Safety Assessment of Dioscorea Villosa (Wild Yam) Root Extract as Used in Cosmetics

Status:	Re-Review for Panel Consideration
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The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Preethi Raj, Senior Scientific Analyst/Writer, CIR.

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Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Preethi S. Raj, M.Sc., Senior Scientific Writer/Analyst, CIR
Date: February 10, 2023
Subject: Re-Review of the Amended Safety Assessment of Dioscorea Villosa (Wild Yam) Root Extract

The Expert Panel for Cosmetic Ingredient Safety (Panel) published a review of the safety of Dioscorea Villosa (Wild Yam) Root Extract in 2004, with a conclusion that this ingredient is safe for use in cosmetic products (*originalreport_WildYam_032023*). In the Discussion of that report, the Panel further clarified that this conclusion is valid only for extracts prepared in a manner that produces a similar chemical profile as that described in the safety assessment, particularly in regard to diosgenin (i.e., an expected upper limit of 3.5%). Additionally, the Panel stated that extracts not prepared in a manner that produces a similar chemical profile would be considered safe if they have a similar safety test profile.

It should be noted that in 1999 a final report was issued, but never published, with an insufficient data conclusion. However, the 1999 report is not included for review because all of the data appear to have been repeated in the 2004 report.

Because it has been 15 years since the final amended report was published, in accordance with Cosmetic Ingredient Review (CIR) Procedures, the Panel should consider whether the safety assessment of Dioscorea Villosa (Wild Yam) Root Extract should be re-opened. An extensive search of the world's literature was performed for studies dated 1999 forward. An historical overview, comparison of original and new use data, search strategy, and relevant data found are enclosed herein (*newdata_WildYam_032023*).

Notable findings include two short-term oral toxicity studies and a 13-wk oral toxicity study in which the NOAEL for both rat sexes was determined to be the maximum received dose of 5000 mg/kg/d. Additionally, studies demonstrating the potential cytotoxicity of *Dioscorea villosa* (wild yam) root extract against breast cancer cell lines, anti-inflammatory effects, and a clinical study in which no significant side-effects or metabolic/endocrinal changes were seen with the 3-mo, topical application of wild yam cream in healthy premenopausal women, were found.

Also included for your review is a table of current and historical use data (*usetable_WildYam_032023*). (As per the Panel's request at the December 2022 meeting, an updated use table format has been implemented. The frequency and concentration of use is presented both cumulatively by likely duration and exposure and individually by product category.) The reported frequency of use has increased from 1 to 43 uses since the last review; however, the reported concentration of use has decreased. The maximum reported use concentration of Dioscorea Villosa (Wild Yam) Root Extract in 1999 was 15% (0.5% maximum solids from wild yam) in moisturizing formulations; in 2022, this ingredient is reported to be used at 0.3% in non-spray moisturizing products.

If upon review of the new studies and updated use data the Panel determines that a re-review is warranted, a Draft Amended Report will be presented at an upcoming meeting.

Re-Review - Dioscorea Villosa (Wild Yam) Root Extract - History and New Data

(Preethi Raj – March 2023 meeting)

Ingredients (1)	Citation	Conclusion	Use - New Data	Results	Use - Existing Data	Results	Notes
Dioscorea Villosa (Wild Yam Root Extract *Changes to Original List* *None*	1999: FR, not published IJT 23(S2): 49-54, 2004	Insufficient data safe as used (amended conclusion)	frequency of use (2022) conc of use (2022)	43 0.3%	frequency of use (1998) conc of use (1999)	1 0.00001 – 15%	frequency of use increased, but concentration of use decreased (was reported at up to 15% in moisturizing creams, lotions, powders, and sprays in 1999, and at 0.3% in non-spray moisturizing body products in 2022) New use categories in 2022: 2 new uses near the eye, 1 non-coloring hair preparation, and 1 mucous membrane use

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
Toxicity			
Lima CM, et al. Bioassay-guided evaluation of Dioscorea villosa - an acute and subchronic toxicity, antinociceptive and anti-inflammatory approach. BMC Complement Altern Med. 2013 Jul 28;13:195.	Acute toxicity	Groups of male and female rats (6/group) received either water (control), or a single dose of up to 5 mg/kg dried extract of *Dioscorea villosa* (wild yam) root extract in water, via gavage. No animals died during the 14-d observation period, and no changes in body weight or internal organ weights was observed. Soft feces was observed in both experimental and control animals, and was not attributed to the acute exposure.	no
Wojcikowski K, et al. Dioscorea villosa (wild yam) induces chronic kidney injury via pro-fibrotic pathways. Food Chem Toxicol. 2008 Sep;46(9):3122-31.	Short-term toxicity, oral	Groups of male Sprague-Dawley rats (n=4) were fed normal rat chow (controls) or chow combined with 0.72% dried ethanolic *Dioscorea villosa* (wild yam) root extract for 7, 14, or 28 d, and specifically observed for effects on the kidney and liver. No acute renal or hepatoxicity was observed. An increase in kidney fibrosis and inflammation in the livers of rats consuming the extract for 28 d was observed.	No short-term oral toxicity in original report
Lima CM, et al. Bioassay-guided evaluation of Dioscorea villosa - an acute and subchronic toxicity, antinociceptive and anti-inflammatory approach. BMC Complement Altern Med. 2013 Jul 28;13:195.	Short-term toxicity, oral	Groups of male and female rats (10/group) received either water (control) or 1000 g/kg/d dried *Dioscorea villosa* (wild yam) root extract, in water, via gavage, for 30 d. Both experimental males and females exhibited weight gain at rates significantly higher than control animals ($p < 0.0001$); this effect was attributed to the possible effect of the extract as a precursor of progesterone and corticoids, which can cause water retention. The extract was not considered toxic under the conditions of this study.	No subchronic oral toxicity in original report
Cha SB, et al. A 13-week subchronic toxicity study of a Dioscorea Rhizome water extract in rats. Regul Toxicol Pharmacol. 2021 Mar;120:104844.	Subchronic toxicity, oral	Rats were administered 0, 800, 2000, or 5000 mg/kg/d of an aqueous *Dioscorea villosa* (wild yam) root extract, via gavage, for 13 wk. At dose levels ≥ 2000 mg/kg/d, an increased incidence of zona glomerulosa hypertrophy and hyperplasia in the adrenal gland was observed in both sexes. No treatment-related adverse effects on clinical signs, body weight, food and water consumption, ophthalmic examination, urinalysis, hematology, or organ weights were observed at any dose. The NOAEL for both sexes was determined to be 5000 mg/kg/d.	No subchronic oral toxicity in original report

