1 Humans						
Humans (age n.p.)	M, number n.p.	saw palmetto extract, 80-90% purity	320 mg/day	n.p.	Saw palmetto exhibited an antiestrogenic effect and blocked progesterone and androgenic receptors.	Lavalle (1997)
Humans with BPH (age n.p.)	**Control**: 17 M **Treatment**: 18M	saw palmetto extract (Permixon®)	480 mg/day orally	3 mo. exposure	Subjects were not treated previously for BPH. 1/18 was positive for estrogen receptors in the nucleus fraction of prostatic cells compared to 14/17 for controls. 12 subjects in both treatment and control groups were positive for estrogen receptors in the cytosolic fraction. The author noted that the antiestrogenic action of the extract, in addition to an antiandrogenic action, may be competitively blocking translocation of cytosolic estrogen receptors to the nucleus.	Di Silverio et al. (1992)
Humans with BPH (age n.p.)	M, number n.p.	saw palmetto extract	saw palmetto extract, CPA, or CPA plus saw palmetto extract (dose n.p.)	n.p.	A multicenter double blind study. A statistically significant difference in prostate volume reduction in the saw palmetto extract plus CPA treatment group compared to either of the monotherapies. The combination of saw palmetto extract plus CPA is thought to be effective due to its antiandrogenic (active on the epithelial component) and antiestrogenic (active on the stromal component) properties.	Di Silverio et al. (1993)
9.2.2 Mice						
Mice (immature)	F, number n.p.	saw palmetto extract or pure β-sitosterol	injected, dose n.p.	n.p.	Both treatments exhibited estrogenic properties. Saw palmetto was 1/1,000 as potent as β--sitosterol.	Tyler (1993; cited by Mendosa, 1997)
Pregnant mice (strain and age n.p.)	n.p.	7-hydroxysitosterol, 7-ketositosterol, or sitosterol, purity n.p.	30 mg/kg/day (0.07 mmol/kg/day), orally, at day 1 or day 6-7 of pregnancy	n.p.	7-hydroxysitosterol was the most effective at inducing abortion; ketositosterol and sitosterol were only slightly effective.	Pakroski and Basak (1976; cited by Finocchiaro and Richardson, 1983)

Abbreviations: BPH = benign prostatic hyperplasia; CPA = cyproterone acetate; F = female; i.p. = intraperitoneal; M = male; n.p. = not provided; s.c. = subcutaneous injection

Table 5. Reproductive Effects of Saw Palmetto and β-Sitosterol (cont.)

Species, Strain, Age	Number and Sex of Animals	Chemical Form, Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
9.2.3 Rats						
Rats (albino, strain n.p, 3-4 month-old)	F, number n.p.	β-sitosterol, purity n.p.	0.5, 1.5, or 2.5 mg/kg/d (0.001, 0.004, or 0.006 mmol/kg/day), s.c.	30 day exposure. Sacrificed 24 h after last injection.	At 1.5 mg, β-sitosterol induced a constant estrus cycle in 60% of the animals. At 2.5 mg, β-sitosterol induced a constant estrus cycle that was prolonged as long as treatment continued and caused a marked increase in ovarian, uterine, and pituitary weights. Estrogenic properties of β-sitosterol were noted.	Malini and Vanithakumari (1988)
Rats (albino Wistar, adult)	10 M per group	β-sitosterol, purity, n.p.	0.5 or 5 mg/kg/day (0.001 or 0.012 mmol/kg/day), s.c.	Low and high dose groups: administered for 16, 32, and 48 days, respectively. Withdrawal group: administered for 16, 32, and 48 days and then treatment was withdrawn for 30 days.	Antifertility effect was observed at the high dose level and was most pronounced after 42 to 48 days of exposure. A significant decrease in sperm concentrations was observed after 48 days of treatment with the low dose and at all 3 periods of treatment with the high dose. After a 30-day withdrawal from treatment, sperm concentrations remained lower. After 32 and 48 days of treatment with the low dose, testicular weight was significantly reduced, respectively. The high dose group showed a significant time-dependent decrease in testicular weight at all 3 lengths of exposure.	Malini and Vanithakumari (1991)
Rats (albino Wistar, 3-4 month-old)	10 ovariectomized F per group	β-sitosterol, purity n.p.	0.5, 2.5, or 5.0 mg/kg/day (0.001, 0.006, or 0.012 mmol/kg/day), s.c.	Administered for 10 days. Killed 24 h after last treatment.	At all dose levels, β-sitosterol caused a dose-dependent increase in glycogen concentration and total lactate dehydrogenase (LDH) activity. At the 2.5 and 5.0 mg/kg/day doses, β-sitosterol significantly elevated glucose-6-phosphate dehydrogenase (G6PDH) and phosphohexose isomerase (PHI) enzyme activity.	Malini and Vanithakumari (1992)
Rats (albino Wistar 3-4 month-old)	10 ovariectomized F per group	β-sitosterol, purity n.p.	0. 5, 2.5, or 5.0 mg/kg/day (0.001, 0.006, or 0.012 mmol/kg/day), s.c. in olive oil	Administered for 10 days.	β-Sitosterol caused a dose-dependent increase in uterine weight. DNA, RNA and protein concentrations were significantly increased following β-sitosterol administration at the 0.25 and 0.5 mg/100g body weight/day dose levels.	Malini and Vanithakumari (1993)

Abbreviations: BPH = benign prostatic hyperplasia; CPA = cyproterone acetate; F = female; i.p. = intraperitoneal; M = male; n.p. = not provided; s.c. = subcutaneous injection

Table 5. Reproductive Effects of Saw Palmetto and β-Sitosterol (cont.)

