Chemical Information Review Document

for

Arbutin [CAS No. 497-76-7] and Extracts from *Arctostaphylos uva-ursi*

Supporting Nomination for Toxicological Evaluation by the National Toxicology Program

January 2006

Prepared by:
Integrated Laboratory Systems, Inc.
Research Triangle Park, NC
Under Contract No. N01-ES-35515

Prepared for:
National Toxicology Program
National Institute of Environmental Health Sciences
National Institutes of Health
U.S Department of Health and Human Services
Research Triangle Park, NC
http://ntp.niehs.nih.gov/

Abstract

Arbutin is found in the dried leaves of a number of different plant species including bearberry (*Arctostaphylos uva-ursi*). The leaves and leaf extracts from uva ursi are used in non-prescription medicinal products mainly to treat urinary tract infection, cystitis, kidney stones, and as a diuretic. The active component, arbutin, is converted to hydroquinone (HQ) which has antimicrobial, astringent, and disinfectant properties. Arbutin is also an inhibitor of melanin formation and is use in some skin-lightening products. It is rapidly metabolized and excreted in rats as unchanged arbutin and in humans as HQ, HQ glucuronide, and HQ sulfate. HQ induced renal tubule adenomas in male rats and liver adenomas and thyroid gland follicular cell hyperplasia in mice. It was negative in the Ames assay but induced mutations and micronuclei in mouse L5178Y lymphoma and chromosome aberrations and sister-chromatid exchange in Chinese hamster ovary cells. Side effects reported from the ingestion of dried uva ursi leaves included nausea and vomiting, irritability, insomnia, and an increased heart rate. Extremely high doses can cause ringing in the ears, shortness of breath, convulsions, collapse, and delirium. Uva ursi has also been linked with albuminuria, hematuria, and urine cast; liver damage is also a risk with long-term use. *In vitro* studies of human melanocytes exposed to arbutin at concentrations below 300 µg/mL reported decreased tyrosinase activity and melanin content with little evidence of cytotoxicity. Arbutin did not induce mutations in hamster V79 cells, except after preincubation with β-glycosidase. Bone marrow micronuclei were not induced in mice after oral treatment with arbutin, and a mixture of uva ursi extracts did not induce micronuclei in cultured human lymphocytes.

Executive Summary

Basis for Nomination

Arbutin is a natural product found in foods, over-the-counter drugs, herbal dietary supplements, or via dermal contact from and cosmetic skin-lightening products. Arbutin was nominated by the National Institute for Environmental Health Sciences (NIEHS) for *in vitro* and *in vivo* metabolism and disposition and genotoxicity studies. The nomination is based on widespread human exposure, insufficient toxicological data, and suspicion of toxicity due to hydrolysis to hydroquinone (HQ). Further studies are needed to clarify rodent and human differences in biological disposition of arbutin and conversion to HQ. Previous National Toxicology Program (NTP) studies demonstrated that HQ induced renal tubule adenomas in male rats and liver adenomas and thyroid gland follicular cell hyperplasia in mice.

Nontoxicological Data

General Information: *Arctostaphylos uva ursi* (also called Arctostaphylos, bearberry, and beargrape) is an evergreen perennial shrub flourishing in humus-rich soil of North America, Europe, and Asia. The leaves are used in medicinal products to treat urinary tract infection, cystitis, and kidney stones. The active compound in uva ursi, arbutin, is converted to HQ, which has antimicrobial, astringent, and disinfectant properties. In several plant species (e.g., *Arctostaphylos* and *Calluna*), quince jam samples, and commercial cream samples, arbutin can be quantitatively determined by high performance liquid chromatography. Uva ursi is commercially available as crushed leaf or powder, and arbutin is available in both natural and synthetic forms. Arbutin can be synthesized from acetobromglucose and HQ or from the reaction of β-D-glucose pentaacetate and HQ monobenzyl ether in the presence of phosphorus oxychloride. It can be formed *in vitro* by the incubation of HQ with enzyme extracts from bean or wheat plants or from the germination of seeds or plant tissues in the presence of HQ. Arbutin is used as a stabilizer for color photographic images and as an anti-infective for the urinary system as well as a diuretic. It is also an inhibitor of melanin formation and a skin-lightening agent. In the United States, uva ursi leaf is used as a urinary antiseptic and diuretic in numerous dietary supplements.

Environmental Occurrence and Persistence: In the leaves of *A. uva-ursi*, the arbutin content generally ranges from 5.95 to 7.16%. In a study of wild populations of *A. uva-ursi* in various regions of Spain, arbutin levels were found to be significantly higher in autumn than in spring. The concentrations also varied based on plant location. Arbutin was a chemical constituent in the aqueous leachate from foliar branches and leaf litter of *A. glandulosa* var. zacaensis, which was toxic to the growth of annual grasses.

Human Exposure: Potential human exposure to arbutin can occur via ingestion, inhalation, or dermal contact. Arbutin-containing foods include marjoram, pears, Japanese pepper, potatoes infected with fungi, honeys in Italy and Sardinia, and beverages. Uva ursi is generally used as crushed leaf or powder, an infusion or cold macerations, and fluidextract, providing 400-840 mg arbutin per day. As the dry extract, 100-210 mg arbutin is supplied to the individual. It is also available as a capsule, tea, and tincture. Exposure can also occur through the use of herbal mixtures that contain bearberry or uva ursi.

In the 1981-1983 National Institute for Occupational Safety and Health (NIOSH) National Occupational Exposure Survey (NOES), an estimated 2629 workers (595 of which were females) were potentially exposed to uva ursi in the Chemicals and Allied Products industry.

Regulations: Bearberry is not generally recognized as safe and effective. In October 1990, the Food and Drug Administrations (FDA) proposed to ban the use of 111 ingredients, including bearberry, in nonprescription diet drug products. When the ban became effective, uva ursi-potassium extract was specifically named. Bearberry (extract of uva ursi) and bearberry fluidextract (extract of bearberry) were included among ingredients in a proposed ban on the use of nonprescription orally administered menstrual drug products. Under §111.100(d), the FDA proposed requiring that no ingredient with a known

stimulant effect, such as uva ursi, be combined in a dietary supplement containing ephedrine alkaloids. As OTC drug products, the use of bearberry and "uva ursi, potassium extract" as anorectics for weight control is regulated under 21 CFR 310.545(a)(20) and the use of bearberry (extract of uva ursi) and bearberry fluidextract (extract of bearberry) as a "menstrual/diuretic" is regulated under 21 CFR 310.545(a)(24).

Toxicological Data
No short-term/subchronic or chronic exposure, carcinogenicity, initiation/promotion, or anti- or co-genotoxicity studies were available.

Human Data: Possible side effects of uva ursi administration are nausea, vomiting, irritability, insomnia, and an increased heart rate. Extremely high doses of uva ursi (i.e., ten times the recommended amount) can cause ringing in the ears, shortness of breath, convulsions, collapse, delirium, and vomiting. Uva ursi has also been linked with albuminuria, hematuria, and urine cast.

Arbutin was found to be extensively absorbed from the gastrointestinal tract and bioavailable as HQ. Volunteers consuming a meal consisting of high levels of arbutin- and HQ-containing foods had significant increases in mean total HQ plasma levels. Urinary total HQ excretion rates were also significantly increased. Four hours after ingestion of a single dose of a preparation containing bearberry leaf extract (945 mg corresponding to 210 mg arbutin), 224.5 µmol/L HQ glucuronide and 182 µmol/L HQ sulfate were recovered in the urine, which represented approximately half the administered arbutin dose. In an open, randomized, two-way crossover study, 16 healthy volunteers received a single oral dose of bearberry leaves dry extract (BLDE) as film-coated tablets (472.5 mg BLDE, corresponding to 105 mg arbutin) or as an aqueous solution (945 mg BLDE, corresponding to 210 mg arbutin). HQ glucuronide and HQ sulfate were recovered in the urine during the first four hours. The total metabolite concentration represented 66.7% of the administered dose in the tablets and 64.8% in the solution; HQ glucuronide accounted for 67.3% and 70.3% of the total arbutin metabolites recovered in each group, respectively.

Absorption, Distribution, Metabolism, and Excretion: Because arbutin is reported to hydrolyze easily in dilute acids to yield D-glucose and HQ, it is expected that ingested arbutin would be hydrolyzed to free HQ by stomach acids.

In female Wistar rats given an aqueous solution of chromatographically pure arbutin, unchanged arbutin was excreted at 82% and 100% after 16 and 30 hours post-treatment, respectively, corresponding to 90.7% of the total arbutin dose administered orally. Oral administration of arbutin (500 mg/kg) to female rats which were overloaded with fluid resulted in a four-fold excess of excreted urine during the second hour of dosing and a total increase of 61% in the first day. No free HQ was detected in the urine samples. Arbutin uptake by the jejunum and ileum of rats with a bile fistula and those that received a restricted diet for five days was increased. The addition of sodium taurocholate to both groups of animals depressed arbutin uptake in both jenunum and ileum towards normal values. Taurocholate also depressed uptake of arbutin in the ileum only of normal animals.

Acute Exposure: In mice orally or intraperitoneally (i.p.) treated with arbutin (50-200 mg/kg), a dose-dependent antitussive effect to ammonia-induced cough was observed. Arbutin had no analgesic or anesthetic effects nor any effects on tracheal smooth muscle contraction, respiratory activity, spontaneous behavior, blood pressure, heart rate or electrical activity. At a dose of 8000 mg/kg administered i.p. for two weeks, no toxic effects were observed.

Synergistic/Antagonistic Effects: *A. uva-ursi* extract or arbutin in combination with prednisolone. indomethacin, or dexamethasone inhibited swelling of contact dermatitis induced by picryl chloride (PC-CD) in mice, sheep red blood cell delayed type hypersensitivity (SRBC-DTH) response, and/or

carrageenin-induced paw edema to a greater extent than did any of the chemicals alone. Uva ursi may also increase the anti-inflammatory effects of nonsteroidal anti-inflammatory drugs, such as ibuprofen and indomethacin. Aloesin (an anti-inflammatory) and arbutin synergistically inhibit tyrosinase activity. In a study of their effects on UV-induced pigmentation in human skin *in vivo*, co-treatment with both chemicals (100 mg/g each) produced an additive effect. Additionally, arbutin inhibited UV-induced nuclear factor-kappaB activation in human keratinocytes. An *A. uva-ursi* extract, considered for use in food preservation, enhanced the antimicrobial activity of nisin. In mouse skin, arbutin counteracted oxidative stress induced by 12-*O*-tetradecanoylphorbol-13-acetate.

Cytotoxicity: Growth of human melanoma cells and normal human melanocytes was not inhibited by exposure to 100 µg/mL arbutin for five days. At 300 µg/mL arbutin treatment for five days, cell toxicity and detachment of cells from the dishes were observed within 48 hours.

Reproductive and Teratological Effects: Daily subcutaneous (s.c.) injection of arbutin (25, 100, or 400 mg/kg) into male and female Sprague-Dawley rats before mating and into females during pregnancy and lactation resulted in a maximum no-effect dose of 100 mg/kg/day for reproduction and for development and growth of their offspring. Oral administration of arbutin (lowest published toxic dose = 13,600 mg/kg) for 14 days prior to copulation and 20 days of pregnancy produced effects (not specified) in the ovaries and fallopian tubes. Fetotoxicity but no deaths was reported.

Anticarcinogenicity: Arbutin (2.5, 12.5, or 50 µg/mL) incubated for four days weakly inhibited the growth of human colon carcinoma HCT-15 cells.

Genotoxicity: Arbutin (up to 0.01 M) did not induce mutations in hamster V79 cells; however, after preincubation of arbutin (≥1 mM) with β-glycosidase, it did have a mutagenic effect. Arbutin (0.5-2 g/kg bw) did not induce micronuclei in the bone marrow of mice, while a mixture of extracts of *Uva ursi* (0.025, 0.05, 0.1, or 0.2 mg/mL) did not affect the level of micronuclei in cultured human blood lymphocytes.

Immunotoxicity: In a murine local lymph node assay, arbutin (5, 10, or 20%), applied to Balb/c female mice after topical treatment with the weak allergen α-hexylcinnamaldehyde, did not produce contact sensitization. Oral application of arbutin (10 or 50 mg/kg) quickly reduced the swelling of PC-CD and SRBC-DTH within 24 hours. Arbutin (1 mg/mL) also inhibited the binding of mouse monoclonal anti-dinitrophenyl immunoglobulin E (IgE[aDNP]) to DNP by 65%. In macrophage cells from male Swiss mice, arbutin (2 mg/mL) failed to induce the release of hydrogen peroxide.

Other Data: Arbutin added to cultured human melanocytes showed a concentration-dependent reduction in tyrosinase activity and significantly reduced melanin content with low cytotoxicity. A 50% methanol extract of *A. uva-ursi* and arbutin isolated from the bearberry leaf also inhibited tyrosinase activity and melanin production *in vitro*. Arbutin exhibited potent inhibitory effects on rat platelet aggregation induced by adenosine diphosphate and collagen.

In unanaesthetized male and female cats administered oral (p.o.) and i.p. doses of arbutin (50 and 100 mg/kg bw) in water, a statistically significant decrease in the number, intensity, and frequency of coughs was observed at the lower dose. An increase in antitussive activity was not seen with the higher dose.