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
Anti-carcinogenicity			
Mazzio E, et al. Effects of wild yam root (*Dioscorea villosa*) extract on the gene expression profile of triple-negative breast cancer cells. Cancer Genomics Proteomics. 2021 Nov-Dec;18(6):735-755.	Anti-carcinogenicity	The cytotoxic potential of a crude ethanolic *Dioscorea villosa* (wild yam) root extract was evaluated in MDA-MB-231 triple-negative breast cancer cell lines at 0, 18.5, 37, 74, 148, 296, or 592 µg/ml when incubated for 24 h. A dose-response relationship was observed; at concentrations > 148 µg/ml there was near complete cell death.	No data on anti-carcinogenicity in the original report
Aumsuwan P, et al. Evaluation of wild yam (Dioscorea villosa) root extract as a potential epigenetic agent in breast cancer cells. In Vitro Cell Dev Biol Anim. 2015 Jan;51(1):59-71.	Anti-carcinogenicity	Two breast cancer cell lines, MCF-7 (estrogen receptor positive) and MDA-MB-231 (estrogen receptor negative) were treated with a methanolic extract of *Dioscorea villosa* (wild yam) root powder (0-50 µg/ml) for 72 h. Treatment with the extract reduced cell viability in both cell lines and altered mRNA and DNA methylation patterns, having an epigenetic effect on promoter regions and expression of the *GATA3* gene (a potential breast cancer biomarker).	No data on anti-carcinogenicity in the original report
Anti-inflammatory			
Lima CM, et al. Bioassay-guided evaluation of Dioscorea villosa - an acute and subchronic toxicity, antinociceptive and anti-inflammatory approach. BMC Complement Altern Med. 2013 Jul 28;13:195.	Anti-inflammatory effects	Mice received a 500 µg/cavity injection of carrageenan in the peritoneal cavity, 1 h after administration of 100, 200, or 400 mg/kg dried *Dioscorea villosa* (wild yam) root extract, in 0.9% saline with two drops of Tween 80, or dexamethasone (positive controls). After injection with phosphate buffered saline, fluid was collected immediately and leukocyte migration was measured. Significant inhibition of carrageenan-induced leukocyte migration to the peritoneal cavity 4 h after exposure was observed in all dose groups (40.41, 32.96, and 31.66%, respectively).	No anti-inflammatory data in original report
Clinical Studies			
Komesaroff PA, et al. Effects of wild yam extract on menopausal symptoms, lipids and sex hormones in healthy menopausal women. Climacteric. 2001 Jun;4(2):144-50.	Hormonal effects	A double-blind, placebo-controlled, cross-over study was conducted to examine the effects of a applying a topical wild yam cream and a placebo in 23 healthy menopausal women. After 3 mo of treatment, no significant side-effects or changes in weight, systolic or diastolic blood pressure, total serum cholesterol, triglycerides, HDL, FSH, glucose, estradiol, or serum and salivary progesterone were observed.	No clinical data in original report

Search (from 1999 on)

PubMed

dioscorea villosa wild yam root extract – 164/5

dioscorea villosa root extract steroid- 58/3

dioscorea villosa root extract estrogen – 6/0

dioscorea villosa root extract progesterone – 1/0

((((((((((((dioscorea villosa extract) OR (dioscorea villosa root extract)) OR (dioscorea villosa rhizome extract)) OR (wild yam extract)) OR (mexican wild yam)) OR (rheumatism root)) OR (devil's bones)) OR (mexican yam)) OR (atlantic yam)) OR (china root)) OR (colic root extract)) OR (extract of colic root)) OR (extract of discorea villosa)) OR (yuma) AND (1999:2023[pdat])– 54,670/5