Species, Strain, Age	Number and Sex of Animals	Chemical Form, Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
Rats (strain n.p., neonatal)	M and F, number n.p.	β-sitosterol, purity n.p.	0.003 or 0.03 mg (0.000007 or 0.00007 mmol) injections	10 day exposure 49 day observation period	Both doses of β-sitosterol caused an estrogenic response (increased basal levels of luteinizing hormones in males and females and both doses altered postpubertal pituitary response to GnRH in females).	Register et al. (1995)
9.2.4 Rabbits						
Rabbits (strain n.p., immature)	M, number n.p.	β-sitosterol, purity n.p.	s.c. (dose n.p.)	n.p.	Caused irregularity in spermiogenesis	Ghannudi et al. (1978; cited by Jones et al., 1997)
9.2.5 Sheep						
sheep (25-wk-old lambs)	6 F per group	β-sitosterol, purity n.p.	0.5, 1.0, 5.0, 10.0, 15.0, or 20.0 mg/day (0.001, 0.002, 0.012, 0.024, 0.036, or 0.048 mmol/day), s.c. in olive oil	2, 4, and 8 wk exposure	Ovarian weight was decreased at all doses for all lengths of exposure, except at 1.0 mg for 2 wks, where ovarian weight was increased. With increasing doses of β-sitosterol, follicular growth was inhibited and follicular distribution did not extend past the 6 and 7 granulosal size layers. The Graaffian follicle exhibited signs of atresia or induced ovulation. In the uterus, there was a significant decrease in alkaline phosphatase distribution at a dose of 20 mg.	El-Sammannoudy et al. (1979)
9.2.6 Fish						
mosquitofish (*Gambusia affinis*, age n.p.)	F (number n.p.)	kraft pulp mill effluent	n.p.	n.p.	Exposed females possessed a modified anal fin resembling a gonopodium (the intromittent organ of males) and exhibited male reproductive behaviors such as mating attempts.	Howell et al. (1980; cited by LeBlanc and Bain, 1997)
white sucker fish (*Catostomus commersoni*, age n.p.)	n.p.				Exposed fish had lower serum 17β-estradiol, testosterone, 17α,20β-dihydroprogesterone, and 11-ketotestosterone compared to fish collected from reference sites.	McMaster et al. (1991); Munkittrick et al. (1991; both cited by LeBlanc and Bain, 1997)

Abbreviations: BPH = benign prostatic hyperplasia; CPA = cyproterone acetate; F = female; i.p. = intraperitoneal; M = male; n.p. = not provided; s.c. = subcutaneous injection

Table 5. Reproductive Effects of Saw Palmetto and β-Sitosterol (cont.)

Species, Strain, Age	Number and Sex of Animals	Chemical Form, Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
lake whitefish (*Coregonus clupeaformis*, age n.p.)						Munkittrick et al. (1992; cited by LeBlanc and Bain, 1997)
rainbow trout (*Oncorhynchs mykiss*, age n.p.)					Exposed fish had a 50% reduction of plasma testosterone levels.	Lindstrom-Suppa et al. (1989; cited by LeBlanc and Bain, 1997)
fish of the Great Lakes region	n.p.	pulp mill effluent	n.p.	n.p.	Exposed fish show a several year delay of time to sexual maturation. The investigators indicated that β-sitosterol, found in high concentrations in the effluent, may be the primary agent responsible for the endocrine effects.	McMaster et al. (1991; cited by Cooper and Kaulock, 1997)
goldfish (presumably *Carassius auratus*) age n.p.	M, F (number n.p.)	β-sitosterol, purity n.p.	β-sitosterol, i.p. (dose n.p.)	n.p.	Plasma testosterone levels were significantly reduced in both males and females. 17β-Estradiol was significantly reduced in females. Findings suggest that β-sitosterol may be a contributing factor to the reproductive dysfunction observed in fish exposed to bleached kraft pulp mill effluent.	MacLatchy and van der Kraak (1995)
trout (age n.p.)	n.p.	β-sitosterol	n.p.	n.p.	β-Sitosterol lowered gonadal steroid production by possibly altering cholesterol availability or inhibiting cytochrome P450 activity.	Mellanen et al. (1996; cited by Cooper and Kaulock, 1997)

Abbreviations: BPH = benign prostatic hyperplasia; CPA = cyproterone acetate; F = female; i.p. = intraperitoneal; M = male; n.p. = not provided; s.c. = subcutaneous injection

In mice, a single 30 mg/kg dose of oxidized sitosterol administered orally to pregnant mice induced abortion; however, oxidized sitosterol was only slightly effective when compared to other oxidized sterols (Pakroski and Basak, 1976; cited by Finocchiaro and Richardson, 1983).

9.2.3 Rats

When inbred female albino rats were administered 1.5 mg/kg/day (0.00362 mmol/kg/day) β-sitosterol s.c. for 30 days, the estrus cycle was disrupted in 60% of the animals (i.e., the rats remained in constant estrus) (Malini and Vanithakumari, 1988). At a dose of 2.5 mg/100 g/day (0.00603 mmol/kg/day), the incidence of persistent estrus was prolonged as long as treatment continued (30 days), and a marked increase in ovarian, uterine, and pituitary weights was induced.

In adult male albino Wistar rats, a low dose (0.5 mg/kg/day; 0.00121 mmol/kg/day) of β-sitosterol significantly decreased sperm concentrations after 48 days of treatment and decreased testicular weight after 32 and 48 days of treatment, respectively (Malini and Vanithakumari, 1991). At the high dose (5 mg/kg/day; 0.0121 mmol/kg/day), fertility was reduced after 42 and 48 days of exposure, sperm concentrations were reduced after 16, 32, and 48 days of exposure, and testicular weight was significantly decreased in a time-dependent manner. Withdrawal from treatment for 30 days did not restore sperm count or testicular weight.

To analyze the effects of β-sitosterol on biological parameters of the uterus, ovariectomized albino Wistar rats were administered 0.5, 2.5, or 5.0 mg/kg/d (0.00121, 0.00603, or 0.0121 mmol/kg/d) β-sitosterol s.c. for 10 days (Malini and Vanithakumari, 1992). β-Sitosterol caused a significant dose-dependent increase in glycogen and total lactate dehydrogenase concentrations. At the median and high dose levels, significant increases in glucose-6-phosphate dehydrogenase and phosphohexose isomerase were observed.

In a similar experiment, β-sitosterol was administered to ovariectomized albino Wistar rats s.c. for 10 days at doses of 0.5, 2.5, and 5.0 mg/ kg/d (0.00121, 0.00603, and 0.0121 mmol/kg/d), respectively (Malini and Vanithakumari, 1993). A significant dose-dependent increase in uterine weight was observed. Uterine DNA, RNA, and protein concentrations were also significantly increased at the median and high dose levels.

β-Sitosterol at doses of 0.003 and 0.030 mg (0.00000723 and 0.0000723 mmol) exhibited an estrogenic response when injected into neonatal male and female rats: postpubertal pituitary response to GnRH was altered in females, and basal luteinizing hormone secretion was altered in both males and females (Register et al., 1995). These doses also altered basal luteinizing hormone secretion in immature male and female rats and postpubertal pituitary response to GnRH in female rats.

9.2.4 Rabbits

Very high doses (dose not provided) of β-sitosterol administered s.c. induced irregularity in spermiogenesis in immature rabbits (Ghannudi et al., 1978; cited by Jones et al., 1997).