Structure-Activity Relationships

Hydroquinone (HQ) [CAS No. 123-31-9]

In a two-year carcinogenesis bioassay, HQ (25 or 50, or 100 mg/kg) administered by gavage gave some evidence of carcinogenicity in male F344/N rats (kidney tubular cell adenoma), female F344/N rats (mononuclear cell leukemia), and female $B6C3F_1$ mice (liver adenoma or carcinoma). Additionally, the

incidence of thyroid follicular cell hyperplasia was increased in female and male mice. In *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, HQ was negative for mutagenicity in the presence and absence of metabolic activation (S9). In Chinese hamster ovary (CHO) cells, it induced sister chromatid exchanges (with and without S9) and chromosomal aberrations (with S9). HQ also induced trifluorothymidine resistance in mouse L5178Y/TK lymphoma cells and was mutagenic in the micronucleus test. Inconclusive results, however, were obtained in *Drosophila*.

Hydroquinone Monomethyl Ether [CAS No. 150-76-5]
HQ monomethyl ether was negative in *Salmonella* mutagenicity assays.

tert-Butylhydroquinone (TBHQ) [CAS No. 1948-33-0]
TBHQ (0.125, 0.25, or 0.5% [1250, 2500, or 5000 ppm]) administered in feed for two years (mice) or lifetime (rats) produced no evidence of carcinogenicity in male and female Fischer 344 rats and $B6C3F_1$ mice. When the rats were placed on a diet restriction (0 or 5000 ppm in feed for 30 months), survival rates increased, while the incidences of neoplasms and nonneoplastic lesions at various sites were maintained. In *S. typhimurium* strains TA97, TA98, TA100, and TA102, negative results were obtained with and without S9. TBHQ was also not mutagenic in the micronucleus test. In CHO cells, it induced sister chromatid exchanges and chromosome aberrations in the presence of S9 only. In immunotoxicity tests, TBHQ (25, 75, or 150 mg/kg) administered via gavage to $B6C3F_1$ mice produced increases in the amount of the third component of serum complement, Fc-mediated adherence and phagocytosis by peritoneal adherent cells, natural killer cell activity, liver and spleen weights, reticulocyte number, and blood glucose and a decrease in the number of neutrophils.

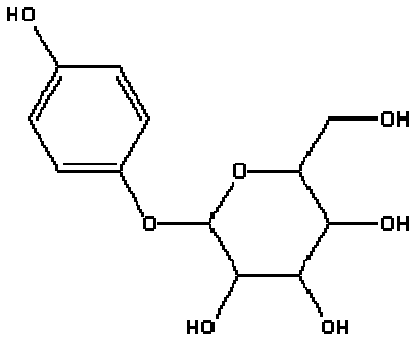
Table of Contents

1.0 Basis for Nomination

Arbutin is a natural product found in foods, over-the-counter drugs, herbal dietary supplements, and cosmetic skin-lightening products. Arbutin was nominated by the National Institute for Environmental Health Sciences (NIEHS) for *in vitro* and *in vivo* metabolism and disposition and genotoxicity studies. The nomination is based on widespread human exposure, insufficient toxicological data, and suspicion of toxicity due to hydrolysis to hydroquinone (HQ). Further studies are needed to clarify rodent and human differences in biological disposition of arbutin and conversion to HQ. Previous National Toxicology Program (NTP) studies demonstrated that HQ induced renal tubule adenomas in male rats and liver adenomas and thyroid gland follicular cell hyperplasia in mice.

2.0 Introduction

Arbutin
[497-76-7]



Arbutin is found in the dried leaves of bearberry (*Arctostaphylos uva-ursi* Spreng., *Ericaceae*), blueberry, cranberry, and pear trees (*Pyrus communis* L., *Rosaceae*); cowberry (*Vaccinium vitis-idaea* L., *Ericaceae*), *Bergenia crassifolia* (L.) Fritsch, and *Saxifragaceae*. It is generally present in plants in combination with methylarbutin, especially plants of the *Ericaceae* family (Budavari, 1996). Arbutin has also been isolated in numerous other plant sources, such as in juvenile foliage of the New Zealand tree *Halocarpus biformis*, *Salvia mexicana* L. var. minor Benth, *Rhodiola sacra* S H Fu, *Onobrychis viciifolia* (Sainfoin), and *Origanum majorana* (Assaf et al., 1987; Delira et al., 2003; Fan et al., 1999; Marais et al., 2000; Perry et al., 1996).

Arctostaphylos uva ursi is an evergreen perennial shrub flourishing in humus-rich soil of North America, Europe, and Asia. Uva ursi contains HQ derivatives (mostly arbutin), polyphenolic tannins, free-form phenolic acids, flavonoids, triterpenes, monotropein, resin, volatile oil, and wax. Commercially available materials derived from uva ursi come entirely from wild plants collected throughout Europe, primarily Spain, Italy, the Balkans, and the USSR. The leaves are used in medicinal products, mainly to treat urinary tract infection, cystitis, and kidney stones. The active compound in uva ursi, arbutin, is converted to HQ, which has antimicrobial, astringent, and disinfectant properties (Am. Botanical Council, 2000; A.D.A.M., Inc., 2004).

2.1 Chemical Identification and Analysis

Arbutin ($C_{12}H_{16}O_7$; mol. wt. = 272.25) is also called:

β-Arbutin
Arbutine
Arbutoside
β-D-Glucopyranoside 4-hydroxyphenyl- (9CI)
Glucopyranoside, *p*-hydroxyphenyl, β-D- (6CI, 7CI)
Hydroquinone-β-D-glucopyranoside
Hydroquinone glucose
4-Hydroxyphenyl-β-D-glucopyranoside
NSC 4036
Ursin
Uvasol

Sources: Budavari (1996); Registry (1984); RTECS (1997)

In several species (e.g., *Arctostaphylos* and *Calluna*), arbutin can be quantitatively determined by high performance liquid chromatography (HPLC) (Parejo et al., 2002; Sticher et al., 1979; Sun et al., 1997). Thin-layer chromatography (TLC) can be used for qualitative analysis (Rychlinska and Gudej, 2003). The main urinary metabolites of arbutin, HQ, HQ glucuronide and HQ sulfate, have been analyzed in human urine by capillary electrophoresis (CE), HPLC, and HPLC with coulometric-array detection (Glockl et al., 2001; Siegers et al., 2003; Süβ et al., 2000 abstr.; Wittig et al., 2001).

Arbutin content in quince jam samples was analyzed by reversed-phase HPLC with a diode array detector, suggesting the jams may be adulterated with pear puree (Silva et al., 2000, 2001).

Commercial arbutin cream samples were analyzed using reversed-phase HPLC with photodiode array detection. The recover range was 98.2-102.2% and the quantitation limit was 15 µg/mL. A storage-stability study was performed on commercial arbutin cream and spiked cream samples stored in 0.05 M KH_2PO_4 buffer (pH 2.5). Arbutin was stable in all samples up to four days of storage. The spiked cream and commercial cream began to hydrolyze after eight days. Arbutin recovery was 73.4% after one month of storage (Huang et al., 2004).

2.2 Physical-Chemical Properties

Property	Information	Reference(s)
Physical State	Needles (from hot ethyl acetate)	Budavari (1996)
Boiling Point (^oC)	561.6±50.0 @ 760 Torr	Registry (1984)*
Melting Point (^oC)	165 (unstable form), 199.5-200 (stable form)	Budavari (1996)
Flash Point (^oC)	293.4±54.2	Registry (1984)*
Vapor Pressure (Torr)	$1.90x10^{-13}$ @ 25 °C	Registry (1984)
Soluble in:	alcohol and water	Budavari (1996)
Molar Solubility	≥0.1 - <1 M @ pH 1-10	Registry (1984)*
Bioconcentration Factor	1 @ pH 1-10	Registry (1984)*
Adsorption Coefficient (K_{OC})	3.67 @ pH 1-10	Registry (1984)*
Octanol-water partition coefficient (log P)	-1.494±0.197	Registry (1984)*

*calculated using Advanced Chemistry Development (ACD/Labs) Software Solaris V4.67(©1994-2004 ACD/Labs)

Arbutin is very hygroscopic. It is readily hydrolyzed by dilute acids or emulsin, producing D-glucose and HQ in a 1:1 mole ratio (Budavari, 1996).

2.3 Commercial Availability

Arbutin is available commercially in both natural and synthetic (hydroquinone-β,D-glucopyranoside) forms (Lewis, 1993). ChemNet.com, a registered trademark of Hangzhou Hi2000 InfoTech Co. Ltd. lists several suppliers of Arbutin in 2-2000 kg quantities. Some websites, including amazon.com, list retail and wholesale suppliers (e.g., Nature's Way, Herb Pharm, Nature's Herbs, Planetary Formulations, Solaray, Nature's Answer, Eclectic Institute). Arbutin assayed at 99% is available from 01Wholesale.com. Uva ursi is commercially available as crushed leaf or powder. The material comes entirely from wild plants collected throughout Europe, primarily Spain, Italy, the Balkans, and the former USSR (Am. Botanical Council, 2000; A.D.A.M., Inc., 2004). American Nutrition Center supplies uva ursi (Bearberry) 4:1, uva ursi bearberry with 20% arbutin, and uva ursi leaf standard with 10% arbutin. Internet searches also found bulk raw material ingredients are supplied by Organic Herbs Inc. and Peakchem in China and Richman Chemicals in the U.S.A.

3.0 Production Processes

Arbutin can be synthesized from acetobromglucose and HQ or from the reaction of β-D-glucose pentaacetate and HQ monobenzyl ether in the presence of phosphorus oxychloride (Budavari, 1996). It can be formed *in vitro* by the incubation of HQ with enzyme extracts from bean or wheat plants or from the germination of seeds (e.g., *Viguna mungo* seeds) or plant tissues in the presence of HQ (Deisinger et al., 1996; Fujiwara and Suzuki, 1995 pat. appl.). Arbutin is also produced by biotransformation of HQ by *Catharanthus roseus* cells (Misawa, 1994).

4.0 Production and Import Volumes

No data were available.

5.0 Uses

Arbutin is used as a stabilizer for color photographic images. Therapeutically, it is used as an anti-infective for the urinary system as well as a diuretic (Budavari, 1996). It is also an inhibitor of melanin formation and a skin-lightening agent that is included in compositions used for treating skin cancer (Fujiwara and Suzuki, 1995 pat. appl.; Patrice, 1998 pat.). In a pilot study of healthy adults exposed to ultraviolet (UV) B radiation followed by topical treatment with arbutin, hyperpigmentation was inhibited in 4/6 volunteers (Kemper et al., 1999; Kim et al., 1994). In a more recent study, UV-induced pigmentation in the skin (forearm) of 15 Korean men (23-27 years old) was suppressed 43.5% by arbutin (100 mg/g [0.367 mmol/g]) (Choi et al., 2002).

In the United States, uva ursi leaf is used as a urinary antiseptic and diuretic in numerous dietary supplements (Am. Botanical Council, 2000). As over-the-counter (OTC) drugs, bearberry and "uva ursi, extract" are used as anorectics for weight control, and bearberry (extract of uva ursi) and bearberry fluidextract (extract of bearberry) are used as a "menstrual/diuretic" (FDA, 2003). *Uva ursi* (bearberry, upland cranberry) can be used for acute treatment and prevention of recurrent cystitis (Bozzuto, 2001). The use of bearberry leaves as a smoking-tobacco substitute was also mentioned. Native Americans combined the leaves with tobacco; they also powdered

the leaves and applied them to sores. Bearberry can be used to make an astringent wash or as an endometrial vasoconstrictor (Crane, 1991; Kemper, 1999; Manybeads et al., 2001).

6.0 Environmental Occurrence and Persistence

Arbutin is found in the dried leaves of a variety of plant species (see Section 2.0). In *A. uva-ursi*, the arbutin content generally ranges from 5.95 to 7.16%. In a study of wild populations of *A. uva-ursi* in various regions of Spain, arbutin levels were found to be significantly higher in autumn than in spring. The concentrations also varied based on plant location: in spring, the highest levels were found in plants from Cloterons (8.05% dry weight) and the lowest ones in those from Adraén Baix (6.30%); in autumn, the concentrations were highest in Adraén Alt (9.10%) and lowest in Guils de Cerdanya (6.97%). The bearberry density was 80-100% in Cloterons and Adraén Baix, 60-80% in Adraén Alt, and 15-20% Guils de Cerdanya, (Parejo et al., 2002).