(Aecopuree Purple Yam) OR (AEC Wild Yam Root Extract Powder) – 26/0

((((((((((((((((((((((((((((((((actigen y) OR (actiphyte of yam BG50)) OR (actiphyte of yam GL50)) OR (actiphyte of yam lipo S)) OR (actiphyte of yam PG50)) OR (activated botanicals estroherb complex)) OR (CO actiphyte of wild yam AL)) OR (CO actiphyte of wild yam GL)) OR (CO actiphyte of wild yam lipo O)) OR (CO actiphyte of wild yam lipo RS)) OR (CO actiphyte of wild yam lipo sun)) OR (dioscorea villosa (wild yam) root extract ies)) OR (FMLT psoriasolve)) OR (FMLT psoriasolve1)) OR (FMLT sebocure)) OR (FMLT sebocure1)) OR (herbex wild yam extract)) OR (multiex lipo phytogenix)) OR (multiex phytomax (lipo))) OR (multiex sapomax)) OR (nlt diosphere 2.0)) OR (premier wild yam root 10% extract)) OR (unisteron y-50)) OR (viazest yam OS)) OR (vt-218 extract of wild yam)) OR (wild yam)) OR (wild yam extract HG 595)) OR (wild yam extract HS 3705 G)) OR (wild yam extract huileux)) OR (wild yam extrait huileux)) OR (wild yam HPT titrated)) OR (wild yam HS)) OR (wild yam LS) AND (1999:2023[pdat]) – 2,530/3

General Web search

dioscorea villosa (wild yam) root extract toxicity- 34,500/2
dermal sensitization dioscorea villosa wild yam root extract – 13,600/0
dermal irritation dioscorea villosa wild yam root extract – 13,200/0
inhalation toxicity dioscorea villosa wild yam root extract – 8,450/0

Table 1. 2022 and historical frequency and concentration of use according to likely duration and exposure and by product category

	# of Uses		*Max Conc of Use (%)*	
	Dioscorea Villosa (Wild Yam) Root Extract			
	2022[1]	**1998[2]**	**2022[3]**	**1999[2]**
Totals	**43**	**1**	**0.3**	**0.00001-15**
summarized by likely duration and exposure*				
Duration of Use				
Leave-On	*39*	*1*	*0.3*	*0.00001-15*
Rinse-Off	*4*	*NR*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*
Exposure Type*				
Eye Area	2	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR
Incidental Inhalation-Spray	25[a]; 10[b]	1[b]	NR	15; 0.00001[b]
Incidental Inhalation-Powder	10[b]	1[b]	NR	0.00001[b]
Dermal Contact	42	1	0.3	0.00001-15
Deodorant (underarm)	NR	NR	NR	NR
Hair - Non-Coloring	1	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR
Nail	NR	NR	NR	NR
Mucous Membrane	1	NR	NR	NR
Baby Products	NR	NR	NR	NR
as reported by product category				
Eye Makeup Preparations				
Eye Lotion	2	NR	NR	NR
Hair Preparations (non-coloring)				
Tonics, Dressings, and Other Hair Grooming Aids	1	NR	NR	NR
Personal Cleanliness Products				
Other Personal Cleanliness Products	1	NR	NR	NR
Skin Care Preparations				
Cleansing	2	NR	NR	NR
Face and Neck (exc shave)	6	NR	NR	NR
Body and Hand (exc shave)	4	1	NR	0.00001 (0.000002% maximum solids from wild yam)
Moisturizing	23	NR	0.3 (not spray)	15 (0.5% maximum solids from wild yam)
Night	1	NR	NR	NR
Paste Masks (mud packs)	1	NR	NR	NR
Other Skin Care Preparations	2	NR	NR	NR

NR – not reported

*likely duration and exposure is derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

**Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.

[b] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories

REFERENCES

1. U.S. Food and Drug Administration Center for Food Safety & Applied Nutrition (CFSAN). 2022. Voluntary Cosmetic Registration Program - Frequency of Use of Cosmetic Ingredients (VCRP). (Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 4, 2022; received January 11, 2022.)

2. Final report of the amended safety assessment of Dioscorea Villosa (Wild Yam) root extract. *Int J Toxicol.* 2004;23 Suppl 2:49-54.

3. Personal Care Products Council. 2022. Concentration of Use by FDA Product Category: Dioscorea Villosa (Wild Yam) Root Extract. (Unpublished data submitted by the Personal Care Products Council on October 31, 2022.)

Final Report of the Amended Safety Assessment of Dioscorea Villosa (Wild Yam) Root Extract[1]