9.2.5 Sheep

Ovarian weight was reduced when β-sitosterol was administered s.c. to 25-week-old female lambs at doses of 0.5, 1.0, 5.0, 10.0, 15.0, and 20.0 mg/d (0.00121, 0.00241, 0.0121, 0.0241, and 0.0482 mmol/d) for 2, 4, or 8 weeks (El Samannoudy et al, 1980). The only exception was in the 1.0-mg dose group at 2 weeks of exposure. With increasing doses, β-sitosterol inhibited follicular growth and distribution did not extend past the 6 and 7 granulosal layers. The large Graaffian follicle exhibited signs of atresia or induced ovulation. At a dose of 20 mg (0.0482 mmol), uterine alkaline phosphatase distribution significantly decreased.

9.2.6 Fish

Fish chronically exposed to kraft pulp mill effluent exhibited a range of reproductive responses: female mosquitofish (*Gambusia affinis*) expressed male anatomical and behavioral characteristics, including a modified anal fin resembling a gonopodium and reproductive behaviors such as mating attempts (Howell et al., 1980; cited by LeBlanc and Bain, 1997); white sucker fish (*Catostomus commersoni*) (McMaster et al., 1991; Munkittrick et al., 1991; both cited by LeBlanc and Bain, 1997) and lake whitefish (*Coregonus clupeaformis*) (Munkittrick et al., 1992; cited by LeBlanc and Bain, 1997) had lower serum 17β-estradiol, testosterone, 17α,20β-dihydroprogesterone, and 11-ketotestosterone levels compared to fish from a reference site; and laboratory exposure of rainbow trout (*Oncorhynchus mykiss*) reduced plasma testosterone levels by approximately 50% (Lindstrom-Suppa et al., 1989; cited by LeBlanc and Bain, 1997). β-Sitosterol, found in high concentrations in the effluent, is believed to be responsible for the toxicological effects (LeBlanc and Bain, 1997). In the presence of bacteria, β-sitosterol degrades into androgens (Conner et al., 1978; cited by LeBlanc and Bain, 1997) thought to be responsible for the masculinizing effects of female fish (Hunsinger and Howell, 1991; cited by LeBlanc and Bain, 1997). Furthermore, the fish downstream of the mills reach maturation several years later than expected (McMaster et al., 1991; cited by Cooper and Kavlock, 1997). Exposure concentrations of the effluent or β-sitosterol were not provided.

In a laboratory experiment on goldfish (presumably *Carassius auratus*, age not provided) injected with β-sitosterol (dose not provided), the same reduction in gonadal weight and hormone levels was observed (MacLatchy and van der Kraak, 1995). Also, significantly reduced concentrations of testosterone were found in both males and females, and estrogen levels were significantly reduced in females. β-Sitosterol-induced reduction in gonadal steroid production may be related to an alteration of cholesterol availability or an inhibition of cytochrome P450 activity (Mellanen et al., 1996; cited by

Cooper and Kavlock, 1997).

9.3 Carcinogenicity

No carcinogenicity studies were located for saw palmetto extract or β-sitosterol. However, several anticarcinogenicity studies with β-sitosterol have been conducted; these are discussed in **Section 9.4**. In none of these studies was an increased incidence of tumors due to treatment with β-sitosterol alone reported.

9.4 Anticarcinogenicity

Details of these studies are presented in **Table 6**.

9.4.1 Mice

In a two-stage skin carcinogenesis study, female ICR mice were initiated with a single topical application of DMBA followed by a twice weekly treatment for 18 weeks with the tumor promoter TPA (Yasukawa et al., 1991). β-Sitosterol (0.005 mmol) was applied topically 30-40 minutes before each TPA treatment. Treatment with β-sitosterol reduced the incidence of tumor-bearing mice by 20% compared to mice treated with DMBA plus TPA. Tumor multiplicity was reduced also; at the end of the treatment period, mice treated with DMBA plus TPA had an average of 21.1 tumors per mouse, whereas the DMBA plus TPA and β-sitosterol treatment group had an average of 11.2 tumors per mouse. In this study, treatment with β-sitosterol markedly inhibited TPA-induced epidermal ornithine decarboxylase (ODC) accumulation.

In a cancer-related study, Mehta and Moon (1991) evaluated the ability of β-sitosterol to alter lesion induction or progression in mammary glands from estrogen and progesterone-treated Balb/c mice. Mammary glands were removed and incubated in the medium with the following treatments: DMBA (initiator) alone; DMBA and TPA (promotor); DMBA, TPA, and β-sitosterol (on days 1-4 prior to treatment); or DMBA,

Table 6. Anticarcinogenicity of Sitosterol

Species, Strain, and Age	Number and Sex of Animals	Chemical Form	Dose	Exposure/ Observation Period	Results/Comments	Reference
9.4.1 Mice						
mice (ICR, 7 wk-old)	20 F in each group (treatment and control)	sitosterol, purity n.p.	**Control**: 0.05 mg 7,12 DMBA applied topically (initiation), 0.003 mg TPA applied twice weekly (promotion) beginning one week after initiation **Treatment**: DMBA and TPA as in control group; 0.005 mmol sitosterol applied topically 30-40 minutes before each TPA treatment	18 wk exposure	DMBA, TPA, and sitosterol were applied to the shaven backs of mice. Number and diameter of skin tumors were measured every other week. Incidence of tumor-bearing mice was 100% in the DMBA/TPA treated group and 80% in the DMBA/TPA/sitosterol treatment group. Mice in the forme group had an average number of 21.1 tumors/mouse; the latter group group had an average of 11.2 tumors/mouse. Sitosterol treatment caused a 40% reduction in the average number of tumors/mouse after 18 wk of treatment.	Yasukawa et al. (1991)
	5 F in each group (treatment and control)		**Control**: vehicle only, followed in 30 min with 5 µg TPA (both applied topically) **Treatment**: 0.005 mmol sitosterol followed in 30 min with 5 µg TPA (both applied topically)	4 h observation	TPA and sitosterol were applied to the shaven backs of mice. The epidermis was separated by brief heat treatment, and ODC activity was determined by measuring the release of $^{14}CO_2$ from [1-^{14}C]ornithine. Induction of epidermal ODC is a characteristic biochemical alteration elicited by TPA and may be representative of the effects of phorbol esters with strong tumor-promoting activity. Sitosterol treatment inhibited ODC accumulation.	
Estrogen plus progesterone -treated mice (Balb/c, age n.p.)	Control Groups: 15 and 13, respectively Treatment Groups: 15 and 14, respectively	β-sitosterol, purity n.p.	**Control**: 2 mg/mL DMBA or 2 mg/mL DMBA plus 25 ng/mL TPA **Treatment**: DMBA and TPA as in control group plus 0.001 mM β--sitosterol (between days 1 and 4) or DMBA and TPA as in control group plus 0.001 mM β--sitosterol during promotional phase (between days 9 and 24)	24 day observation	Thoracic pair of mammary glands were removed and incubated in medium with compounds specified under dose. β--Sitosterol inhibited induction of lesions by 76% as compared with the DMBA plus TPA treatment group.	Mehta and Moon (1991)
9.4.2 Rats						
Rats (Fischer CD, 6 wk-old)	**Control**: 71 M **Treatment**: 48 M	β-sitosterol (95% pure)	**Control**: 2 mg MNU by intracolonic injection at 4 separate intervals (days 1, 4, 7, and 10) **Treatment**: MNU as in control group plus 0.2% β-	28 wk exposure	Incidence of animals with MNU-induced tumors was significantly suppressed by 38% with β-sitosterol treatment (16/48 animals had tumors in the treatment group compared to 38/71 in the control group). Number of tumors per animal was also significantly decreased with β-sitosterol treatment (0.44 tumor per animal in treatment group compared to 1.1 in the control group).	Raicht et al. (1980)