7.0 Human Exposure

Potential human exposure to arbutin is most likely to occur occur via ingestion or dermal contact. Arbutin-containing foods include marjoram, pears (fruit, juice, possible adulterant in quince products), Japanese pepper, potatoes (*Solanum tuberosum*) infected with fungi, foods of Native Canadians, honeys in Italy and Sardinia, and beverages (Deisinger et al., 1996; Keller et al., 1996; Schmidt, 1969 diss.). Significant amounts of arbutin levels were found in wheat products (1-10 ppm [4-37 µmol/kg]), pears (4-15 ppm [0.01-0.055 mmol/kg]), and coffee and tea (0.1 ppm [0.4 µM]) (Deisinger et al., 1996). Uva ursi is generally taken by adults as crushed leaf or powder, an infusion or cold macerations, and fluidextract, providing 400-840 mg [1.47-3.09 mmol] arbutin per day. As the dry extract, 100-210 mg [0.367-0.771 µmol] arbutin are supplied to the individual (Am. Botanical Council, 2000). It is also available as a capsule, tea, and tincture (A.D.A.M., Inc., 2004). It has been estimated that an individual who drinks up to 3 g of tea from uva ursi leaves (which contain up to 6% HQ glycosides) four times a day consumes ~720 mg arbutin (2.64 mmol; ~12 mg/kg bw) (Müller and Kasper, 1996 abstr.). Exposure can also occur through the use of herbal mixtures that contain bearberry or uva ursi (see Section 5.0).

In the 1981-1983 National Institute for Occupational Safety and Health (NIOSH) National Occupational Exposure Survey (NOES), an estimated 2629 workers (595 of which were females) were potentially exposed to uva ursi in the Chemicals and Allied Products industry (Standard Industrial Classification [SIC] 28). Workers in the packaging area had the highest potential exposure. In a separate survey, which is apparently a subset of the uva ursi data, the total number of employees exposed to uva ursi powdered extract was approximated at 1339; this was also in SIC 28 (NIOSH, undated).

8.0 Regulatory Status

Bearberry is not generally recognized as safe and effective. In October 1990, the Food and Drug Administration (FDA) proposed to ban the use of 111 ingredients, including bearberry, in nonprescription diet drug products (FDA, 1990 press release). When the ban became effective, uva ursi-potassium extract was specifically named (FDA, 1991 press release). Bearberry (extract of uva ursi) and bearberry fluidextract (extract of bearberry) were included among ingredients in a proposed ban on the use of nonprescription orally administered menstrual drug products (FDA, 1992 press release). In 1999, the OTC drug e-Ludesô Capsules, containing uva ursi, was

recalled due to its being marketed without an approved new drug application (FDA, 1999). Under §111.100(d), the FDA proposed requiring that no ingredient with a known stimulant effect, such as uva ursi, be combined in a dietary supplement containing ephedrine alkaloids (FDA, 2000b). In 2000, dietary supplements manufactured by Hillestad Pharmaceuticals (Woodruff, WI) containing uva ursi and other ingredients such as saw palmetto were seized due to their status as unapproved new drugs and their failure to display adequate directions for use (FDA, 2000a). In the OTC Ingredient List, the use of bearberry and uva ursi, potassium extract as anorectics for weight control is regulated under 21 CFR 310.545(a)(20) and the use of bearberry (extract of uva ursi) and bearberry fluidextract (extract of bearberry) as a "menstrual/diuretic" is regulated under 21 CFR 310.545(a)(24); marketing of these products for these uses requires an approved new drug application (FDA, 2003). *A. uva-ursi* is an unapproved herbal product currently marketed for use in animals as a diuretic and urinary antiseptic (FDA CVM, 2000).

9.0 Toxicological Data

Note: Several toxicity studies in the following sections were published in their original non-English languages. These are noted in the citations in brackets. When available, translated abstracts were used. Therefore, full details were not provided in most cases.

9.1 General Toxicology

9.1.1 Human Data

In persons with sensitive stomachs, nausea and vomiting are possible side effects from the ingestion of dried uva ursi leaves (as low as 15 g [0.055 mol]); other symptoms that have been reported are irritability, insomnia, and an increased heart rate (Am. Botanical Council, 2000; A.D.A.M., Inc., 2004). Extremely high doses of uva ursi (i.e., ten times the recommended amount) can cause ringing in the ears, shortness of breath, convulsions, collapse, delirium, and vomiting. Liver damage is a risk with long-term use (Murray, 1997; Whole Foods Market, 2003).

A 78-year-old woman, experiencing recurrent edematous pruritic erythema on the periorbital areas and cheeks over a nine-month period, patch-tested positive for arbutin which was found in one of the cosmetics she had been using for two years (Sugawara et al., 2002).

Uva ursi use has also been linked to albuminuria, hematuria, and urine cast (Adesunloye, 2003). *Uva ursi* extract, used in a treatment study of 57 women with recurrent cystitis, produced no side effects. At the end of one year of treatment, 5/27 women in the placebo group had a recurrence compared to 0/30 women in the treatment group. Neither group reported side effects (Larsson et al., 1993 [cited by Kemper, 1999 and Murray, 1997]).

Several case reports exist where herbal supplements or mixtures containing uva ursi have been implicated as the cause. In a 56-year-old female who drank tea containing uva ursi regularly for three years to prevent recurrent urinary tract infection, bull's eye maculopathy, paracentral scotomas, reduction in electroretinography amplitude, and retinal thinning on optical coherence tomography were observed. The maculopathy was suspected to result from uva ursi because of its ability to inhibit melanin synthesis (Wang and Del Priore, 2004). In another case report, a 52-year-old woman who ingested the herbal remedy CKLS (a colon, kidney, liver, and spleen

purifier), which includes uva ursi among its ingredients, developed acute renal failure; however, aloe vera and cascara sagrada also present in the remedy were more likely the contributory factors (Adesunloye, 2003).

Absorption, Distribution, Metabolism, and Excretion: Arbutin was found to be extensively absorbed from the gastrointestinal (GI) tract and bioavailable as HQ. Volunteers (two males and two females, 36-45 years old) consuming a meal consisting of high levels of arbutin- and HQ-containing foods (coffee or tea, wheat cereal, whole wheat bread, wheat germ, and Bosc pears) had significant increases in mean total HQ (i.e., HQ and its conjugated metabolites) plasma levels; after two hours, it was five times the background concentration (at 0.15 µg/g [0.55 nmol/g]). Urinary total HQ excretion rates were also significantly increased; after two-three hours, levels were 12 times background levels. A low-HQ diet (corn cereal, 2% milk, cantaloupe, black cherry yogurt, and soft drink) resulted in a slight decrease in the mean levels of HQ in human plasma and urine (Deisinger et al., 1996).

Four hours after ingestion of a single dose of a preparation containing bearberry leaf extract (945 mg corresponding to 210 mg [0.771 mmol] arbutin), 224.5 µmol/L HQ glucuronide and 182 µmol/L HQ sulfate were recovered in the urine, which represented approximately half the administered arbutin dose (Glockl et al., 2001). In an open, randomized, two-way crossover study, 16 healthy volunteers (8 males, 8 females, mean age of 25.4-years-old) received a single oral dose of bearberry leaves dry extract (BLDE) as film-coated tablets (472.5 mg BLDE, corresponding to 105 mg [0.386 mmol] arbutin) or as an aqueous solution (945 mg BLDE, corresponding to 210 mg [0.771 mmol] arbutin). HQ glucuronide and HQ sulfate were recovered in the urine during the first four hours but no metabolites were detected after 24 hours. The rate of metabolism was faster in the group given BLDE in an aqueous solution. The total metabolite concentration represented 66.7% of the administered dose in the tablets and 64.8% in the solution. HQ glucuronide accounted for 67.3% and 70.3% of the total arbutin metabolites recovered in each group, respectively (Schindler et al., 2002).

Urine samples collected at four-hour intervals from volunteers who were kept on a xanthine- and arbutin-free diet and given bearberry leaf extract were used to develop an HPLC and coulometric-array detection method for measuring HQ in human urine. Specificity and selectivity were carried out by separation experiments involving the prodrug arbutin and its metabolites (Wittig et al., 2001). Urine samples collected from four volunteers given 6 dragees Cystinol akut (an antiseptic; 420 mg [1.54 mmol] arbutin, equivalent to 168 mg HQ) were assayed with or without added glusulase or an *E. coli* suspension, and analyzed by HPLC for HQ. Free HQ from the conjugation of HQ glucuronide and sulfate was 2.3-fold higher in the *E. coli*-suspension compared to the glusulase incubate. The HQ concentration in bacteria following separation from the urine was 20-fold higher than that in the supernatant (Siegers et al., 2003).

9.1.2 Chemical Disposition, Metabolism, and Toxicokinetics

Because arbutin is reported to hydrolyze easily in dilute acids to yield D-glucose and HQ, it is expected that ingested arbutin would be hydrolyzed to free HQ by stomach acids (Deisinger et al., 1996).

Female Wistar rats were given an aqueous solution of chromatographically pure arbutin, isolated from *Arctostaphylos uva-ursi*, and their excreted urine was evaluated by TLC, HPLC and spectral analysis. Unchanged arbutin was excreted at 82% and 100% after 16 and 30 hours post-treatment, respectively, corresponding to 90.7% of the total arbutin dose administered orally (Johdodar et al., 1983).

Oral administration of arbutin (500 mg/kg [1.84 mmol/kg]) to female rats which were overloaded with fluid resulted in a fourfold excess of excreted urine during the second hour of dosing and a total increase of 61% in the first day. No free HQ was detected in the urine samples. Diuretic activity from treatment with 200 mg/kg HQ was greater than that observed in arbutin-treated animals (Temple et al., 1971).

The relationship of bile salts to intestinal sugar transport was investigated *in vitro* and *in vivo* in adult Wistar rats. The rate of sugar uptake (with 5 mM [1 mg/mL] arbutin as substrate) *in vitro* was severely impaired by bile salts throughout the incubation period, persisting for 72 hours; the rate of absorption of arbutin *in vivo* was significantly depressed by bile salts for 48 hours and returned to normal after 72 hours (Gracey et al., 1973).

Arbutin uptake by the jejunum and ileum of rats with a bile fistula and those that received a restricted diet for five days was increased. The addition of sodium taurocholate to both groups of animals depressed arbutin uptake in both jenunum and ileum towards normal values. Taurocholate also depressed uptake of arbutin in the ileum only of normal animals. Since the results in the bile fistula rats (which only consumed approximately one-third as much food as normal rats over the first five days) and rats on a restricted diet were similar, the authors suggested monosaccharide uptake was related to reduced food intake and not the effect of bile salts (Burke et al., 1978).

The effect of microorganisms isolated from the upper GI tract of malnourished Indonesian patients (10 boys and 10 girls, 1 month to 4 years of age) on intestinal sugar transport was also studied in rats *in vivo*. Specimens of intestinal contents were obtained by pernasal intubation of the duodenum after four hours of fasting, cultured in selective media, and grown overnight in a nutrient broth. The resulting supernatant containing microorganisms in similar numbers to those found in the patients was used for the perfusion experiment in anesthetized Wistar rats. Gram-positive cocci (*Staphylococcus saprophyticus*) and the Enterobacteriaciae organisms (*Salmonella paratyphi* B, a *Shigella* and *Proteus* species) did not adversely affect intestinal absorption of arbutin (10 mM [2.7 mg/mL]); however, the gram-positive rod, *Lactobacillus* and all the species of *E. coli* inhibited arbutin absorption. Many organisms not generally considered enteropathogenic inhibited intestinal absorption when present in the lumen of the GI tract (Gracey et al., 1975).

Albino Wistar rats (normal and protein-deprived) were fed *Candida albicans* ($1x10^9$ organisms/mL) by intragastric inoculation for three days. In an *in vivo* absorption study, arbutin uptake was decreased in both groups of animals. Specimens from the small intestine were also incubated for 30 minutes with Krebs Henseleit (KH) buffer containing 3 mM arbutin and 2 mM 2-deoxy-glucose. Arbutin uptake *in vitro* decreased throughout the incubation period for samples from both normal and protein-deprived animals (Burke and Gracey, 1980).

A method for screening glycosides such as arbutin, which delayed the time-course effect of sugar absorption in mice, was reported. Male Std ddY mice were given a glucose solution with or without phlorizin or various glycosides via a stomach sonde. Arbutin delayed the postprandial blood glucose rise following glucose ingestion suggesting it may be a useful additive in treating diabetic subjects (Takii et al., 1997).

9.1.3 Acute Exposure

In mice orally or intraperitoneally (i.p.) treated with arbutin (50-200 mg/kg [0.18-0.735 mmol/kg]), a dose-dependent antitussive effect to ammonia-induced cough was observed. The antitussive effect of arbutin (200 mg/kg [0.735 mmol/kg]) was as potent as that of codeine phosphate (30 mg/kg), but arbutin had no analgesic or anesthetic effects. Additionally, arbutin had no effect on tracheal smooth muscle contraction, respiratory activity, spontaneous behavior, blood pressure, heart rate or electrical activity. At a dose of 8000 mg/kg [29.38 mmol//kg] administered i.p. for two weeks, no toxic effects were observed (Li et al., 1982 [Chin.]).

9.1.4 Short-term and Subchronic Exposure

No data were available.

9.1.5 Chronic Exposure

No data were available.