Dioscorea Villosa (Wild Yam) Root Extract is an extract of the rhizomes of the wild yam, *D. villosa*. A manufacturing process was described in which cut up and ground rhizomes are combined with an eluant (e.g., oleyl alcohol), the plant material precipitated with addition of a miscible solvent, washed, and redissolved in the original eluant. The extract contains glycoside and steroidal saponins (≤0.4%), diosgenin (≤3.5%), alkaloids, tannins, phytosterols, and starch. Levels of heavy metals, 1,4-dioxane, chloroform, methylene chloride, trichloroethylene, and benzene are reported to be below limits of detection. Although only one use was reported to the U.S. Food and Drug Administration (in a body and hand preparation), industry reported uses in body and hand creams, lotions, powders, and sprays at a concentration of 0.00001% (equivalent to 0.000002% plant solids), and in moisturizing creams, lotions, powders, and sprays at concentrations up to 15% (equivalent to 0.5% plant solids). Preparations from *D. villosa* are used in herbal medicine for treatment of a variety of ailments and by the pharmaceutical industry in the preparation of steroids. Using Dioscorea Villosa (Wild Yam) Root Extract prepared via a specified process, it is possible to produce a stable extract with a narrow range of diosgenin content. The extract produced using this methodology was tested in acute and short-term toxicity tests, dermal irritation tests, a sensitization test, an ocular irritation test, a rat uterotropic assay, and genotoxicity tests. An acute oral toxicity test produced hypoactivity, piloerection, and dyspnea and a death in 1 of 10 rats at 2 g/kg using the specified extract, but no toxicity in rats given 0.5 g/kg. A dermal toxicity test using the specified extract demonstrated no acute toxicity in rats. Both a 7-day local tolerance test and a 28-day dermal toxicity test in rats produced no significant adverse effects at the maximum tested concentration of 10%. A single application of undiluted extract to the intact and abraded skin of rabbits produced sufficient irritation for the test material to be rated "irritant," but a 10% dilution was not irritating. Undiluted extract was only mildly irritating to the conjuctiva of the rabbit eye; irritation in the iris and cornea was mild and transient. Undiluted extract was not irritating during the induction phase of a guinea pig sensitization study, nor did challenge with a 25% dilution elicit any sensitization. The specified extract at concentrations up to 500 mg/kg/day did not have any estrogenic activity in the juvenile rat uterotrophic assay. Genotoxicity assays in bacterial and mammalian systems were negative, except that Ames test strain TA 1537 was positive at one dose level using the plate incorporation method, but not using a preincubation method. Although the concentration at which the actual plant extract is used in cosmetic products is low, one of the primary safety concerns with this plant extract is the possible metabolic/endocrine activity, e.g., estrogen-like or progesterone-like activity as a result of the presence of small amounts of plant phytosterols such as diosgenin. Extracts prepared as described in this safety assessment, with an upper limit of 3.5% diosgenin, did not have any estrogenic activity, demonstrating that it is possible to produce material that does not present this specific safety concern. Although extracts from pesticide-free plants were not considered genotoxic and it was the view of the Cosmetic Ingredient Review (CIR) Expert Panel that there do not appear to be any components that could be carcinogenic, pesticide residues could raise this issue. It was urged that manufacturers limit pesticide residues to the limit previously used for lanolin of not more than 40 ppm (with not more than 10 ppm for any one residue). Based on these data, it was concluded that Dioscorea Villosa (Wild Yam) Root Extract is safe as used in cosmetic formulations. This conclusion regarding safety, however, is valid only for extracts prepared in a manner that produces a similar chemical profile as that described in this report, particularly as regards diosgenin. Extracts not prepared in a manner that produces a similar chemical profile would be considered safe if they have a similar safety test profile.

INTRODUCTION

Dioscorea Villosa (Wild Yam) Root Extract is an extract of the rhizomes of the wild yam, *Dioscorea villosa* (Pepe, Wenninger, and McEwen 2002). The safety of this ingredient was initially reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel with the conclusion that the available data were not sufficient to support the safety of this ingredient in cosmetic products (CIR 1999), but additional data were provided by one supplier, including safety test data on a well-characterized product. On the basis of these new data, the CIR Expert Panel has reached an amended conclusion regarding the safety of Dioscorea Villosa (Wild Yam) Root Extract in cosmetic products.

CHEMISTRY

Definition

As described by the Cosmetic, Toiletry, and Fragrance Association (CTFA) and listed in the *International Cosmetic Ingredient Dictionary and Handbook*, Dioscorea Villosa (Wild Yam) Root Extract (CAS no. 90147-49-2) is an extract of the rhizomes of the wild yam, *D. villosa* (CTFA 1999a; Pepe, Wenninger, and

Received 12 February 2004; accepted 4 June 2004.

[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. This report was prepared by Eric Hooker, Scientific Analyst and Writer. Address correspondence to Eric Hooker, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA. E-mail: info@cir-safety.org

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McEwen 2002). Dioscorea Villosa (Wild Yam) Root Extract is also known as Colic Root Extract; Extract of Colic Root; Dioscorea Villosa Extract; Extract of Dioscorea Villosa; Wild Yam Extract; and Extract of Wild Yam (Pepe, Wenninger, and McEwen 2002). *D. villosa* is also known as wild yam (Polunin and Robbins 1992), Mexican wild yam (Ritchason 1995; Ody 1993), colic root, rheumatism root (Ritchason 1995; Polunin and Robbins 1992), and devil's bones (Ritchason 1995). Other common names include Mexican yam, Atlantic yam, China root, and yuma (CTFA 1999a).

Physical and Chemical Properties

Only limited data are available describing the properties of this ingredient. According to CTFA (1999a) Dioscorea Villosa (Wild Yam) Root Extract has a pH of 4.0 to 6.8, refractive index of 1.362 to 1.47 (25°C), and specific gravity of 0.90 to 1.06 (25°C).

Manufacture and Production

Dioscorea Villosa (Wild Yam) Root Extract is prepared by grounding and cutting the dried rhizomes/root and extracting this material with water/alcohol (denatured) (CTFA 1999a). The plant material is further extracted by maceration or percolation with solvents, and the maceration process generally lasts 3 or more days. The solvents and extractibles are filtered. The water and alcohol can be removed by distillation and the remaining material is diluted to strength with the solvent of choice (usually propylene glycol, but butylene glycol, glycerin, water, vegetable oil, or alcohol can be used).