Abbreviations: AOM = azoxymethane; DMBA = 7,12-dimethyl[*a*]anthracene; F = female; M = male; MNU = *N*-methyl-*N*-nitrosourea; n.p. = not provided; ODC = ornithine decarboxylase; TPA = 12-*O*-tetradecanoylphorbol-13-acetate

Table 6. Anticarcinogenicity of Sitosterol

Species, Strain, and Age	Number and Sex of Animals	Chemical Form	Dose	Exposure/ Observation Period	Results/Comments	Reference
			sitosterol administered in the diet			

Table 6. Anticarcinogenicity of Sitosterol (cont.)

Species, Strain, and Age	Number and Sex of Animals	Chemical Form	Dose	Exposure/ Observation Period	Results/Comments	Reference
Rats (Fischer, age n.p.)	**Control**: 3 M **Treatment**: 5 M	β-sitosterol, purity n.p.	**Control**: 2 mg MNU administered intrarectally at four separate intervals (days 1, 4, 7, & 10) **Treatment**: MNU as specified for controls plus 0.2% β-sitosterol in the diet	28 wk exposure	Half of the rats in the control and treatment groups were injected with tritiated thymidine 1 h prior to killing. The remaining half of the rats in these groups were reserved for killing until 24 h after isotopic administration. 25 crypts of the distal colon were analyzed for the number and position of ^{3}HTdR-labeled cells and the total number of epithelial cells/crypt column. β-Sitosterol treatment effectively reduced the size of the MNU-induced colonic proliferative compartment and depressed MNU-induced colonic epithelial cell proliferation: Control: 15 labeled cells in a single crypt column. Mean number of labeled cells/crypt column, 5.4 Treatment: 9 labeled cells in a single crypt column. Mean number of labeled cells/crypt column, 3.3	Deschner et al. (1982)
Rats (Sprague-Dawley, 6 wk-old)	**Control**: 30 M **Both treatment groups**: 28 M	β-sitosterol (95% pure)	**Control**: 8 mg/kg AOM for 8 wks **Treatment A**: AOM as specified for controls plus 2000 mg/kg (4.82 mmol/kg) β-sitosterol in the diet **Treatment B**: AOM as specified for controls, plus 2 ppm H_2SeO_3 in the drinking water, 50 mg/kg 13-*cis*-retinoic acid in the diet, and 2000 mg/kg (4.82 mmol/kg) β-sitosterol in the diet	26 wk exposure	All animals were killed and necropsies were performed. Tissues were examined for tumors, recording number, size, and location of intestinal tumors. Treatment A did not significantly reduce the total number of intestinal tumors/rat. However, Treatment B significantly reduced the tumor incidence/rat (Treatment B, 2.75 tumors/rat; Control, 5.07 tumors/rat).	Nigro et al. (1982)

Abbreviations: AOM = azoxymethane; DMBA = 7,12-dimethyl[*a*]anthracene; F = female; M = male; MNU = *N*-methyl-*N*-nitrosourea; n.p. = not provided; ODC = ornithine decarboxylase; TPA = 12-*O*-tetradecanoylphorbol-13-acetate

Table 6. Anticarcinogenicity of Sitosterol (cont.)

Species, Strain, and Age	Number and Sex of Animals	Chemical Form	Dose	Exposure/ Observation Period	Results/Comments	Reference
Rats (Fischer-344, young)	n.p.	β-sitosterol, purity n.p.	40 or 80% of the maximum tolerated dose in an AIN-76A semi-synthetic diet	Fed one wk prior to receiving two 15 mg/kg injections of AOM one week apart Animals sacrificed after 5 weeks	After the animals are sacrificed, the rat colons are assayed for frequency of aberrant crypts by staining them with methylene blue. β-Sitosterol was one of 29 compounds tested, but it was not mentioned among the most active aberrant crypt-inhibiting agents. Numbers or discussion not provided for β-sitosterol.	Wargovich et al. (1992)
Rats (Fischer-344, 6 wk-old)	40 M randomized into four groups of 10 (see dose for groups).	β-Sitosterol of highest available purity	**Control**: AOM **Treatment Groups**: 3200 and 6400 mg/kg (7.72 and 15.4 mmol/kg) β-sitosterol with AOM	4 wks	Colons were removed, flushed with cold PBS, cut open along the longitudinal median, and fixed flat in 10% buffered formalin for 24 h. The number of carcinogen-induced aberrant crypt foci (ABF) was evaluated in the 0.3% methylene blue-stained colon. The results were reported as a percentage of the control. 3200 mg/kg (7.72 mmol/kg) β-sitosterol: Induced 138+/-10 ACF/colon., 165% percent of that of the control. Negative for inhibition. Number of ACF/colon was significantly greater than the control at $p<0.05$. 6400 mg/kg (15.4 mmol/kg) β-sitosterol: Induced 137+/-7 ACF/colon., 165% of that of the control. Negative for inhibition. Number of ACF/colon was significantly greater than the control at $p<0.05$.	Wargovich et al. (1996)

Abbreviations: AOM = azoxymethane; DMBA = 7,12-dimethyl[*a*]anthracene; F = female; M = male; MNU = *N*-methyl-*N*-nitrosourea; n.p. = not provided; ODC = ornithine decarboxylase; TPA = 12-*O*-tetradecanoylphorbol-13-acetate

TPA, and β-sitosterol (on days 9-24 following treatment). The results indicated that β-sitosterol was an effective inhibitor of the initiation of mammary lesions, inhibiting the induction of lesions by 76% as compared with the DMBA plus TPA treatment group.