9.1.6 Synergistic/Antagonistic Effects

Arbutin and *A. uva-ursi* extract increased the inhibitory potency of prednisolone against swelling due to contact dermatitis in mice (Kubo et al., 1990 [Jpn.]). In mice, *A. uva-ursi* extract or arbutin in combination with prednisolone or dexamethasone inhibited swelling of contact dermatitis induced by picryl chloride (PC-CD) and sheep red blood cell delayed type hypersensitivity (SRBC-DTH) response to a greater extent than either of the two chemicals alone (Kubo et al., 1990 [Jpn.]; Matsuda et al., 1990 [Jpn.]). Water extracts from the leaf of *A. uva ursi* also increased the inhibitory effect of dexamethasone ointment on PC-CD- and carrageenin-induced paw edema (Matsuda et al., 1992b [Jpn.]). Arbutin plus indomethacin produced the same results in these models and showed a stronger inhibitory effect than indomethacin alone in carrageenin-induced edema and adjuvant-induced arthritis (Matsuda et al., 1991 [Jpn.]). Uva ursi may also increase the anti-inflammatory effects of nonsteroidal anti-inflammatory drugs (e.g., ibuprofen and indomethacin) (A.D.A.M., Inc., 2004).

Aloesin (an anti-inflammatory) and arbutin synergistically inhibit tyrosinase activity. In a study of their effects on UV-induced pigmentation in human skin *in vivo*, co-treatment with both chemicals (100 mg/g each) produced an additive effect; 63.3% suppression of pigmentation versus 34% with aloesin and 43.5% with arbutin alone (Choi et al., 2002). Additionally, arbutin inhibited UV-induced nuclear factor-kappaB activation in human keratinocytes (Ahn et al., 2003).

An *A. uva-ursi* extract, considered for use in food preservation, enhanced the antimicrobial activity of nisin; bearberry extract alone had no effect (Dykes et al., 2003). Arbutin counteracted

oxidative stress induced by 12-*O*-tetradecanoylphorbol-13-acetate in mouse skin (Nakamura et al., 2000).

9.1.7 Cytotoxicity

Growth of human melanoma cells and normal human melanocytes was not inhibited by exposure to 100 µg/mL [0.367 mM] arbutin for five days. At 300 µg/mL [1.10 mM] arbutin treatment for five days, cell toxicity and detachment of cells from the dishes were observed within 48 hours (Chakraborty et al., 1998).

Arbutin (5-50 µM [1-14 µg/mL]) inhibited the growth of the roots of *Allium sativum* L. and produced anti-mitotic effects. At 10 µM, the anti-mitotic effect was already visible within 24 hours and ended at 48 hours. At 20 µM [5.4 µg/mL], arbutin was very toxic, completely stopping root growth after day 1. The effects were similar to those seen with HQ at 5 µM (Deysson and Truhaut, 1957).

9.2 Reproductive and Teratological Effects

Only one reproductive toxicity study was found [Japanese]. Daily subcutaneous (s.c.) injection of arbutin (25, 100, or 400 mg/kg [0.092, 0.367, or 1.47 mmol/kg]) to male and female Sprague-Dawley rats before mating and to females during pregnancy and lactation resulted in a maximum no-effect dose of 100 mg/kg/day for reproduction and for development and growth of their offspring. No specifics were provided in the abstract; however, the following parameters were measured: viability, fertility, and mortality; musculoskeletal system growth; sex ratio; behavior and psychologic processes; mutigenerational study; reproductive toxicity; and maternal weight changes (Itabashi et al., 1988). The RTECS (1997) record states the following regarding the Itabashi et al. (1988) study: Oral (route of administration appears to be incorrectly annotated in RTECS record) administration of arbutin (lowest published toxic dose = 13,600 mg/kg [49.954 mmol/kg]) for 14 days prior to copulation and 20 days of pregnancy produced maternal effects (not specified) in the ovaries and fallopian tubes. Fetotoxicity (e.g., stunted fetus) but no deaths was reported.

9.3 Carcinogenicity

No data were available.

9.4 Initiation/Promotion Studies

No data were available.

9.5 Anticarcinogenicity

Arbutin (2.5, 12.5, or 50 µg/mL [9.2, 45.9, 180 µM]) incubated for four days weakly inhibited the growth of human colon carcinoma HCT-15 cells (Kamei et al., 1998).

9.6 Genotoxicity

Arbutin (up to 0.01 M [2.73 mg/mL]) did not induce mutations in hamster V79 cells; however, after preincubation of arbutin (≥1 mM [0.3 mg/mL]) with β-glycosidase, it did have a mutagenic effect. In mice orally treated with arbutin (0.5-2 g/kg [2-7 mmol/kg] bw), there was no induction of bone marrow micronuclei (Müller and Kasper, 1996 abstr.).

A mixture of extracts of *Uva ursi* (0.025, 0.05, 0.1, or 0.2 mg/mL [0.092, 0.2, 0.4, or 0.7 mM]) did not affect the level of micronuclei in cultured human blood lymphocytes (Joksic et al., 2003).

9.7 Cogenotoxicity
No data were available.

9.8 Antigenotoxicity
No data were available.

9.9 Immunotoxicity
In a murine local lymph node assay (LLNA), arbutin (5, 10, or 20%), applied to Balb/c female mice after topical treatment with the weak allergen α-hexylcinnamaldehyde, did not produce contact sensitization (Lee et al., 2003 [Korean]). Oral application of arbutin (10 or 50 mg/kg [0.037 or 0.18 mmol/kg]) quickly reduced the swelling of PC-CD and SRBC-DTH within 24 hours (Matsuda et al., 1990, 1991 [Jpn.]). (See also Section 9.1.6.) Arbutin (1 mg/mL [4 mM]) inhibited the binding of mouse monoclonal anti-dinitrophenyl immunoglobulin E (IgE[aDNP]) to DNP by 65% (Varga et al., 1991). In macrophage cells from male Swiss mice, arbutin (2 mg/mL 7 mM]) failed to induce the release hydrogen peroxide (Moreira et al., 2001).

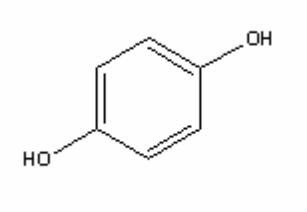
9.10 Other Data
Arbutin added to cultured human melanocytes showed a concentration-dependent reduction in tyrosinase activity and significantly reduced melanin content with low cytotoxicity (Ding et al., 2001; Maeda and Fukuda, 1991, 1996; Maeda and Naganuma, 1996; Miyazaki et al., 1998; Nishimura et al., 1995). The effects on melanocyte pigmentation at an arbutin concentration of 0.5-8 mM were evaluated by a cell-blotting assay. Cells treated with arbutin increased the pigmentation and decreased the tyrosinase activity of these cells. No changes were detected in the expression of the tyrosinase gene in melanocytes treated with arbutin (Nakajima et al., 1998). A 50% methanol extract of *A. uva-ursi* and arbutin isolated from the bearberry leaf also inhibited tyrosinase activity and melanin production *in vitro* (Matsuda et al., 1992a [Jpn.]).

Arbutin exhibited potent inhibitory effects on rat platelet aggregation induced by adenosine diphosphate (ADP; IC_{50}=0.12 mM) and collagen (IC_{50}=0.039 mM) and displayed the same inhibitory activities as the positive control, tetramethylene glutaric acid, on rat lens aldose reductase (Lim et al., 2003 [Korean]).

Unanaesthetized male and female cats were administered oral (p.o.) and i.p. doses of 50 and 100 mg/kg [0.18 or 0.367 mmol/kg] bw arbutin in water and observed at 0.5-, 1-, 2-, and 5-hour intervals. Cough parameters evaluated were as follows: number of efforts, frequency, intensity of maximum expiration and inspiration, cough effort, and intensity of cough attack in expiration and inspiration. Arbutin at 50 mg/kg bw (i.p. and p.o.) elicited a statistically significant decrease in the number, intensity, and frequency of coughs. Similar results were recorded for the 100 mg/kg bw i.p. and p.o. doses; an increase in antitussive activity was not observed at this dose (Strapkova et al., 1991).

10.0 Structure-Activity Relationships
In this section, the results from NTP studies are summarized.

Hydroquinone (HQ) [CAS No. 123-31-9]

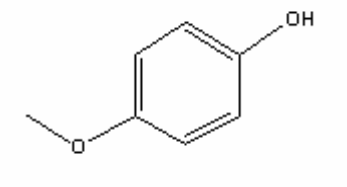


The health effects of HQ have been extensively reviewed in IPCS Environmental Health Criteria No. 157 and summarized in IPCS Health and Safety Guide No. 101 (IPCS, 1994, 1996).

In a two-year carcinogenesis bioassay, HQ (25 or 50, or 100 mg/kg) administered by gavage gave some evidence of carcinogenicity in male F344/N rats (kidney tubular cell adenoma), female F344/N rats (mononuclear cell leukemia), and female $B6C3F_1$ mice (liver adenoma or carcinoma). There was no evidence of carcinogenicity in male mice. Additionally, the incidence of thyroid follicular cell hyperplasia was increased in female and male mice (NTP, 1989, 2001a, 2004a).

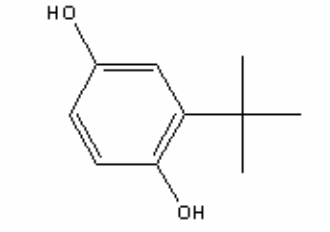
In *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, HQ was negative for mutagenicity in the presence and absence of metabolic activation (S9). In Chinese hamster ovary (CHO) cells, it induced sister chromatid exchanges (with and without S9) and chromosomal aberrations (with S9). HQ also induced trifluorothymidine resistance in mouse L5178Y/TK lymphoma cells and was mutagenic in the micronucleus test. Inconclusive results, however, were obtained in *Drosophila* (NTP, 1989, 2004a).

Hydroquinone Monomethyl Ether [CAS No. 150-76-5]



HQ monomethyl ether was negative in *Salmonella* mutagenicity assays (NTP, 2004b).

tert-Butylhydroquinone (TBHQ) [CAS No. 1948-33-0]



TBHQ (0.125, 0.25, or 0.5% [1250, 2500, or 5000 ppm]) administered in feed for lifetime produced no evidence of carcinogenicity in male and female Fischer 344 rats and $B6C3F_1$ mice (NTP, 2001b, 2004c). When the rats were placed on a diet restriction (0 or 5000 ppm in feed for

30 months), survival rates increased, while the incidences of neoplasms and nonneoplastic lesions at various sites were maintained (NTP, 1997a. 2001c).

In *S. typhimurium* strains TA97, TA98, TA100, and TA102, negative results were obtained with and without S9. TBHQ was also not mutagenic in the micronucleus test. In CHO cells, it induced sister chromatid exchanges and chromosome aberrations in the presence of S9 only (NTP, 1997b, 2004c).

In immunotoxicity tests, TBHQ (25, 75, or 150 mg/kg) administered via gavage to $B6C3F_1$ mice produced increases in the amount of the third component of serum complement, in Fc-mediated adherence and phagocytosis by peritoneal adherent cells, and in natural killer cell activity. Increases were also observed in liver and spleen weights, reticulocyte number, and blood glucose increased, while a decrease in the number of neutrophils was seen (NTP, 2004c).

11.0 Online Databases and Secondary References

11.1 Online Databases

STN International Files

AGRICOLA	IPA
BIOSIS	MEDLINE
BIOTECHNO	NIOSHTIC
CABA	NTIS
CANCERLIT	Registry
EMBASE	RTECS
ESBIOBASE	TOXCENTER

National Archives and Records Administration
Code of Federal Regulations (CFR)

In-House Databases
Current Contents on Diskette®
The Merck Index, 1996, on CD-ROM

Other Databases
ChemIDplus
Dr. Duke's Phytochemical and Ethnobotanical Database

11.2 Secondary References

Budavari, S., Ed. 1996. The Merck Index, 12th ed. Merck and Company, Inc., Whitehouse Station, NJ. CD-ROM version 12:1 1996, Chapman & Hall Electronic Publishing Division.

Lewis, R.J. 1993. Hawley's Condensed Chemical Dictionary, 12th ed. New York, NY: Van Nostrand Reinhold Co., p. 94.

12.0 References

A.D.A.M., Inc. 2004. Uva Ursi. Internet address: http://www.healthandage.com/html/res/com/ConsHerbs/Uvarursich.html. Last reviewed in April 2002. Last accessed on July 7, 2004.

Adesunloye, B.A. 2003. Acute renal failure due to the herbal remedy CKLS. American Journal of Medicine, 9343(03):00427-3.

Ahn, K.S., Moon, K.-Y., Lee, J., and Kim, Y.S. 2003. Downregulation of NF-kappaB activation in human keratinocytes by melanogenic inhibitors. J Dermatol Sci, 31(3):193-201. Abstract from PubMed 12727023.

American Botanical Council. 2000. Uva Ursi leaf. Excerpt from Herbal Medicine: Expanded Commission E Monographs (published in 1994). Internet address: http://www.herbalgram.org/iherb/expandedcommissione/he098.asp. Last accessed on July 7, 2004.

Assaf, M.H., Ali, A.A., Makboul, M.A., Beck, J.P., and Anton, R. 1987. Preliminary study of phenolic glycosides from *Origanum majorana*; quantitative estimation of arbutin; cytotoxic activity of hydroquinone. Planta Med, 53(4):434-345. Abstract from PubMed 3671554.