Active Organics (2000a) provided additional detail to this general process. Rhizomes grown pesticide-free are ground and combined 1:1 (*w*/*w*) with a specified eluant. The phytochemical constituents in the eluant are precipitated by adding a miscible solvent in which the phytochemicals are not soluble. The precipitate is washed with water and redissolved in the specified eluant. Assays are conducted for particular phytochemical constituents (e.g., diosgenin), then diluted to the desired concentration with the specified eluant. The maximum concentration of diosgenin is stated to be 3.5% (CTFA 2000). Measured values of diosgenin were 3.1% with hexyldecanol as the eluant (Active Organics 2000b) and 2.7% with oleyl alcohol as the eluant (Active Organics 2000c). Preservatives may be added.

Table 1 lists the eluants and miscible solvents which may be used in the above procedure.

Analytical Methods

This ingredient may be characterized by high-performance liquid chromatography (HPLC) or gas chromatography (GC) (Active Organics 2000d, 2000e). Data using these techniques demonstrate that most of the peaks detected relate to the eluant (hexyldecanol and oleyl alcohol were used), that plant material can be determined from overlays of the eluant alone and the extract, and that the plant materials do not degrade with time at 45°C (Active Organics 2000d, 2000e). Stability of the ingredient in oleyl alcohol was demonstrated by a comparison of a lot prepared in 1998 (stored at room temperature) and reanalyzed in 2000; showing close agreement in distribution of peaks, with many of the differences related to the oleyl alcohol peaks (Active Organics 2000e).

TABLE 1
Eluants and miscible solvents used in preparing Dioscorea Villosa (Wild Yam) Root Extract (Active Organics 2000a)

Eluant	Miscible solvent
Oleyl alcohol	Propylene glycol (± water)
Isocetyl alcohol	Butylene glycol (± water)
Isostearyl alcohol	Glycerin (± water)
Ethanol	Water
Hexyldecanol	Safflower oil

Composition

Wild yam (*D. villosa*) contains glycoside saponins (Mowrey 1986), steroidal saponins, diosgenin, alkaloids, tannins, phytosterols, and starch (Ody 1993).

At a concentration of 1% to 2% plant material, Dioscorea Villosa (Wild Yam) Root Extract contained 0.4% steroidal saponins (CTFA 1999a). The concentration of other components of raw material in Dioscorea Villosa (Wild Yam) Root Extract as sold to the trade is 97% to 99.5% solvents. The other components included the solvents water:alcohol, propylene glycol, propylene glycol:water, butylene glycol, butylene glycol:water, glycerin, glycerin:water, safflower oil, and vegetable oil and 1% of the preservatives phenonip or phenoxyethanol. Other contaminants are not known, but the following are below the limit of detection: 1,4-dioxane (<50 ppm), benzene (<50 ppm), chloroform (<25 ppm), methylene chloride (<50 ppm), trichloroethylene (<50 ppm), heavy metals, i.e., lead (<20 ppm), arsenic (<3 ppm), and iron (<100 ppm).

Ultraviolet Absorption

Dioscorea Villosa (Wild Yam) Root Extract was stated to have very low absorption at short wavelengths (CTFA 1999a). Actual absorption between 200 and 700 nm was determined using a double beam spectrophotometer (Centre Internationale de Toxicologie [CIT] 2000a); λ_{max} was 232 nm (in the UVC region) and the absorbance (λ_{max}) was 0.41 AU for a 0.106 g/L solution. The peak trailed up to and past 300 nm, but no significant absorption occurred above 250 nm.

USE

Cosmetic

Dioscorea Villosa (Wild Yam) Root Extract is reported to function as skin-conditioning agent; other uses are "trade secret"

in cosmetic formulations (CTFA 1999a). The product formulation data submitted to the Food and Drug Administration (FDA) in 1998 reported that Dioscorea Villosa (Wild Yam) Root Extract was used in one cosmetic formulation, a body and hand preparation (FDA 1998).

Data submitted to CTFA reported the concentration of plant material in raw material as sold to the trade as 0.5% to 3% (CTFA 1999a). Concentration of use information stated that the maximum concentration of Dioscorea Villosa (Wild Yam) Root Extract used in body and hand creams, lotions, powders, and sprays (excluding shaving preparations) was 0.00001% (0.000002% maximum solids from Wild Yam) and of the Extract used in moisturizing creams, lotions, powders, and sprays was 15% (0.5% maximum solids from Wild Yam) (CTFA 1999b).

Dioscorea Villosa (Wild Yam) Root Extract does not appear in Annex II (list of substances which must not form part of the composition of cosmetic products) or Annex III (list of substances which cosmetic products must not contain except subject to the restrictions and conditions laid down) of the *Cosmetics Directive of the European Union* (European Economic Community 2000).

Dioscorea Villosa (Wild Yam) Root Extract is not included in the list of Japanese cosmetic ingredients (Rempe and Santucci 1997).

Noncosmetic

D. villosa is used in herbal medicine for treatment of rheumatic diseases, colic, inflammation of the colon, cramps, intermittent claudication, menstrual cramps, and ovarian and uterine pain (Polunin and Robbins 1992). *D. villosa* root is used in the preparation of steroids by the pharmaceutical industry.

Wild yam root and wild yam extract are included as components in a patent for pharmaceutical compositions and methods for protecting and treating sun damaged skin. The patent states that wild yam contains glycoside saponins and diosgenins, which are hormonal precursors to cortical steroids that are stated to reduce pain. These materials are present in the composition at between 0.5% and 8% by weight (Murad 1998).