9.4.2 Rats

β-Sitosterol significantly reduced the incidence of colon tumors (predominantly adenomatous polyps) induced in male Fischer CD rats by the direct-acting carcinogen MNU (Raicht et al., 1980). Rats were fed 0.2% β-sitosterol in the diet for 28 weeks. MNU was injected into the colon on days 1, 4, 7, and 10 of the experiment. In a subsequent study by Deschner et al. (1982), the anticarcinogenicity of β-sitosterol was related to its ability to decrease MNU-induced colonic epithelial cell proliferation.

In a study using outbred male Sprague-Dawley rats, β-sitosterol supplemented in the diet (2000 mg/kg; 4.82 mmol/kg) did not significantly inhibit the number of azoxymethane (AOM)-induced tumors per rat (Nigro et al., 1982). However, when β-sitosterol (at the same dose) was given in combination with 13-*cis*-retinoic acid and selenous acid (H_2SeO_3), the number of AOM-induced tumors per animal were significantly decreased.

β-Sitosterol in the diet of male F344 rats did not inhibit, but actually promoted, AOM-induced aberrant crypt foci formation in the colon (Wargovich et al., 1996). At both low and high doses (3200 and 6400 mg/kg; 7.72 and 15.4 mmol/kg), β-sitosterol significantly increased aberrant crypt formation by 165% over that of the control.

9.5 Genotoxicity

The genotoxicity studies discussed in this section are presented in **Table 7**.

9.5.1 Acellular Assays

β-Sitosterol, at 1000 µM, was negative for the induction of strand breaks in λDNA (Osada et al., 1993). Incubation was for 1 hour at 37°C, with the induction of strand breaks identified by electrophoresis under neutral conditions.

9.5.2 Prokaryotic Assays

β-Sitosterol was not mutagenic at concentrations up to 600 µL/plate (1.4 µmol/plate) in *S. typhimurium* strain TA98 with metabolic activation or in TA100 without metabolic activation (Lawson et al., 1989). Autoxidized β-sitosterol was not mutagenic when tested at doses up to 5000 µg/plate in the absence of metabolic activation in *S. typhimurium* strains TA98, TA100, TA1535, and TA1538 (Ansari et al., 1982).

Table 7: Genotoxicity of β-Sitosterol

Test System	Biological Endpoint	Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
9.5.1 Acellular Assays							
γDNA	DNA strand breaks	n.p.	β-sitosterol, purity n.p.	1000 µM	Negative	Incubation was for 1 h at 37°C, with the induction of strand breaks identified by electrophoresis under neutral conditions.	Osada et al. (1993)
9.5.2 Prokaryotic Systems							
Salmonella typhimurium strains TA98 and TA100	gene mutations	+	β-sitosterol extracted from dried savoy chieftain cabbage leaves by isolating the acetone-insoluble residue	150, 300, and 600 µg/plate (0.36, 0.72, and 1.4 µmol/plate)	Negative		Lawson et al. (1989)
S. typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538	gene mutations	-	commercially available β-sitosterol, allowed to autoxidize	Up to 5000 µg/plate (12 µmol/plate)	Negative		Ansari et al. (1982)
S. typhimurium strains TA98 and TA100	gene mutations	+/-	β-sitosterol as a pyrolysis product	1000 µg/plate (2.4 µmol/plate)	Negative with and without S9	pyrolysis product prepared at 450°C.	Malaveille et al. (1982)
S. typhimurium strains TA97, TA98, and TA100	gene mutations	+/-	β-sitosterol, purity n.p.	400 µg/plate β-sitosterol (0.96 µmol/plate)	Significant increase in mutagenic activity in all three strains, with and without metabolic activation.	pyrolysis product prepared at 700°C. Higher mutagenic activity was exhibited toward the TA97 strain as compared to either TA98 or TA100 strains. Mutagenic activity was also higher in the presence of S9 activity for the TA97 strain.	Kuroda et al. (1985)

Abbreviations: n.p. = not provided

A pyrolysis product of β-sitosterol (prepared at 450°C) was not mutagenic when tested up to 1000 µg/plate in the presence or absence of metabolic activation in *S. typhimurium* strains TA98 and TA100 (Malaveille et al., 1982). However, in another study, a pyrolysate of β-sitosterol (formed at 700°C) was mutagenic when tested at 400 µg/plate to *S. typhimurium* strains TA97, TA98, and TA100 in the presence and absence of metabolic activation (Kuroda et al., 1985). The pyrolyzate product was more mutagenic in strain TA97 than strains TA98 or TA100.

9.6 Antigenotoxicity

The studies presented in this section are also outlined in **Table 8**.

9.6.1 Acellular Assays

β-Sitosterol at 1000 µM did not inhibit the ability of ascorbic acid (250 µM) to induce strand breaks in λDNA (Osada et al., 1993). Incubation was for 1 to 6 hours at 37°C, with the induction of strand breaks identified by electrophoresis under neutral conditions.

9.6.2 Prokaryotic Systems

In *S. typhimurium*, β-sitosterol at concentrations up to 600 µL/plate (1.4 µmol/plate) inhibited in a dose-dependent manner the mutagenic activity of MNU in TA100 in the absence of metabolic activation, and of 2-AA in TA98 in the presence of metabolic activation (Lawson et al., 1989). In contrast, β-sitosterol (0.01 to 1000 µg/plate; 0.000024 to 2.4 µmol/plate)did not suppress the mutagenicity of 0.1 nM *Trp-P-2* in *S. typhimurium* strain TA98 in the presence of metabolic activity (Kanazawa, 1995).

9.6.3 *In Vitro* Mammalian Systems

In a V79 mammalian mutagenicity assay, β-sitosterol at 50 and 250 µg/mL (0.12 and 0.60 µM) completely inhibited the induction of oubain-resistance mutants by 2-AA at 25 mg/mL in the presence of hamster hepatocytes but was inactive against MNU (50 µg/mL)-induced mutations (Lawson et al., 1989).

In a study screening potential chemopreventative agents, β-sitosterol (dose n.p.) did not inhibit the binding of B[*a*]P to DNA in human bronchial epithelial cells (Sharma et al., 1994).