Bozzuto, T.M. 2001. Antimicrobial Herbs. Internet Address: http://dcmsonline.org/jax-medicine/2001journals/Feb2001/herbs.htm. Last updated in February 2001. Last accessed on July 14, 2004

Burke, V., and Gracey, M. 1980. An experimental model of gastrointestinal candidiasis. J Med Microbiol, 13(1):103-110.

Burke, V., Malajczu, A., Gracey, M., Speed, T.P., and Thornett, M.L. 1978. Intestinal transport of monosaccharide after biliary diversion in the rat. Aust J Exp Biol Med Sci, 56(3):253-263.

Chakraborty, A.K., Funasaka, Y., Komoto, M., and Ichihashi, M. 1998. Effect of arbutin on melanogenic proteins in human melanocytes. Pigment Cell Res, 11(4):206-212.

Choi, S., Park, Y.-I., Lee, S.-K., Kim, J.-E., and Chung, M.-H. 2002. Aloesin inhibits hyperpigmentation induced by UV radiation. Clin Exp Dermatol, 27(6):513-515.

Chou, C.-H., and Muller, C.H. 1972. Allelopathic mechanisms of *Arctostaphylos glandulosa var. zacaensis*. Am. Midland Nat, 88(2):324-347. Abstract from TOXCENTER 1973:53737.

Crane, M.F. 1991. *Arctostaphylos uva-ursi*. In: Fire effects Information System, U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory. Internet Address: http://www.fs.fed.us/database/feis/plants/shrub/arcuva/all.html. Last updated in February 1991. Last accessed on July 7, 2004.

Deisinger, P.J., Hill, T.S., and English, J.C. 1996. Human exposure to naturally occurring hydroquinone. J Toxicol Environ Health, 47(1):31-46.

Delira, R.A., Parra-delgado, H., Apan, M.R.T. Camacho Nieto, A., and Martinez-vazquez, M. 2003. Isolation and chemical transformations of some anti-inflammatory triterpenes from *Salvia mexicana* L. var. minor Benth. Rev Soc Quim Mexico 47(2):167-172. Abstract from Chem. Abstr. 140:318007.

Deysson, G., and Truhaut, R. 1957. Influence of glucoside formation on radiomimetic activity; comparison of hydroquinone and its glucoside, arbutoside. Comp Rendus Seanc Soc Biol Fil, 151:1719-1722. Abstract from NIOSHTIC 1997:60913.

Ding, G., Chen, B., and Tang, C. 2001. Effects of arbutin on cultured human melanocytes. Disi Junyi Daxue Xuebao, 22(20): 1846-1848. Abstract from TOXCENTER 2002:813.

Dykes, G.A., Amarowicz, R., and Pegg, R.B. 2003. Enhancement of nisin antibacterial activity by a bearberry (*Arctostaphylos uva-ursi*) leaf extract. Food Microbiol, 20(2):211-216. Abstract from AGRICOLA 2003:17700.

Fan, W., Tezuka, Y., Komatsu K., Namba, T., and Kadota, S. 1999. Prolyl endopeptidase inhibitors from the underground part of *Rhodiola sacra* S H Fu. Biol Pharm Bull, 22(2):157-161. Abstract from PubMed 10077434.

FDA (Food and Drug Administration). 1990 press release. 111 Ingredients. News 10/29/1990. Internet address: http://www.fda.gov/bbs/topics/NEWS/NEW00035.html. Last accessed on July 6, 2004.

FDA. 1991 press release. FDA to ban 111 diet product ingredients. News 08/07/1991. Internet address: http://www.fda.gov/bbs/topics/NEWS/NEW00003.html. Last accessed on July 6, 2004.

FDA. 1992 press release. Banning 415 ingredients from seven categories of non prescription drugs. News 08/25/1992. Internet address: http://www.fda.gov/bbs/topics/NEWS/NEW00298.html. Last accessed on July 6, 2004.

FDA. 1999. Enforcement report for December 29, 1999. Internet address: http://www.fda.gov/bbs/topics/ENFORCE/ENF00622.html. Last accessed on July 6, 2004.

FDA. 2000a. Enforcement report for July 19, 2000. Internet address: http://www.fda.gov/bbs/topics/ENFORCE/ENF00651.html. Last accessed on July 6, 2004.

FDA. 2000b. 12 CFR Part 11. Dietary supplements containing ephedrine alkaloids; withdrawal in part. Fed Reg, 65(64):17474-17476. Internet address: http://www.fda.gov/OHRMS/DOCKETS/98fr/040300a.pdf.

FDA. 2003. OTC Ingredient List (updated July 2003). Internet address: http://www.fda.gov/cder/Offices/OTC/Ingredient_List_A-C_July_2003.pdf.

FDA CVM (Food and Drug Administration, Center for Veterinary Medicine). FDA Veterinarian. November/December 2000. Vol., XV, No. VI. Internet address: http://www.fda.gov/cvm/index/fdavet/2000/november.pdf.

Fujiwara, N., and Suzuki, I. 1995 pat. appl. Arbutin extracted from plants for therapeutic and cosmetic uses. Japan Kokai Tokkyo Koho, 4 pp. Abstract from TOXCENTER 1995:212731.

Glöckl, I., Blaschke, G., and Vei, M. 2001. Validated methods for direct determination of hydroquinone glucuronide and sulfate in human urine after oral intake of bearberry leaf extract by capillary zone electrophoresis. J Chromatogr B Biomed Sci Appl, 761:261-266.

Gracey, M., Papadimitriou, J., Burke, V., Thomas, J., and Bower, G. 1973. Effects on small-intestinal function and structure induced by feeding a deconjugated bile salt. Gut 14(7):519-528.

Gracey, M., Burke, V., Thomas, J., and Stone, D. 1975. Effect of microorganisms isolated from the upper gut of malnourished children on intestinal sugar absorption *in vivo*. Am J Clin Nutr, 28(8):841-845.

Huang, S.-C., Lin, C.-C., Huang, M.-C., and Wen, K.-C. 2004. Simultaneous determination of magnesium ascorbyl phosphate, ascorbyl gucoside, kojic acid, arbutin, and hydroquinone in skin whitening cosmetics by HPLC. J Food Drug Anal, 12(1):13-18.

IPCS (International Programme on Chemical Safety). 1994. Hydroquinone. Environmental Health Criteria 157. Geneva, Switzerland: World Health Organization. Internet address: http://www.inchem.org/documents/ehc/ehc/ehc157.htm. Last accessed on May 12, 2004.

IPCS. 1996. Hydroquinone. Health and Safety Guide No. 101. Geneva, Switzerland: World Health Organization. Internet address: http://www.inchem.org/documents/hsg/hsg/hsg101.htm. Last accessed on May 12, 2004.

Itabashi, M., Hiroyuki, A., Inoue, T., Yamate, J., Sadako, S., Masanori, T., Chiaki, T., and Yoshikuni, W. 1988. Reproductive studies with arbutin in rats by subcutaneous administration (Jpn.). Iyakuhin Kenkyu, 19(2):282-297. Abstract from TOXCENTER 1988:133285. Search result from DART Special (Document No. ETICBACK/46035). Also cited by RTECS (1997).

Jahodar, L., Leifertova, I., and Lisa, M. 1983. Elimination of arbutin from the organism. Pharmazie, 38(11):780-781.

Joksic, G., Stankovic, M., and Novak, A. 2003. Antibacterial medicinal plants Equiseti herba and Ononidis radix modulate micronucleus formation in human lymphocytes in vitro. J Environ Pathol Toxicol Oncol, 22:41-48.

Kamei, H., Koide, T., Kojima, T., Hashimoto, Y., and Hasegawa, M. 1998. Inhibition of cell growth in culture by quinones. Cancer Biother Radiopharm, 13:185-188.

Keller, H., Hohlfeld, H., Wray, V., Hahlbrock, K., Scheel, D., and Strack, D. 1996. Changes in the accumulation of soluble and cell wall-bound phenolics in elicitor-treated cell suspension cultures and fungus-infected leaves of Solanum tuberosum. Phytochemistry, 42(2):389-396. Abstract from AGRICOLA 96:44946.

Kemper, K. 1999. Uva ursi (*Arctostaphylos uva-ursi*). The Longwood Herbal Task Force. Internet address: http://www.mcp.edu/herbal/default.htm. Last updated on August 19, 1999. Last accessed on July 14, 2004.

Kim, S., Suh, K., Chae, Y., and Eom, S. 1994. The effect of arbutin, glycolic acid, kojic acid, and pentadecanoic acid on the *in vitro* and *in vivo* pigmentry system after ultraviolet B (UVB) irradiation. Korean J Dermatol, 32:977-989.

Kubo, M., Ito, M., Nakata, H., and Matsuda, H. 1990. Pharmacological studies on leaf of *Arctostaphylos uva-ursi* (L.) Spreng. I. Combined effect of 50% methanolic extract from *Arctostaphylos uva-ursi* (L.) Spreng. (bearberry leaf) and prednisolone on immuno-inflammation (Jpn.). Yakugaki Zasshi, 110(1):59-67. Abstract from PubMed 1693958.

Larsson, B., Jonasson, A., and Fianu, S. 1993. Prophylactic effect of UVA-E in women with recurrent cystitis: A preliminary report. Curr Ther Res, 53:441-443. Cited by Murray (1997) and Kemper (1999).

Lee, J.W., Park, S.H., Park, J.H., Kim, H.S. Eom, J.H., Nam, K.T., Han, B.S., Jang, D.D., Choi, K.W., Oh, H.Y. 2003. Evaluation of skin sensitization potential of skin whitening agents by local lymph node assay (Korean). J Toxicol Public Health 19(4):285-291. Abstract from TOXCENTER 2004:44008.

Li, S., Liu, G., Zhang, Y., and Xu, J. 1982. Experimental study on antitussive effect or arbutin (Chin.). Yaoxue Tongbao, 17(12):720-722. Abstract from TOXCENTER 1983:102710.

Lim, S.S, Joo, W.K, Jung, S.H, Cho, S., and Shin, K.H. 2003. Effects of Vaccinium koreanum on rat lens aldose reductase and platelet aggregation (Korean). Korean J Pharmacol, 34(4):314-317. Abstract from CABA 2004:39752.

Maeda, K., and Fukuda, M. 1991. *In vitro* effectiveness of several whitening cosmetic components in human melanocytes. J Soc Cosmet Chem, 42:361-368.

Maeda, K., and Fukuda, M. 1996. Arbutin: Mechanism of its depigmenting action in human melanocyte culture. J Pharmacol Exp Ther, 276(2):765-769.

Maeda, K., and Naganuma, M. 1996. Depigmenting action of arbutin and its new aspects in cosmetic science (Jpn.). Nippon Koshohin Kagakkaishi, 20(1):12-16. Abstract from TOXCENTER 1996:172593.

Manybeads, E., Lamm, Y., and Davis, T. 2001. *Arctostaphylos uva ursi*. Internet address: http://medplant.nmsu.edu/ursi/htm. Last accessed on July 7, 2004.

Marais, J.P., Mueller-Harvey, I., Brandt, E.V., and Ferreira, D. 2000. Polyphenols, condensed tannins, and other natural products in *Onobrychis viciifolia* (Sainfoin). J Agric Food Chem, 48(8):3440-3447. Abstract from PubMed 10956131.

Matsuda, H., Nakata, H., Tanaka, T., and Kubo, M. 1990. Pharmacological study on *Arctostaphylos uva-ursi* (L.) Spreng. II. Combined effects of arbutin and prednisolone or dexamethasone on immuno-inflammation (Jpn.). Yakugaku Zasshi, 119(1):68-76. Abstract from PubMed 2355310.

Matsuda, H., Tanaka, T., and Kubo, M. 1991. Pharmacological studies on leaf of *Arctostaphylos uva-ursi* (L.) Spreng. III. Combined effect of arbutin and indomethacin on immuno-inflammation (Jpn.). Yakugaku Zasshi, 111(4-5):253-258. Abstract from PubMed 1875280.

Matsuda, H., Nakamura, S., Shiomoto, H., Tanaka, T., and Kubo, M. 1992a. Pharmacological studies on leaf of *Arctostaphylos uva ursi* (L.) Spreng. IV. Effect of 50% methanolic extract from *Arctostaphylos uva ursi* (L.) Spreng (bearberry leaf) on melanin synthesis (Jpn.). Yakugaku Zasshi, 112(4):276-282. Abstract from PubMed 19921026.

Matsuda, H., Nakamura, S., Tanaka, T., and Kubo, M. 1992b. Pharmacological studies on leaf of *Arctostaphylos uva-ursi* (L.) Spreng. V. Effect of water extract from *Arctostaphylos uva-ursi* (L.) Spreng. (bearberry leaf) on the antiallergic and anti-inflammatory activities of dexamethasone ointment (Jpn.). Yakugaku Zasshi, 112(9):673-677. Abstract from PubMed 1469616.

Misawa, M. 1994. Approaches to increase productivity (Chapter 6). In: Plant Tissue Culture: An Alternative for Production of Useful Metabolite. FAO Agricultural Services Bulletin No. 108. FAO of the United Nations Rome. Internet address: http://www.fao.org/docrep.t0831e/t0831e07.htm. Last accessed on July 8, 2004.