GENERAL BIOLOGY

Published data on the absorption, distribution, metabolism, and excretion of Dioscorea Villosa (Wild Yam) Root Extract (normally included in this section) were not found.

ANIMAL TOXICOLOGY

Acute Oral Toxicity

CIT (2000b) reported results of a single dose of Dioscorea Villosa (Wild Yam) Root Extract (oleyl alcohol eluant) in corn oil delivered by gavage to five male (182 ± 13 g) and five female (141 ± 6 g) Sprague-Dawley rats. Two dose levels were used: 500 mg/kg and 2000 mg/kg (10 animals each). Historical controls were available. Weight gain was monitored. Clinical signs were evaluated at days 1, 7, and 14. Animals were subjected to necropsy on day 15; macroscopic examination of internal organs (stomach, interstines, heart, kidneys, liver, lungs, pancreas, spleen) was performed.

Hypoactivity, piloerection, and dyspnea were observed in all animals given 2000 mg/kg. One animal (male) in the high-dose group died on day 2, but all others recovered. No clinical signs were observed in animals given 500 mg/kg and there was no mortality. Weight gain was not affected by exposure to either dose level. Macroscopic examination of internal organs found no abnormalities in any animal, including the one male in the high dose group that died on day 2 (CIT 2000b).

Acute Dermal Toxicity

Dioscorea Villosa (Wild Yam) Root Extract (oleyl alcohol eluant) in corn oil was applied undiluted to the closely clipped skin of five male (245 ± 4 g) and five female (216 ± 7 g) Sprague-Dawley rats at a dose of 2000 mg/kg (CIT 2000c). The exposed dorsum was covered with a semi-occlusive dressing for 24 h and the animals were observed for two weeks. Animals were necropsied on day 15 and a macroscopic examination of internal organs was made as described above. No effects were seen in the treated animals, except for a reduced weight gain in female rats between day 1 and day 8 (compared to historical controls). The weight gain between day 8 and 15 was not different from historical controls.

Short-Term Dermal Toxicity

The local tolerance after cutaneous applications of Dioscorea Villosa (Wild Yam) Root Extract (oleyl alcohol eluant) in corn oil for 7 days in Sprague-Dawley rats was determined (CIT 2000d). Groups of five male (341 to 457 g) and five female (219 to 293 g) rats received either 0% (control), 1%, 3%, or 10% concentration of the test material at a volume of 1 ml/kg/day. Protective collars were worn after each application, for the duration of the exposure period (6 h on weekdays, 4 h on weekends). After the exposure period the area was washed with water and dried. Clinical signs, body weight, food consumption, and macroscopic evaluation of internal organs was performed as described above. Some desquamation and very slight erythema were seen in some female animals but in none of the males. No signs of systemic toxicity were seen. The authors concluded that the test substance was not irritating in male and practically not irritating in female rats.

A 4-week study was conducted by CIT (2000e) in which the closely clipped skin of Sprague-Dawley rats were exposed to Dioscorea Villosa (Wild Yam) Root Extract (oleyl alcohol eluant) in corn oil. Groups of five male (343 to 374 g) and five female (211 to 262 g) rats received either 0% (control), 1%, 3%, or 10% concentration of the test material at a volume of 1 ml/kg/day for a period of 29 days on the entire clipped dorsum. Protective collars were worn after each application, for the duration of the exposure period (6 h on weekdays, 4 h on weekends). After the exposure period the area was washed with water and dried.

Clinical signs, including erythema and edema; body weight; food consumption; hematological parameters; urinalysis; and macroscopic and microscopic pathology of skin and major internal organs were recorded. No clinical signs, other than effects on the skin, were observed. No differences between any exposure level and controls were seen in body weight and food consumption, hematology (including blood chemistry profiles), urinalysis, macroscopic, or microscopic examination of internal organs. Desquamation and a slight to well-defined erythema at the site of application was seen in both control and exposed animals; there was no relationship of severity/frequency of these endpoints as a function of dose and the effects were attributed to individual animals reactions to the repeated clipping, washing, and drying of the application site.

Dermal Irritation

CIT (2000f) evaluated the acute dermal irritation of 10% Dioscorea Villosa (Wild Yam) Root Extract (oleyl alcohol eluant) in corn oil applied in a single topical application to three New Zealand white rabbits. A single topical application (0.5 ml) was applied a gauze pad, which was held to the clipped flank of each animal with a semiocclusive dressing. The exposed site was evaluated at 1, 24, 48, and 72 h for erythema and edema. In two animals, a very slight erythema developed but disappeared with time. In one animal, an initial well defined erythema faded to a very slight erythema. No edema was seen. The authors concluded that a 10% dilution of the test material was non-irritating to intact rabbit skin.

The primary skin irritation index of Actiphyte of Mexican Yam concentrate SP60 was determined in New Zealand albino rabbits (Laboratoire de Recherche et D'Experimentation 1998). A single application of undiluted material (0.5 ml) was deposited on each of two gauze patches; one was applied to an abraded area and the other to unabraded, healthy skin of five male and one female rabbits. The patches were removed at 24 h. Observations were carried out between 30 min and 1 h, 48 h, and 5 days after removal of the dressing. Erythema and edema in intact and abraded skin were evaluated at the first and second examinations and used to calculate a primary skin irritancy index. The authors stated that the test material was in the "irritant" range. There did not appear to be a difference in response between intact and abraded skin.