Table 8: Antigenotoxicity of β-Sitosterol

Test System or Species, Strain, and Age of Animal	Biological Endpoint	Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
9.6.1 Acellular Assays							
γDNA	inhibition of ascorbic acid -induced DNA strand breaks	-	β-sitosterol, purity n.p. plus ascorbic acid (250 µM	1000 µM	Negative for inhibiting the induction of DNA damage by ascorbic acid.	Incubation was for 1 to 6 hours at 37°C, with the induction of strand breaks identified by electrophoresis under neutral conditions.	Osada et al. (1993)
9.6.2 Prokaryotic Systems							
S. typhimurium strains TA98 and TA100	inhibition of 2-AA - induced mutagenicity in TA98 or MNU-induced mutagenicity in TA100	+ for 2-AA - for MNU	β-sitosterol extracted from dried savoy chieftain cabbage leaves by isolating the acetone-insoluble residue	150, 300, and 600 µg/plate (0.36, 0.72, and 1.4 µmol/plate)	Inhibited 2-AA- and MNU-induced mutagenicity		Lawson et al. (1989)
S. typhimurium strain TA98	inhibition of Trp-P-2 - induced mutagenicity	+	β-sitosterol, purity n.p.	0.01-1000 µg/plate (0.000024-2.4 µmol/plate)	Negative for inhibiting induced mutagenicity.		Kanazawa (1995)
9.6.2 *In Vitro* Mammalian Systems							
V79 cells	inhibition of 2-AA or MNU-induced mutagenicity at oubain locus	+ (hamster hepatocytes) for 2-AA - for MNU	β-sitosterol extracted from dried savoy chieftain cabbage leaves by isolating the acetone-insoluble residue	50 and 250 µg/mL (0.12 and 0.60 µmol/mL)	2-AA-induced mutagenicity: Positive for inhibition at both doses MNU-induced mutagenicity: Negative at 50 µg/mL; β-sitosterol toxic at 250 µg/mL		Lawson et al. (1989)
Human bronchial epithelial cells	inhibition of B[*a*]P-DNA binding	n.p.	β-sitosterol, purity n.p.	1 :M [^{3}H]B[*a*]P plus β-sitosterol (dose n.p.) for 2 h	negative		Sharma et al. (1994)
Rat tracheal epithelial cells	inhibition of B[*a*]P-induced cell transformation	n.p.	β-sitosterol, purity n.p.	10 µg B[*a*]P pluse β-sitosterol (dose	positive. 2.41 µM inhibited by 43% the induction of transformed Class II and III foci		Arnold et al (1995)

Abbreviations: 2-AA = 2-aminoanthracene; B[*a*]P = benzo[*a*]pyrene; DMBA = 7,12-dimethylbenz[*a*]anthracene; ID_{50} = Inhibitory dose for 50% reduction of mutagenic activity; i.p. = intraperitoneal; MNU = *N*-methyl-*N*-nitrosourea; n.p. = not provided.

Table 8: Antigenotoxicity of β-Sitosterol

Test System or Species, Strain, and Age of Animal	Biological Endpoint	Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
				range n.p.)			

Table 8: Antigenotoxicity of β-Sitosterol (cont.)

Test System or Species, Strain, and Age of Animal	Biological Endpoint	Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
9.6.3 *In Vivo* Mammalian Systems							
$B6C3F_1$ female mice	inhibition of DMBA-induced micronuclei induction in bone marrow polychromatic erythrocytes	n.p.	β-sitosterol	150 mg/kg/day (0.36 mmol/kg/day) β-sitosterol injected i.p. for 2 days before injecting 25 mg/kg DMBA i.p.	Positive (60% inhibition)	*In vivo* bone-marrow micronucleus assay was used.	Raj and Katz (1984)

Abbreviations: 2-AA = 2-aminoanthracene; B[*a*]P = benzo[*a*]pyrene; DMBA = 7,12-dimethylbenz[*a*]anthracene; ID_{50} = Inhibitory dose for 50% reduction of mutagenic activity; i.p. = intraperitoneal; MNU = *N*-methyl-*N*-nitrosourea; n.p. = not provided.

β-Sitosterol, at 2.41 μM, inhibited by 43% the induction of transformed Class II and III foci in cultured rat tracheal epithelial cells by B[*a*]P (Arnold et al., 1995).

9.6.4 *In Vivo* Mammalian Systems

β-Sitosterol was reported to inhibit (by 60%) the ability of DMBA to induce micronucleated polychromatic erythrocytes in $B6C3F_1$ mice using the *in vivo* bone marrow micronucleus assay (Raj and Katz, 1984). β-Sitosterol was administered by i.p. injection at 150 mg/kg/d for 2 days prior to an i.p. injection of DMBA at 25 mg/kg; bone marrow was sampled for analysis at 24, 48, and 72 hours after treatment.

9.7 Immunotoxicity

The studies presented in this section are summarized in **Table 9**.

β-Sitosterol enhanced the *in vitro* proliferative response of T-cells stimulated by suboptimal concentration of phytohemagglutinin (PHA) (Bouic et al., 1996). Higher stimulating activity was noted when a ratio (by mass) of 100 β-sitosterol to 1 β-sitosterol glucoside was administered at the same dosage. One microgram per milliliter of 100:1 β-sitosterol/β-sitosterol glucoside also significantly enhanced the expression of CD25 and HLA-Dr activation antigens on T-cells *in vitro*, increased the secretion of IL-2 and γ interferon into the medium, and increased NK-cell activity. When the same 100:1 ratio was ingested by volunteers for 4 weeks, proliferation of PHA-stimulated T-cells was enhanced.

Soybean dust (which contained β-sitosterol) originating from harbor activities in Barcelona, Spain, was concluded to have contributed to asthma outbreaks in the city (Aceves et al., 1991). The result was confirmed using an immunochemical assay of specific soybean allergens. Concentrations of soybean dust in the air corresponded to a 24-hour average level of 25 $\mu g/m^3$.

β-Sitosterol demonstrated antiinflammatory and antipyretic effects in rats, but not

in mice (Gupta et al., 1980). In another study using female ICR mice, sitosterol had a slight, but significant, inhibitory effect on TPA-induced inflammation when applied to the ear 30 minutes before topical application of TPA to the same area (Yasukawa et al., 1991).

9.8 Other Data

9.8.1 Cultured Tumor and Nontumor Cell Toxicity

Cultured PC3 human prostatic cells exposed to saw palmetto extract (Permixon®) at a concentration of 25 - 400 µg/mL exhibited a dose-dependent increase in cell mortality over a 72 hour period (Ravenna et al., 1996). Increased toxicity of LNCaP human prostatic cells was observed after 50 µg/mL Permixon® treatment over 24-, 72-, and 144-hour observation periods.