Miyazaki, K., Nishida, Y., and Ichioka, M. 1998. Inhibitory effects of melanogenic inhibitors on dendricity of cultured B16 mouse melanoma cells (Jpn.). Nippon Koshohin Kagakkaishi, 22(3):182-186. Abstract from TOXCENTER 1998:215088.

Moreira, R.R., Carlos, I.Z., and Vilega, W. 2001. Release of intermediate reactive hydrogen peroxide by macrophage cells activated by natural products. Biol Pharm Bull, 24:201-204.

Müller, L., and Kasper, P. 1996 abstr. The mutagenic potential of arbutine, a naturally occurring hydroquinone glycoside. Mutat Res, 360(3):291-292.

Murray, M.T. 1997. The emerging problems of antibiotic overuse. Am J Nat Med, 4(5):5-12.

Nakajima, M., Shinoda, I., Fukuwatari, Y., and Hayasawa, H. 1998. Arbutin increases the pigmentation of cultured human melanocytes through mechanisms other than the induction of tyrosinase activity. Pigment Cell Res, 11(1):12-17.

Nakamura, Y., Torikai, K., Ohto, Y., Murakami, A., Tanaka, T., and Ohigashi, H. 2000. A simple phenolic antioxidant protocatechuic acid enhances tumor promotion and oxidative stress in female ICR mouse skin: Dose- and timing-dependent enhancement and involvement of bioactivation by tyrosine. Carcinogenesis, 21(10):1899-1907. Abstract from PubMed 11023549.

NIOSH (National Institute for Occupational Safety and Health). Undated. National Occupational Exposure Survey (1981-1983). Search results for uva ursi and uva ursi powdered extract. Internet address: http://www.cdc.gov/noes/noes4/80569sco.html and http://www.cdc.gov/noes/noes5/x35109ctr.html, respectively. Both last accessed on July 15, 2004.

Nishimura, T., Kometani, T., Okada, S., Ueno, N. and Yamamoto, T. 1995. Inhibitory effects of hydroquinone-alpha-glucoside on melanin synthesis (Jpn.). Yakugaku Zasshi, 115(8):626-632. Abstract from PubMed 19951207.

NTP (National Toxicology Program). 1989. Toxicology and carcinogenesis studies of hydroquinone (CAS No. 123-31-9) in F344/N rats and B6C3F1 mice (gavage studies). Technical Report No. 366. Internet address: http://ntp-server.niehs.nih.gov/htdocs/LT-studies/tr366.html. Last accessed on May 27, 2004.

NTP. 1997a. Effect of dietary restriction on toxicology and carcinogenesis studies in F344/N rats and B6C3F1 mice. Technical Report No. 460. Internet address: http://ntp-server.niehs.nih.gov/htdocs/LT-studies/tr460.html. Last accessed on August 18, 2004.

NTP. 1997b. Toxicology and carcinogenesis studies of t-butylhydroquinone (CAS No. 1948-33-0) in F344/N rats and B6C3F1 mice (feed studies). Technical Report No. 459. Internet address: http://ntp-server.niehs.nih.gov/htdocs/LT-studies/tr459.html. Last accessed on August 18, 2004.

NTP. 2001a. Target organs and levels of evidence. Technical Report No. 366. (Produced from Chemtrack Database 09/19/01.) Internet address: http://ntp-server.niehs.nih.gov/htdocs/Levels/Tr366levels.Html. Last accessed on May 27, 2004.

NTP. 2001b. Target organs and levels of evidence. Technical Report No. 459. (Produced from Chemtrack Database 09/19/01.) Internet address: http://ntp-server.niehs.nih.gov/htdocs/Levels/Tr459levels.Html. Last accessed on May 27, 2004.

NTP. 2001c. Target organs and levels of evidence. Technical Report No. 460. Feed restriction studies. (Produced from Chemtrack Database 09/19/01.) Internet address: http://ntp-server.niehs.nih.gov/htdocs/Levels/Tr460levels.Html. Last accessed on May 27, 2004.

NTP. 2004a. Testing status: Hydroquinone. Internet address: http://ntp-server.niehs.nih.gov/htdocs/Results_Status/Resstath/10022-H.Html. Last updated on May 13, 2004. Last accessed on May 27, 2004.

NTP. 2004b. Testing status: Hydroquinone monomethyl ether. Internet address: http://ntp-server.niehs.nih.gov/htdocs/Results_Status/Resstath/10602-Y.Html. Last updated on May 13, 2004. Last accessed on May 27, 2004.

NTP. 2004c. Testing status: *t*-Butylhydroquinone. Internet address: http://ntp-server.niehs.nih.gov/htdocs/Results_Status/Resstatb/1948330.Html. Last updated on May 13, 2004. Last accessed on May 27, 2004.

Parejo, I., Viladomat, F., Bastida, J., and Codina, C. 2002. Variation of the arbutin content in different wild populations of *Arctostaphylos uva-ursi* in Catalonia, Spain. J Herbs Spices Med Plants, 9(4):329-333.

Patrice, T. [assignee: Centre Hospitalier Universitaire de Nantes]. 1998 pat. Depigmenting agent in compositions for treating skin cancer. French Patent No. 2759289. Issue date: August 14, 1998. Fr. Demande, 17 pp. Abstract from Chem. Abstr. 129:298382.

Perry, N.B., Benn, M.H., Foster, L.M., Routledge, A., and Weavers, R.T. 1996. The glycosidic precursor of (*Z*)-5-ethylidene-2(5*H*)-furanone in *Halocarpus biformis* juvenile foliage. Phytochemistry, 42(2):453-459.

RTECS (Registry of Toxic Effects of Chemical Substances). 1997. Arbutin. RTECS No. CE8863000. Internet address: http://www.cdc.gov/niosh/rtecs/ce873d18.html. Last updated in September 1997. Last accessed on July 8, 2004.

Rychlinska, I., and Gudej, J. 2003. Qualitative and quantitative chromatographic investigation of hydroquinone derivatives in *Pyrus communis* L. flowers. Acta Pol Pharm, 60(4):309-312. Abstract from PubMed 14714860.

Schindler, G., Patzak, U., Brinkhaus, B., von Nieciecki, A., Wittig, J., Krähmer, N. Glöckl, I., and Veit, M. 2002. Urinary excretion and metabolism of arbutin after oral administration of *Arctostaphylos uvae ursi* extract as film-coated tablets and aqueous solution in healthy humans. J Clin Pharmacol, 42(8):920-927.

Schmidt, A. 1969 diss. Hydroxyzimtsäureamide in Zellkulturen von Kartoffel: Isolierung und Charakterisierung eines THT cDNA-Klons aus *Solanum tuberosum*. Internet address: http://webdoc.gwdg.de/ebook/w/2002/pub/bio/00H004/prom.pdf.

Siegers, C., Bpdinet, C., Ali, S.S., and Siegers, C.P. 2003. Bacterial deconjugation of arbutin by *Escherichia coli*. Phytomedicine, 10(Suppl. 4):58-60. Abstract from PubMed 12807345.

Silva, B.M., Andrade, P.B., Mendes, G.C., Valentao, P., Seabra, R.M., and Ferreira, M.A. 2000. Analysis of phenolic compounds in the evaluation of commercial quince jam authenticity. J Agric Food Chem, 48(7):2883-2887. Abstract from PubMed 20001006.

Silva, R.M., Andrade, P.B., Seabra, R.M., and Ferreira, M.A. 2001. Determination of selected phenolic compounds in quince jams by solid-phase extraction and HPLC. J Liq Chromatogr Related Technol, 24(18):2861-2872. Abstract from EMBASE 2001376045.

Sticher, O., Soldati, F., and Lehmann, D. 1979. High-performance liquid chromatographic separation and quantitative determination of arbutin, methylarbutin, hydroquinone and hydroquinone-monomethylether in *Arctostaphylos*, *Bergenia*, *Calluna* and *Vaccinium* species (Ger.). Planta Med, 35(3):253-261. Abstract from PubMed 432304.

Strapkova, A., Jahodar, L. and Nosalova, G. 1991. Antitussive effect of arbutin. Pharmazie, 46:611-612.

Süβ, F., Blaschke, G., and Veit, M. 2000 abstr. Direct CE analysis of hydroquinone conjugates after oral application of *Arctostaphylos* preparations in humans. Abstract No. P-137. Phytomedicine, 7(Suppl. 2):118.

Sugawara, K., Kobayashi, H, Teramae, K., Ishii, M., and Kato, A. 2002. A case of contact dermatitis due to arbutin. Environ Dermatol 9:146-148. Abstract available the Japanese Society for Contact Dermatitis at Internet address: http://www.fujita-hu.ac.jp/JSCD/all_folder/text_folder/vol9/94-14602.htm. Last accessed on July 8, 2004.

Sun, H., Wang, X., Huang, R., and Yuan, C. 1997. Determination of arbutin in the herbs of *Vaccinium vitis-idaea* L. by RP-HPLC (Chin.). Zhongguo Zhong Yao Za Zhi, 22(9):555. Abstract from PubMed 11038950.

Takii, H., Matsumoto, K., Kometani, T., Okada, S., and Fushili, T. 1997. Lowering effect of phenolic glycosides on the rise in postprandial glucose in mice. Biosci Biotech Biochem, 61(9):1531-1535.

Temple, A., Gal, F., and Reboul, C. 1971. The phenolic glucosides of certain *Ericaceae*. The elimination of arbutin and hydroquinone (Fr.). Trav Soc Pharm Montepellier, 31(1):5-12. Abstract from NIOSHTIC 1997:61443.

Varga, J.M., Kalchschmid, G., Klein, G.F., and Fritsch, P. 1991. Mechanism of allergic cross-reactions—I. Multispecific binding of ligands to a mouse monoclonal anti-DNP IgE antibody. Mol Immunol, 28(6):641-654.

Wang, L., and Del Priore, L.V. 2004. Bull's-eye maculopathy secondary to herbal toxicity from uva ursi. Am J Ophthalmol, 137:1135-1137.

Whole Foods Market. 2003. Uva Ursi. Internet address: http://www.wholefoods.com//healthinfo/wholehealth/uvaursi.html. Last accessed on July 7, 2004.

Wittig, J., Wittemer, S., and Veit, M. 2001. Validated method for the determination of hydroquinone in human urine by high-performance liquid chromatography-coulometric-array detection. J Chromatogr B, 761:125-132.

13.0 References Considered But Not Cited

Alvarado, F. 1965. The relationship between Na+ and the active transport of arbutin in the small intestine. Biochim Biophys Acta, 109:478-494.

Annuk, H., Hirmo, S., Turi, E., Mikelsaar, M., Arak, E., and Wadstrom, T. 1999. Effect on cell surface hydrophobicity and susceptibility of *Helicobacter pylori* to medicinal plant extracts. FEMS Microbiol Lett, 172(1):41-45. Abstract from PubMed 19990415.

Danner, H. 1940. The physiology of arbutin. Botan Arch, 41:168-202. Abstract from TOXCENTER 2001:285182.

Frohne, D. 1970. Urinary antiseptic effect of bearberry leaf extracts. Planta Med, 19:1-25. Abstract from IPA 70:4278.

Funayama, M., Arakawa, H., Yamamoto, R., Nishino, T., Shin, T., and Murao, S. 1995. Effects of α- and β-arbutin on activity of tyrosinases from mushroom and mouse melanoma. Biosci Biotechnol Biochem 59(1):143-144.

Hardin, J.W., and Brownie, C.F. 1994. Plants poisonous to livestock and pets in North Carolina. North Carolina Agric Res Service Bull, 0(414 Rev.):1-154. Search results from TOXLINE (Secondary Source ID: BIOSIS/97/12858).

Ito, M., Nogami, M., and Kubo, M. 1987 abstr. Anti-inflammatory effect of crude drug "*Uvae-ursi*." J Pharmaceut Sci, 76(11):S200.

Kinoshita, K., Morikawa, K., Fujita, M., and Natori, S. 1992. Inhibitory effects of plant secondary metabolites on cytotoxic activity of polymorphonuclear leukocytes. Planta Med, 58(2):137-145.

Morganti, P., Fabrizi, G., and James, B. 1999. An innovative cosmeceutical with skin whitening activity: Note I. J Appl Cosmetol 17(4):144-153. Abstract from EMBASE 2000109645.

Paper, D.H., Koehler, J., and Franz, G. 1993 abstr. Bioavailability of drug preparations containing a leaf extract of *Arctostaphylos uva-ursi* (Uvae Ursi Folium). Planta Med, 59(7 [Suppl. Issue]):A589.

Rosatti, T.J. 1988. Pollen morphology of *Arctostaphylos-uva-ursi Ericaceae*. Grana, 27(2):115-122. Abstract from BIOSIS 1988:457822.

Saario, M., Koivusalo, S., Laakso, I, and Autio, J. 2002. Allelopathic potential of lingonberry (*Vaccinium vitis-idaea* L.) litter for weed control. Biol Agric Horticult Int J, 20(1):11-28. Abstract from AGRICOLA 2003:10587.

Saeki, K., Shoji, Y., Noro, T., Miyase, T., Nakamura, Y., Funayama, M., and Isemura, M. 1999. Inhibitory effects of tetragalloylglucose and digalloylhamamelose on adhesion and *in vitro* invasion of mouse lung carcinoma cells. Planta Med, 65(3):227-229.