Ocular Irritation

Three male rabbits from the above test were used to evaluate the ocular irritation potential of Actiphyte of Mexican Yam concentrate SP60 (Laboratoire de Recherche et D'Experimentation 1998). Undiluted test substance (0.1 ml) was instilled into one eye without washing. Evaluation of the conjunctiva, iris, and cornea were made at 1, 24, and 48 h. Although conjunctival irritation persisted, effects on the iris and cornea had resolved at 48 h. The authors stated that the ocular irritation was in the "slightly irritant" range.

Sensitization

The potential of Dioscorea Villosa (Wild Yam) Root Extract (oleyl alcohol eluant) to induce delayed contact hypersensitivity was evaluated in Hartley Crl: (HA) BR guinea pigs (CIT 2000g). Fifteen male (354 ± 18 g) and 15 female (347 ± 14 g) animals were used. On days 1, 8, and 15 the treatment group of 20 animals received an application of the undiluted test substance to the induction site which was held in place with a waterproof plaster (controls received only corn oil) for 6 h. After the last induction and a rest period of 14 days, the vehicle and test substance (25% *w/w* in corn oil) were applied to a site different from the site of induction and held in place with a waterproof plaster for 6 h. Little erythema was seen during induction and what was seen did not appear cumulative. No sensitization was seen.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

In a study on the content and estrogen receptor of phytoestrogens in various foods, herbs, and spices, Zava, Dollbaum, and Blen (1998) did not find estrogen receptor binding in the "herb" wild yam (Dioscorea villosa).

Eagon et al. (1999) presented, in an abstract, results of an estrogen-binding assay and a reporter-gene assay. In the binding assay, an ethanol extract of wild yam roots, at the highest concentration, did interact slightly with estrogen receptors. Dilutions did not interact. In the reporter-gene assay, there was a $3.3\times$ enhancement of reporter-gene product activity at the maximum concentration, and there was a dose dependence (Eagon 2000).

The estrogenic activity of Dioscorea Villosa (Wild Yam) Root Extract (oleyl alcohol eluant) in corn oil was evaluated in a uterotrophic assay in juvenile female rats (CIT 2000h). Twenty-two-day-old female Sprague-Dawley rats were divided into six groups of six animals and treated by gavage for 4 days. The corn oil vehicle was given to one group, 17-α-ethynylestradiol (EE) and diethylstilbestrol (DES) were each used as positive controls in a group, at 0.010 and 0.015 mg/kg day^{-1}, respectively. The test material was given at doses of 50, 150, or 500 mg/kg day^{-1} to the final three groups. Animals were killed on day 5. In addition to uterine and vaginal parameters, a complete macroscopic examination of the abdominal cavity was made, focused on the reproductive tract. A microscopic examination of the uterus and the vagina was done. Positive controls demonstrated the expected vaginal epithelial cell hyperplasia and hyperkeratosis and uterine lumen dilation, endometrial epithelial cell hypertrophy/hyperplasia and myometrial hypertrophy. All doses of the test material were well tolerated. No differences from controls were noted in the uterus or vagina in animals receiving the test material, in sharp contrast to animals receiving EE and DES, which had clear evidence of estrogenic activity.

GENOTOXICITY

A bacterial reverse mutation assay of Dioscorea Villosa (Wild Yam) Root Extract (oleyl alcohol eluant) diluted with dimethylsulfoxide (DMSO) was conducted using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538,

and TA102 with and without S9 metabolic activation (Phoenix International 2000). Using the plate incorporation method, dose levels of 52, 164, 512, 1600, and 5000 μg/plate were used. A precipitate was noted at 164 μg/plate and above. Using the preincubation method, dose levels of 5, 9, 16, 29, and 52 μg/plate were used. A precipitate was noted from 16 μg/plate upwards. Sodium azide was used as a positive control for TA100 and TA1535, 2-nitrofluorene was the positive control for TA98 and TA1538, t-butyl hyperperoxide was the positive control for TA102, and 9-aminoacridine was the positive control for TA1537. In all metabolic activation studies, 2-aminoanthracene was the positive control. DMSO alone served as the negative control.

All positive and negative controls produced the expected results. Weak to moderate cytotoxicity was seen in strain TA1538 in the preincubation series at test concentrations of 9 μg/plate and above, but this was not seen with metabolic activation or in any of the plate incorporation series tests. No increase in revertants was seen with any of the strains at any test material concentration in the absence of metabolic activation. There was a statistically significant increase in TA1537 revertants (frameshift mutation) at the 52 μg/plate in the plate incorporation series compared to vehicle controls, but not in TA1538 or TA98 (also frameshift mutations) or in any other strains. At the 52 μg/plate level in the preincubation series, there was a decrease in the number of TA98 (frameshift) revertants when compared to the vehicle control. The authors concluded that the test material was not mutagenic in the bacterial reverse mutation assay.