Table 9. Immunotoxicity of Sitosterol

Test System or Species, Strain, and Age of Animal	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
T-cells stimulated by PHA	β-sitosterol, purity n.p.	n.p.	β-Sitosterol enhanced the proliferative response of PHA; it also increased the NK-cell activity. When given in a mass ratio of 100 β-sitosterol to 1 β-sitosterol glucoside, higher stimulating activity was noted. 1 µg/mL of the 100:1 ratio significantly enhanced the expression of CD25 and HLA-Dr activation antigens on T-cells and increased the secretion of IL-2 and γ interferon into the medium.		Bouic et al. (1996)
Exposed humans	Soybean particulates which include β-sitosterol, purity n.p.	25 µg/m^3 expressed as an average 24 hour exposure	The dust has contributed to asthma outbreaks. The result was confirmed using an immunochemical assay of the specific soybean allergens.	Soybean dust originated from harbor activities in Barcelona, Spain.	Aceves et al. (1991)
Rats, strain and age n.p.	β-sitosterol, purity n.p.	n.p.	Antiinflammatory and antipyretic activities were observed.		Gupta et al. (1980)
Mice, strain and age n.p.			Antiinflammatory or antipyretic activities were not observed.		
ICR mice (7 wk-old)	Sitosterol, purity n.p.	5 µmol applied topically to the ear 30 min. before TPA (5µg) treatment	A slight, but significant inhibitory effect on TPA-induced inflammation was observed with sitosterol treatment.	Mice were killed 4 hr after TPA treatment. The epidermis was separated by brief heat treatment and ODC activity was determined my measuring the release of $^{14}CO_2$ from [1-^{14}C]ornithine.	Yasukawa et al. (1991)

Abbreviations: F = female; n.p. = not provided; ODC = ornithine decarboxylase; PHA = phytohemagglutinin; TPA = 12-*O*-tetradecanoylphorbol-13-acetate

In vitro exposure of human umbilical vein endothelial cells to 700 μM sitosterol for 72 hours caused contraction of the endothelial cells and increased the release of intracellular lactate dehydrogenase (Boberg et al., 1991). After 96 hours of exposure, the cells were partly detached from the substrate.

β-Sitosterol was also effective in inhibiting the growth of human prostate cancer cells (von Holtz et al., 1991). After 3 days of incubation with 16 M β-sitosterol, cell growth was 72% of the cell growth of cultures treated with equimolar cholesterol (no comparison was made with untreated cultures). This percentage was reduced to 66% after 5 days of incubation.

In a study screening potential chemopreventative agents using biochemical markers of carcinogenesis, β-sitosterol was highly effective in inhibiting TPA-induced tyrosine kinase activity in HL-60 cells, TPA-induced ornithine decarboxylase (ODC) activity in rat tracheal epithelial cells, and poly(ADP-ribose) polymerase activity in propane sultone-treated primary human fibroblasts (Sharma et al., 1994). In contrast, β-sitosterol did not induce a reduction of glutathione in Buffalo rat liver cells, or TPA-induced free radical formation in primary human fibroblasts or HL-60 cells.

When tested on cultured T-47D breast cancer cells, β-sitosterol was weakly estrogenic (i.e., significantly enhanced cell proliferation); no effect was noted on the growth of MCF-7 breast cancer cells (Mäkelä et al., 1995).

The ability of β-sitosterol to inhibit the *in vitro* growth of HT-29 human colon cancer cells was evaluated by Awad et al. (1996). Five concentrations of β-sitosterol were delivered to the cultured cells as a sterol-cyclodextrin complex (0.05, 0.5, 2.0, 8.0, and 16.0 μM). Cell cultures were observed on days 3, 5, 7, and 9. When compared to the control cultures, the cell cultures with 8-μM β-sitosterol supplementation were effective at cell growth inhibition after 7 or 9 days of treatment. The 16-μM β-sitosterol supplementation effectively inhibited cell growth at all treatment lengths (3 to 9 days). At 16-μM β-sitosterol supplementation, membrane cholesterol was reduced by 26% and

membrane sphingomyelin was reduced by 50%, suggesting an active role of sphingomyelin in inhibition of tumor growth by sterols. Since incorporation of β-sitosterol into tumor membranes did not influence phospholipid content, the authors noted that the observed inhibition of growth is not mediated through alterations in the phospholipid membrane parameters.

9.8.2 Hypocholesterolemic Action

The ability of sitosterol to lower cholesterol levels was noted in the early 1950s, when sitosterol addition to the diet of cholesterol-fed chickens or rabbits lowered cholesterol levels in both species; sitosterol addition to the diet inhibited atherogenesis in rabbits (Pollak and Kritchevsky, 1981; cited by Howard and Kritchevsky, 1997). In a study of laying hens, a diet including 4% plant sterols reduced cholesterol absorption by 40% (Kudchodkar et al., 1976).

β-Sitosterol inhibited cholesterol absorption, decreased liver cholesterol concentration, and decreased the synthesis of bile acids when administered at 1% in the diet of mice (Uchida et al., 1984). Additionally, in a study of rats dosed with 3% cholesterol in the diet, β-sitosterol was effective in lowering liver cholesterol, triglyceride, and fatty acid levels (Tabata et al.,1980). β-Sitosterol was administered for 5 days by intravenous injection as an emulsion with saline-albumin.

Human studies have also found sitosterols to be effective in lowering cholesterol levels: a dose of 6000 mg (14.5 mmol) β-sitosterol per day (route not specified) decreased cholesterol levels by 9% (Weisweiler et al., 1984; cited by Ling and Jones, 1995) and 722 mg/day (route not specified) decreased cholesterol levels by 11% (Heinemann et al., 1993; cited by Ling and Jones, 1995). Children treated with 6000 mg/day (14.5 mmol/day) β-sitosterol for 3 months experienced a 17% reduction in total cholesterol (Becker et al., 1992, 1993; both cited by Jones et al., 1997), a 19.5% reduction in low-density lipoprotein (LDL) cholesterol, and no change in high-density lipoprotein (HDL) cholesterol levels (Becker et al., 1993; cited by Jones et al., 1997).

Men with myocardial infarction were pretreated with varying amounts of fat and cholesterol for 6 to 12 weeks and were then given sitosterol at doses of 12,000 to 18,000 mg/day for 12 to 24 weeks (Farquhar et al., 1956; cited by Jones et al., 1997). A 17% reduction in total cholesterol levels was noted.

Also, 2000 mg sitosterol per day effectively reduced LDL cholesterol by 20% when used as treatment for familial hypercholesterolemia (Denke, 1995; cited by Ling and Jones, 1995). Familial-type hypercholesterolemic children had a 6% reduction in total cholesterol, a 7% LDL cholesterol reduction, a 15% HDL cholesterol reduction, and a 23% increase in triglycerides when treated with 12,000 mg/day (28.9 mmol/day) β-sitosterol for 3 months (Schlierf et al., 1978; cited by Jones et al., 1997). In hypercholesterolemia treatment, phytosterol was able to alter lipid metabolism (i.e., reduce liver acetyl-CoA carboxylase and malic enzyme activities) (Laraki et al., 1993; cited by Ling and Jones, 1995).