Saeki, K., Hayakawa, S., Noro, T., Miyase, T., Nakamura, Y., Tanji, K.I., Kumazawa, S., Nakaywama, T., and Isemura, M. 2000. Apoptosis-inducing activity of galloyl monosaccharides in human histiocytic lymphoma U937 cells. Planta Med, 66(2):124-126.

Acknowledgements

Support to the National Toxicology Program for the preparation of Chemical Information Review Document for Arbutin and Extracts from *Arctostaphylos uva-ursi* was provided by Integrated Laboratory Systems, Inc., through NIEHS Contract No. N01-ES-35515. Contributors included: Scott A. Masten, Ph.D. (Project Officer, NIEHS); Marcus A. Jackson, B.A. (Principal Investigator, ILS, Inc.); Bonnie L. Carson, M.S. (ILS, Inc.); Claudine A. Gregorio, M.A. (ILS, Inc.); Yvonne H. Straley, B.S. (ILS, Inc.); Nathanael P. Kibler, B.A. (ILS, Inc.); and Barbara A. Henning (ILS, Inc.).

Appendix A: Units and Abbreviations

ºC = degrees Celsius
µg/L = microgram(s) per liter
µg/m^3 = microgram(s) per cubic meter
µg/mL = microgram(s) per milliliter
µM = micromolar
BLDE = bearberry leaves dry extract
bw = body weight
FDA = Food and Drug Administration
g = gram(s)
g/mL = gram(s) per milliliter
GI = gastrointestinal
h = hour(s)
HPLC = high performance liquid chromatography
HQ = hydroquinone
i.g. = intragastric
i.p. = intraperitoneal(ly)
kg = kilogram(s)
L = liter(s)
lb = pound(s)
LC_{50} = lethal concentration for 50% of test animals
LD_{50} = lethal dose for 50% of test animals
M = molar
mg/kg = milligram(s) per kilogram
mg/m^3 = milligram(s) per cubic meter
mg/mL = milligram(s) per milliliter
min = minute(s)
mL/kg = milliliter(s) per kilogram
mm = millimeter(s)
mM = millimolar
mmol = millimole(s)
mmol/kg = millimoles per kilogram
mol = mole(s)
mol. wt. = molecular weight
NIEHS = National Institute of Environmental Health Services
NIOSH = National Institute for Occupational Safety and Health
n.p. = not provided
NTP = National Toxicology Program
OTC = over-the-counter
PC-CD = picryl chloride-induced contact dermatitis
p.o. = per oral(ly)
ppm = parts per million
RTECS = Registry of Toxic Effects of Chemical Substances
SRBC-DTH = sheep red blood cell delayed type hypersensitivity
TBHQ = *tert*-butylhydroquinone
UV = ultraviolet

Appendix B: Description of Search Strategy and Results

Arbutin (4-Hydroxyphenyl-β-D-glucopyranoside; Hydroquinone-β-D-glucopyranoside; Arbutoside) (CAS RN 497-76-7; ILS CODE X0110) and Extracts from *Arctostaphylos uva-ursi* (Bearberry, Upland Cranberry, etc.)

Nomination

Arbutin is a common ingredient in dietary supplements, cosmetics and dermatological preparations for skin lightening (inhibits conversion of tyrosine to melanin), and pharmaceutical preparations, particularly for urinary tract infections (in other countries). It occurs in certain foods and beverages of a normal North American diet, being found in the leaves of many other species and in other plant parts (e.g., pear fruits). Arbutin hydrolysis gives hydroquinone (HQ) (excreted by mammals primarily in the urine as the sulfate and glucuronide) from enzymatic cleavage in mammals and certain bacteria (capability of arbutin hydrolysis is commonly used in a battery of tests for characterization of bacterial species). Additional cleavage by *Escherichia coli* to hydroquinone may occur in the urinary tract (primarily during symptomatic urinary tract infections) and increase the concentration of free HQ in urine. In NTP testing in rodents, HQ induced renal tubule and liver adenomas and thyroid gland follicular cell hyperplasia. [IARC (vol. 71, 1999) stated HQ has limited evidence for carcinogenicity in animals and assigned it to Group 3.]

Search Strategy

An initial search in PubMed (free MEDLINE) for arbutin OR uva-ursi (and plant synonyms) was done on May 27, 2004, retrieving 274 records. The Chemical Information System, searched with the CAS RN on May 18 resulted in a few additional records from SANSS, DATALOG, PHYTOTOX, and AQUIRE. On July 9, 2004, a simultaneous search of MEDLINE, CANCERLIT, AGRICOLA, NIOSHTIC, EMBASE, CABA, ESBIOBASE, BIOTECHNO, BIOSIS, IPA, TOXCENTER, and NTIS was done using as search terms the CAS RN (889 records) and several chemical synonyms listed in the Registry file record. Several synonyms for *Arctostaphylos uva-ursi* were also used. The search strategy for the online session is shown in Attachment A. The numbers of records per STN International database were as follows (numbers of records printed in full from the database are in parentheses; practically every patent in the first 700 alphabetically sorted titles was printed):

```
268    ANSWERS '1-268' FROM FILE MEDLINE (3)
  3    ANSWERS '269-271' FROM FILE NIOSHTIC (3)
136    ANSWERS '272-407' FROM FILE AGRICOLA (40)
284    ANSWERS '408-691' FROM FILE CABA (68)
164    ANSWERS '692-855' FROM FILE EMBASE (69)
  5    ANSWERS '856-860' FROM FILE ESBIOBASE (0)
 33    ANSWERS '861-893' FROM FILE IPA (20)
316    ANSWERS '894-1209' FROM FILE BIOSIS (58)
160    ANSWERS '1210-1369' FROM FILE TOXCENTER (72)
  6    ANSWERS '1370-1375' FROM FILE NTIS (2)
```

Several Internet searches with the Google search engine were done using multiple strategies. Specific Internet searches included the Phytochemical and Ethnobotanical Databases, the Fire

Effects Information System (FEIS) (Forest Service), CFR titles 21, 29, and 40, the FDA and USDA websites, PubMed Central, ChemIDplus, TOXLINE core, and DART. The bulk of the information retrieved from the Internet was on commercial dietary supplements and cosmetics and reviews from various health- and plant-related web sites. The URLs for many documents retrieved during the Internet searches are listed in Attachment B.

Search Results

Search results are grouped numerically by ILS subject code (shown by the major headings in the discussion below) and by subgroups (with or without ILS subject codes). Within the groups, the database records and other pages are organized alphabetically (first author surname, company name, or publication name).

Reviews (Subject Codes 05/11)

About 25 items are in this group; some are only citations from reviews found on the Internet. Some of the more notable reviews included are the American Botanical Council (2000) Expanded Commission E Monograph; Clifford (2000), who provided a critical review of phenols in foods and beverages; Crane (1991); ESCOP (European Scientific Cooperative on Phytotherapy) (1997); Kemper (1999) (15 pp., 63 refs.); Rook (2004); and Simonet (2000). ESCOP recommended that uva ursi be used for "uncomplicated infections of the urinary tract such as cystitis when antibiotic treatment is not considered essential" (Sharp Labs., Inc., USA, 2002).

Chemical Identification (13a)

This group contains about 50 items, including 18 from the Internet, 19 from STN International biomedical databases, and 9 from PubMed. ChemIDplus, SANSS, and Registry records and pages from the Phytochemical and Ethnobotanical Databases are included. Topics in the biomedical database records include other *A. uva-ursi* constituents, descriptions of plant morphology and growth habits, and photographic images. Other constituents of *A. uva-ursi* leaves and their extracts include methylarbutin (also found in the roots), gallic acid, tannins, gallotannins, flavonoids, piceoside, and ursolic acid. See especially the Phytochemical Database listing of multiple constituents besides arbutin.

Chemical-Physical Properties (13b)

This group contains 16 items (7, STN Int.; 6, PubMed; 2, Internet; and 1, TOXLINE). Properties included are taste, polymorphism, and hydration. Reactions studied included hydrolysis, thermodegradation, photostability, storage stability of extracts, color reactions, and reduction.

Analytical Methods (13c)

This group contains 27 STN International records and 14 PubMed records. Other papers including mention of analytical methods are distributed throughout the package. No directed search for analytical methods was done in CAPLUS. Modern methods for arbutin determination in crude drugs, plant tissues and fluidextracts, and cosmetics include HPLC with UV or diode array detectors, planar and [?] high performance thin layer chromatography, spectrophotometry, and capillary zone electrophoresis.

Commercial Availability (01a/b) [Producers (01a)/Suppliers (01b)]
This group contains 33 items from Internet searches. Many are from individual suppliers of consumer dietary supplements. Some websites such as amazon.com returned lists of retail or wholesale suppliers. Some of the 133 uva-ursi health products listed by amazon.com were from the following suppliers (number of products in parentheses):

Eclectic Institute (23)
Gaia Herbs (14)
Nature's Way (12)
Nature's Herbs (4)
Nature's Answer (4)
Herb Pharm (4)
Planetary Formulas (3)
Solaray (3)
betterlife com (3)
Planetary Formulations (2)
Health From The Sun (2)
NATURE'S WAY (2)
Nature's Way Herbal Singles (1)
Nature'S Herbs (1)
Nature'S Way (1)

Searches for bulk suppliers found companies such as Peakchem and Organic Herbs Inc. in China and Richmond Chemicals in the USA. Searches of CHEMCATS, PROMT, CIN, and CEN would provide more trade information.

Production Processes (01d)
Approximately 70 database records or other items are included in several subgroups:

- ***01d: Arbutin Synthesis from Hydroquinone Added to Tissue Cultures of Other Species*** (16 records; 4 from PubMed, and 12 from fee-based databases)
- ***01d: Pharmaceutical Extractions*** (15 records)
- ***01d: Arbutin Enzymatic Synthesis (3), Arbutin Biosynthesis (3), and α-arbutin synthesis (3)***
- ***01d: Other Arbutin Preparation Processes: Preparation from Acylated Arbutins (2 patents), Reducing Microbiological Contamination (1), Bergenia Leaves as A. uva-ursi Substitute Source for Arbutin (1), and Drying and Shredding A. uva-ursi Leaves (1)***
- ***01d: Agricultural Production and Harvesting from the Wild (3)*** [See also Musil et al. (1988) in Group 13a.]
- ***01d: Plant Propagation Methods: Hydroponics (1); Tissue Culture (2); Seminatural Cultivation (1); Habitat Revival/Restoration (3); and 15 on Cultivation for Ornamental Use, Growth in Greenhouses, Use of Cuttings, and Effects of Irradiation***

The origins for the first 50 records (all but the last subgroup) were tallied: 34 records from STN International databases, 1 from TOXLINE, 7 from PubMed, and 8 from the Internet.

Production and Import Volumes (01c)
Traffic (the Wildlife Trade Monitoring Network) (undated) reported that *A. uva-ursi* has not been cultivated, indicating that plant material in trade is from wild sources. No statistics are available for annual demand for the leaves (sold dry, cut or whole).

Other Processes (01e)
One to three papers in other groups have been coded 01e. No specific group is included in this package.

Uses (01f)
Industrial uses of arbutin such as in photography and polymer science [not yet confirmed by ILS search of CAPLUS] are mentioned in *Hawley's Condensed Chemical Dictionary* (Lewis, 1993). The ~70 items in this group are primarily from searches of the biomedical databases and the

Internet. Treatment for urinary tract infections and skin-lightening dermatological and cosmetic formulations predominate. Uva-ursi is used in naturopathic (e.g., Abascal and Yarnell, 2002) and homeopathic remedies (e.g., abchomeopathy.com, 2002). Arbutin is used in biochemical identification of strains of microorganisms [arbutin hydrolysis test] (e.g., Aldova et al., 1994). (Only a few examples of this use from the search results are included in the package.) Permitted food additive use of arbutin or *A. uva-ursi* extracts was not confirmed (e.g., ILS would need to see Anonymous, 2002 [Swiss food legislation] and DaVincenzi et al., 1997); but a few articles studied the antioxidant abilities against food-contaminating microorganisms (e.g., Amarowicz et al., 2004). α-Arbutin is produced synthetically and has been found to be an order of magnitude more effective than arbutin as a skin-lightening compound for skin care products (e.g., Aoyama, 2001).

Environmental Releases, Occurrence, and Fate (04)

Approximately 60 records are included in this group. Time did not permit actual subgrouping. A large fraction of these studies report finding arbutin in other plants. (This is not a comprehensive collection; many records in the STN International results were not selected for printing.) Arbutin was a metabolite of the herbicide ioxynil (1,6-diiodo-4-cyanophenol) in barley roots, but not spinach leaves (Berűter, 1971). Its rate of photooxidation in aqueous solution has been determined (Buxton et al., 1988). It occurs in soils and leaf litter and is phytotoxic (allelopathic) (e.g., Chou and Muller, 1972; Saario et al., 2002). It is hydrolyzed by fungi (e.g., Demetriades and Emmanouil, 1971) and snails (e.g., Hryk et al., 1992), which suggests possible biodegradation when released to soil. Several sources described the geographical distribution of *A. uva-ursi* in the USA (e.g., Douglas and Bliss, 1977; Johnson and Wyatt, 2004; Rosatti, 1987, 1988; USDA NRCS, 2002). Arbutin has been reported in air particulates, presumably in pollen (Inglett and Lodge, 1959; Li et al., 2002). Malovic (1953) reported that "addition of arbutine substances to the soil increased the acidity of soil and exhibited on [*sic*] adverse effect upon the growth of higher plants." The Phytochemical and Ethnobotanical Databases list plants with arbutin (edible plant parts include rosemary leaves and bilberry fruits). A report by Russian authors suggests that arbutin may be found in sewage [or industrial effluents discharged to sewers?] (Stom et al., 1992).