Chromosome damage or damage to the mitotic apparatus was determined in the bone marrow micronucleus test (CIT 2000i). Sprague-Dawley rats (~5 weeks old) were given two oral treatments of Dioscorea Villosa (Wild Yam) Root Extract (oleyl alcohol eluant) in corn oil at dose levels of 0, 500, 1000, and 2000 mg/kg separated by 24 h. A positive-control group received one oral dose of cyclophosphamide at 15 mg/kg. A preliminary toxicity test produced no adverse effects. Animals were killed 24 h after the last treatment. Bone marrow smears were prepared and the number of micronucleated polchromatic erythrocytes (MPE) were determined in a standard count of 2000 polchromatic erythrocytes (PE). The polychromatic and normal erythrocyte (NE) ratio was determined in a standard count of 1000 erythrocytes. The mean values of MPE and the PE/NE ratio were not statistically different from controls and all the data were consistent with historical controls. Cyclophosphamide produced the expected increase in MPE frequency.

CARCINOGENICITY

Published data on the carcinogenicity of Dioscorea Villosa (Wild Yam) Root Extract were not found.

CLINICAL ASSESSMENT OF SAFETY

Published data on the irritation nor the sensitization potential of Dioscorea Villosa (Wild Yam) Root Extract were not found. Anecdotal information on the use in herbal medicine was not considered.

SUMMARY

Dioscorea Villosa (Wild Yam) Root Extract, an extract of the rhizomes of the wild yam, *D. villosa*, was reported in 1998 to be used in one body and hand preparation. Concentration of use data submitted by industry reported that the maximum concentration of use of Dioscorea Villosa (Wild Yam) Root Extract in body and hand creams, lotion, powders, and sprays and in moisturizing creams, lotions, powders, and sprays was 0.00001% and 15%, respectively (0.000002% and 0.5% maximum solids, respectively, from wild yam.) Wild yam (*D. villosa*), which is used in herbal medicine, contains diosgenin, steroidal saponins, glycosides saponins, alkaloids, tannin, phytosterols, and starch.

HPLC and GC techniques can be used to identify both the eluants and the plant material in Dioscorea Villosa (Wild Yam) Root Extract. Using a specified process of manufacture/production, one manufacturer demonstrated the ability to produce a stable extract with a narrow range of diosgenin content.

The extract produced using this methodology was tested in acute and short-term toxicity tests, dermal irritation tests, a sensitization test, an ocular irritation test, a rat uterotrophic assay, and genotoxicity tests.

An acute oral toxicity test produced hypoactivity, piloerection, and dyspnea and a death in one of ten rats at 2 g/kg using the specified extract, but no toxicity in rats given 0.5 g/kg. A dermal toxicity test using the specified extract demonstrated no acute toxicity in rats. Both a 7-day local tolerance test and a 28-day dermal toxicity test in rats produced no significant adverse effects at the maximum tested concentration of 10%. A single application of undiluted extract to the intact and abraded skin of rabbits produced sufficient irritation for the test material to be rated "irritant," but a 10% dilution was not irritating.

Undiluted extract was only mildly irritating to the conjuctiva of the eye. Irritation in the iris and cornea was mild and transient.

Undiluted extract also was not irritating during the induction phase of a guinea pig sensitization study. Challenge with a 25% dilution did not elicit any sensitization.

The specified extract at concentrations up to 500 mg/kg/day did not have any estrogenic activity in the juvenile rat uterotrophic assay.

Genotoxicity assays in bacterial and mammalian systems were negative, except that Ames test strain TA 1537 was positive at one dose level using the plate incorporation method, but not using a preincubation method.

DISCUSSION

In reviewing the additional data provided by one supplier, including safety test data on a well-characterized product, the CIR Expert Panel concluded that it is possible to produce an extract from the rhizome of the Mexican wild yam plant that is safe for use in cosmetics. The technique of eluant extraction using solvents, precipitation of plant extract material, and resolubilization in the original solvent described in this safety assessment can effectively produce a material that is mostly solvent, but which has a clearly identifiable plant component. The expected

upper limit of concentration of diosgenin, a plant phytosterol, by this method is 3.5%. Use of material extracted in this manner in safety tests demonstrated that Dioscorea Villosa (Wild Yam) Root Extract is minimally irritating to the skin and eye, does not present any systemic toxicity, does not have estrogenic activity, and is not genotoxic.

Although the Panel recognizes that the concentration at which the actual plant extract is used in cosmetic products is low, one of the primary safety concerns with this plant extract is the possible metabolic/endocrine activity, e.g., estrogen-like or progesterone-like activity as a result of the presence of small amounts of plant phytosterols such as diosgenin. Extracts prepared as described in this safety assessment, with an upper limit of 3.5% diosgenin, did not have any estrogenic activity, demonstrating that it is possible to produce material that does not present this specific safety concern.

Concern, however, was expressed about alternative approaches to extraction that might not produce material with the same safety profile described in this safety assessment, especially if pesticides were used on the plants. Although extracts from pesticide-free plants were not considered genotoxic and there do not appear to be any components that could be carcinogenic, pesticide residues could raise this issue. The Panel urged that manufacturers limit pesticide residues to the limit previously used for lanolin of not more than 40 ppm (with not more than 10 ppm for any one residue).

The conclusion regarding safety is valid only for extracts prepared in a manner that produces a similar chemical profile as that described in this report, particularly as regards diosgenin. Prepared in this manner, the Panel's conclusion is that these extracts do not have significant estrogenic activity. Extracts not prepared in a manner that produces a similar chemical profile would be considered safe if they have a similar safety test profile.

CONCLUSION

On the basis of the chemical and animal data included in this safety assessment, the CIR Expert Panel concludes that Dioscorea Villosa (Wild Yam) Root Extract is safe for use in cosmetic products.

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