Treatment of hypercholesterolemia with a combination of β-sitosterol and lovastatin was found to be significantly more effective in decreasing LDL cholesterol than treatment with lovastatin alone. Thirty patients with background LDL cholesterol between 5.89 and 12.26 mmol/L were investigated. The combination treatment lowered LDL cholesterol levels by an additional 12.8 to 15.1% over the treatment group administered lovastatin alone (Richter et al., 1996).

The mode of action is thought to involve inhibition of cholesterol absorption, even though plant sterols are very poorly absorbed (Tilvis and Miettinen, 1986; cited by Howard and Kritchevsky, 1997). Ingestion of 1000 mg of β-sitosterol reduced absorption of a 500 mg cholesterol-containing meal by 42%. The mechanism is thought to involve crystallization and co-precipitation of cholesterol (Mattson et al., 1982; cited by Howard and Kritchevsky, 1997).

9.8.3 Hormonal Responses

Saw palmetto is known to exhibit an antiandrogenic action (experimental model not specified), although the compound responsible for this action has not been identified (Tyler, 1993; cited by Mendosa, 1997).

The cause of the antiandrogenic effects of saw palmetto extracts are debated. The effects are thought to be caused by a direct action on the androgen receptor (Briley et al., 1983; Carilla et al., 1984; both cited by Champault et al., 1984; Ravenna et al., 1996), the inhibition of the enzyme testosterone-5-α-reductase (Sultan et al., 1984; cited by Champault et al., 1984), and/or competitive inhibition of dihydrotestosterone (DHT) binding to both cytosolic and nuclear receptors (Sultan et al.,1984; cited by Lowe and Ku, 1996). However, studies by Rhodes et al. (1993; cited by Lowe and Ku, 1996) found that saw palmetto berry extract (Permixon®) did not demonstrate any inhibition of DHT binding or inhibition of 5-α-reductase activity. Strauch et al. (1994) also did not observe inhibition of 5-α-reductase activity when determined by measuring serum DHT levels. Délos et al. (1995) found that Permixon® inhibited the formation of all the testosterone metabolites studied (DHT; androst-4-ene-3,17-dione; and 5α-androstane-3,17-dione) in both epithelial and fibroblast cells from BPH and prostate cancer tissues. Lehle et al. (1995) also found that the saw palmetto extract markedly inhibited both isoforms of human 5-α-reductase in the baculovirus-directed insect cell expression system, but the inhibition was noncompetitive. Permixon® inhibited DHT and testosterone binding in 11 different human tissue specimens (el-Sheikh et al., 1988). In humans, the antiandrogenic effect is achieved without significantly influencing systemic hormone levels, including testosterone, follicle-stimulating hormone, and luteinizing hormone (Casarosa et al., 1988).

Bourbon concentrate (containing β-sitosterol) induced an estrogenic response (decreased luteinizing hormone (LH) levels and increased sex hormone binding of globulin and HDL cholesterol) in normal post-menopausal women (Gavaler et al., 1991; van Thiel

et al., 1991; both cited by Rosenblum et al., 1993).

β-Sitosterol, added to cell cultures of *Saccaromyces cerevisae* strain BJ3535 containing a human estrogen receptor, demonstrated weak estrogenic activity (Gaido et al., 1997).

9.8.4 Analgesic Effects

β-Sitosterol (3-100 mg/kg; 0.00723-0.241 mmol/kg) administered i.p. to mice caused a dose-dependent inhibition of acetic acid-induced abdominal constriction; the ID_{50} was 9 mg/kg (0.0217 mmol/kg) (Santos et al., 1995). β-Sitosterol was equipotent with aspirin in its analgesic effects.

10.0 STRUCTURE-ACTIVITY RELATIONSHIPS

To compare the effect of β-sitosterol and cholesterol on growth and differentiation of human prostate cancer cells, von Holtz et al. (1991) cultured the cancer cells in medium supplemented with up to 16 μM sterol delivered by a cyclodextrin vehicle. Growth was measured by counting the cells in each culture on days 1, 3, and 5 of treatment, and culture media and cell lysates were assayed for total (ACP) and prostatic acid phosphatase (PAcP). β-Sitosterol was more effective in inhibiting the growth of human prostate cancer cells than cholesterol. After 3 days of incubation with 16 M β-sitosterol, cell growth was 72% of the cell growth of cultures treated with equimolar cholesterol. In examining the 4-μM sterol groups, PAcP in the cell lysate was significantly higher in the cholesterol-treated group than in the β-sitosterol-treated group. At the 16-μM level, the concentration of PAcP remained constant in the cholesterol group and increased significantly in the β-sitosterol-treated group, suggesting that differentiation may be involved in the inhibition of prostate cancer cell growth.

11.0 ONLINE DATABASES AND SECONDARY REFERENCES

11.1 Online Databases

Chemical Information System Files

SANSS
TSCAPP
TSCATS (Toxic Substances Control Act Test Submissions)

DIALOG Files

Foods ADLIBRA
Chem. Econ. Hdbk.
FOODLINE: Current Food Legislation
FOODLINE: Food Science and Technology
FOODLINE: International Food Market Data
NIOSHTIC
KIRK-OTHMER ENCYLOPEDIA OF CHEM. TECHNOL.

National Library of Medicine Databases

EMIC and EMICBACK (Environmental Mutagen Information Center)

Internet

Phytochemeco database cited in Section 12 as Beckstrom-Sternberg and Duke (1997)

STN International Files	CHEMLIST	IPA
	CROPB	LIFESCI
AGRICOLA	CROPU	MEDLINE
BIOSIS	CSNB	NAPRALERT
CA File	DDFB	PHIN
CABA	DDFU	PROMPT
CANCERLIT	DRUGLAUNCH	RTECS
CAPLUS	EMBASE	TOXLINE
CBNB	FSTA	TOXLIT
CEN	HODOC	

TOXLINE includes the following subfiles:

Toxicity Bibliography	TOXBIB
International Labor Office	CIS
Hazardous Materials Technical Center	HMTC
Environmental Mutagen Information Center File	EMIC
Environmental Teratology Information Center File (continued after 1989 by DART)	ETIC
Toxicology Document and Data Depository	NTIS
Toxicological Research Projects	CRISP
NIOSHTIC7	NIOSH
Pesticides Abstracts	PESTAB
Poisonous Plants Bibliography	PPBIB
Aneuploidy	ANEUPL
Epidemiology Information System	EPIDEM
Toxic Substances Control Act Test Submissions	TSCATS
Toxicological Aspects of Environmental Health	BIOSIS
International Pharmaceutical Abstracts	IPA
Federal Research in Progress	FEDRIP
Developmental and Reproductive Toxicology	DART

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