Exposure Potential (02)

Items are grouped as follows:

- ***Subgroup 02: Natural Foods*** [19 records (PubMed and STN). Arbutin-containing foods include marjoram; pears (fruit, juice, possible adulterant in quince products); multiple foods and beverages (Deisinger et al., 1996); foods of Native Canadians; Japanese pepper; potatoes (*Solanum tuberosum*) infected with fungi (Keller et al., 1996; Schmidt, 1969 diss.); and honeys in Italy and Sardinia. McDonald et al. (2001) hypothesized that "Phenol and hydroquinone derived mainly from diet and gastrointestinal flora activity are causal factors of leukemia."]
- ***Subgroup 02: Potential as Food Additive*** [5 records (4 STN, 1 Internet). Two of the studies were from the Department of Applied Microbiology, University of Saskatchewan (Dykes et al., 2003b; Pegg et al., 2003 abstr.) [See also Dykes et al. (2003b) in Group 22.] The antioxidant and antimicrobial properties of *A. uva-ursi* extracts are discussed in the 4 journal articles.]
- ***Subgroup 02: Dietary Supplements, Pharmaceuticals, Skin Care Products, and Internal Cleansers*** [5 items (2 STN, 1 PubMed). Representatives on the first three topics are, of course, in other groups in this package. Renew Life Formulas, Inc., USA, has patented an

herbal intestinal tract cleanser containing uva ursi, several other herbs, and magnesium caprylate (Watson and Watson, 2003 patent). This is probably a laxative taken orally or perhaps as a suppository, not an irrigation product. Oral and dermal appear to be the major routes of consumer exposure to arbutin.]

- ***Subgroup 02: Occupational Exposure*** [This group comprises multiple web pages from the National Occupational Exposure Survey (1981-1983), a CDC website. About 2600 workers (595 female) were potentially exposed to uva ursi in the Chemicals and Allied Products industry (SIC 28). A breakdown by occupation indicates that nearly half of the workers operated packaging and filling machines. Another product exposure category is uva ursi powdered extract, which is apparently a subset of the uva ursi data. For example, the number involved in operating grinding and similar machines (357) was the same in each category. Dermal, inhalation, and unwashed hand-to-mouth contact are likely occupational exposure routes.]
- ***Subgroup 02: Free Hydroquinone in Urinary Tract*** [3 PubMed records. Siegers et al. (2003) reported that *E. coli* takes up, enriches, and metabolizes HQ glucuronides and sulfates to hydroquinone. Thus, urine of persons with urinary tract infections caused by *E. coli* (common) and treated with arbutin-containing preparations would be expected to have a higher ratio of free to conjugated HQ than that of persons eating a normal arbutin-containing diet. Note that viable *E. coli* have been found in the urine of humans and mice without urinary tract infections (Anderson et al., 2004; Schlager et al., 2003). The urine was drawn directly from the bladders of the mice.]

Regulations (24)

Bearberry is not generally recognized as safe and effective. In October 1990, FDA proposed to ban the use of 111 ingredients, including bearberry, in nonprescription diet drug products (FDA, 1990 press release). When the ban became effective, uva ursi-potassium extract was specifically named (FDA, 1991 press release). The use of bearberry (extract of uva ursi) and bearberry fluid extract (extract of bearberry) was included among ingredients in a proposed ban from nonprescription orally administered menstrual drug products (FDA, 1992 press release). FDA (1999, 2000) list product recalls for distribution without approved new drug applications. In the OTC Ingredient List Updated July 2003, the use of bearberry as an anorectic for weight control is regulated under 21 CFR 310.545(a)(20) and the use of bearberry (extract of uva ursi) and bearberry fluidextract (extract of bearberry) as a "menstrual/diuretic" is regulated under 21 CFR 310.545(a)(24)(Shustov and I). [Attempts to retrieve pdf copies of the CFR sections have not been successful.] *The FDA Veterinarian* (FDA CVM, 2000) noted that *A. uva-ursi* is an unapproved herb currently marketed for use in animals as a diuretic and urinary antiseptic.

Human Data (18) (Including Human Metabolism)

This group contains 24 records (9 PubMed, 12 STN Int., 3 Internet). The studies include clinical efficacy (10), metabolism (9), adverse effects/case studies (4), and a study of the antioxidant activity (biopsy specimens?).

ADME (12)

About 40 items are in this group (24 PubMed, 10 STN Int., 3 Internet). Human metabolism studies are in Group 18. Studies in Group 12 include studies of intestinal absorption and arbutin transport (e.g., Alvarado, 1965; Lostao et al., 1994); bacterial utilization (e.g., Brown and Thomson, 1998);

effects of bile salts on sugar uptake (e.g., Gracey et al., 1973); erythrocyte permeability (Matsumoto et al., 1989, 1991, 1993); dermal absorption (Takeoka et al., 2003 pat. appl.); and rat urinary metabolites (Temple et al., 1971 [French]).

Acute Toxicity (03)

One Chinese study of the antitussive effect of arbutin at oral and i.p doses up to 200 mg/kg in mice apparently included some short-term pharmacological experiments (Li et al., 1982). The TOXCENTER/CA abstract stated that its antitussive effect was as potent as that of codeine phosphate, but arbutin had no analgesic or anesthetic effects. Arbutin had no effect on tracheal smooth muscle contraction, respiratory activity, spontaneous behavior, blood pressure, or heart rate and electrical activity.

Short-Term and Subchronic Toxicity (06a)

No group 06a is in the package. When Li et al. (1982) (in Group 03) dosed mice with 8 g arbutin/kg i.p. (acute study?) and dosed monkeys with 50 mg/kg/day for 2 weeks, they did not observe any toxicity. Some other subchronic studies may have been done with animal models of disease, but these are placed in a separate code 14 subgroup with other short-term studies of therapeutic actions.

Chronic Toxicity (06b)

No group 06b is in the package. No long-term studies were found.

Antagonisms and Synergisms (22)

Four studies are in this group (3 PubMed, 1 STN Int.). Ahn et al. (2003) studied *in vitro* inhibition of UV-induced NF-kappaB activation. An *A. uva-ursi* extract, considered for food preservation use, enhanced the antimicrobial activity of nisin (Dykes et al., 2003a). Arbutin and *A. uva-ursi* extract increased the potency of prednisolone in inhibition of swelling due to contact dermatitis in mice (Kubo et al., 1990). [See other anti-inflammatory studies in Group 08.] Arbutin counteracted oxidative stress induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in mouse skin (Nakamura et al., 2000).

Reproductive and Developmental Toxicity (10)

Itabashi et al. (1988) (Japanese) studied the effect of arbutin in subcutaneous doses up to 400 mg/kg/day on the reproductive performance of parent rats and the development and reproductive performance of F1 rats. The maximum no-effect dose was 100 mg/kg/day. According to the RTECS record, some fetotoxicity and reproductive effects on the dams and/or offspring were noted.

Carcinogenicity (07a) and Co-Carcinogenicity (07b)

No studies were found.

Anticarcinogenicity (07c)

Several *in vitro* studies are in this group of 7 records from STN International databases. One study used tannins isolated from *A. uva-ursi* (Kakiuchi et al., 1985) and another used its galloyl glycosides (Saeki et al., 2000). Extracts were tested for antitumor activity in numerous *in vitro* screening systems by Itokawa et al. (2000) as well as in mice with Sarcoma 180A and P388 lymphocytic leukemia.

Genotoxicity (09a)

Two of the 3 records in this group are from STN International databases. Deysson and Truhaut (1957) studied cytotoxicity, growth inhibition and stimulation, and DNA damage in arbutin-treated garlic (*Allium sativum*) roots. Uva-ursi extracts were negative in micronucleus tests with human peripheral blood lymphocytes (Joksic et al., 2003). Műller and Kasper (1996 abstr.) studied micronucleus formation in mice and mutations in hamster V79 cells.

Immunotoxicity (08)

Half of the 10 records in this group are from STN International databases and the rest are from PubMed. Studies include effects on polymorphonuclear leukocytes and peritoneal macrophages; skin sensitization potential using the murine local lymph node assay (LLNA); synergistic anti-inflammatory effects in combination with prednisolone, dexamethasone, or indomethacin in mice; and the mechanism of allergic cross-reactions.

Other Biological Activities (14)

The folders in these subgroups contain a total of 92 biomedical database records (50 from PUBMED, 3 from TOXLINE, and 39 from STN International databases). Total records in each folder are shown in parentheses.

- ***Subgroup 14-23. Effects on Membranes (3)***
- ***Subgroup 14-28. Effects on Enzymes: Tyrosinase and Inhibition of Melanogenesis (37)*** [These studies are on the skin-lightening effect. Arbutin is often used as a positive control when comparing other skin-lightening agents.]
- ***Subgroup 14-28. Effects on Enzymes: P-450 (1)***
- ***Subgroup 14-30. Effects on Blood: Anticoagulant and Hypoglycemic (6)***
- ***Subgroup 14. Antioxidant and Free Radical Scavenger (7)***
- ***Subgroup 14. Therapeutic Efficacy in Animal Models, Especially Models of Disease (Other Than Diabetes and Arthritis/Inflammation) (9)***
- ***Subgroup 14. Cholegogue and Choloretic Activity (3)*** ["MESH Database definition: Gastrointestinal agents that stimulate the flow of bile into the duodenum (cholagogues) or stimulate the production of bile by the liver (choleretic)."]
- ***Subgroup 14. Uterotonic Activity [Bearberry leaf infusions did not show uterotonic activity in isolated guinea pig and rabbit uterine horn (Shipochliev, 1981).]***
- ***Subgroup 14. Antimicrobial Activity (9)***
- ***Subgroup 14. Aquatic Toxicity (3)*** [Tests on brine shrimp and *Daphnia* are used to screen phytochemicals for biological activity. See tests with algal species in the next subgroup.]
- ***Subgroup 14. Phytotoxicity and Other Effects on Plants (14)***

Structure-Activity Relationships (25)

Subgroup 25: Hydroquinone

IPCS (1994) reviewed HQ health effects in Environmental Health Criteria 157. IPCS (1996) is a Health and Safety Guide for HQ. In a two-year carcinogenesis bioassay, HQ administered by gavage gave some evidence of carcinogenicity in male (MR) and female rats (FR) and female mice (FM) (NTP HQ testing status report, 1994). Neoplastic lesions were kidney tubular cell adenoma (MR), mononuclear cell leukemia (FR), and liver adenoma and some carcinoma (FM). MM and FM exhibited thyroid gland follicular cell hyperplasia (NTP Target Organs, 2001; NTP TR-366,

1989). The RTECS record for HQ lists several reproductive effects studies, primarily in rats, and numerous *in vitro* and *in vivo* genotoxicity assays. DeCaprio (1999) published a 48-page review on the toxicology of hydroquinone; the most serious effect in humans is pigmentation of the eye, which may cause corneal damage.

Subgroup 25: Hydroquinone Monomethyl Ether; p-Hydroxyanisole
The compound was negative in NTP-sponsored *Salmonella* mutagenicity assays (NTP testing status report, 2004).

Subgroup 25: tert-Butylhydroquinone (TBHQ)
ILS prepared an NTP-sponsored genotoxicity review for this compound ca. 1994. TBHQ gave positive results in *in vitro* studies of chromosome aberrations and sister chromatid exchanges. There was no evidence of carcinogenicity in a 2-year bioassay (dosed-feed) (NTP testing status report, 2004; NTP TR-489, 1997). In an NTP-sponsored immunology study, mice showed elevated natural killer cell activity and increased phagocytosis and adherence of cRBC (NTP, undated).

Subgroup 25: Analogs from Budavari (1996)

Subgroup 25: Analogs from ChemIDplus
Only one analog, α-arbutin, was returned with the 4-hydroxyphenyl moiety in the top 15 results of a similarity search. 4-Hydroxyphenyl-*O*-xyloside was within the top 50. Some other arbutin glycosides were mentioned from another plant (the record is not in this group).

Subgroup 25: Analogs from the STN International REGISTRY File

Subgroup 25: Other Studies
Many of the studies on the effects on tyrosinase/melanogenesis compared arbutin with other plant constituents. Some were glycosides, but they did not have a 4-hydroxyphenyl moiety. Hamid et al. (2004) studied the effect of deoxyarbutin on melanogenesis in mice. Yamashita et al. (1993) found methoxy analogs of arbutin in cane molasses and compared their tyrosinase inhibition activity with that of arbutin. Glycoside binding and translocation studies compared arbutin and phenylglucose (Diez-Sampedro et al., 2000) and arbutin and other phenylglucosides (Lostao et al., 1994).