Final Report on the Safety Assessment of Biotin[1]

Biotin is a water-soluble vitamin used as a hair-conditioning agent and a skin-conditioning agent in many cosmetic products at concentrations ranging from 0.0001% to 0.6%. Although Biotin does absorb some ultraviolet (UV) radiation, the absorption shows no peaks in the UVA or UVB region. Biotin is rapidly metabolized and excreted in urine. Little acute oral toxicity is seen in animal tests. Short-term and subchronic toxicity studies likewise found no evidence of toxicity. Although intradermal injection of a small quantity of Biotin (0.1 ml) into guinea pig skin did not produce skin irritation, Biotin (0.1% at pH 7.3) did produce slight, transient ocular irritation in rabbit eyes. Biotin was not mutagenic in bacterial tests, but positive results were found in a Tradescantia micronucleus test. There was evidence of an increase in the number of resorptions in rats receiving Biotin by subcutaneous injection, with concomitant decreases in fetal, uterine, and placental weights. Another study of mice receiving Biotin orally or by subcutaneous injection found no differences between control and treatment groups. Although there is one case study reporting an urticarial reaction in the literature, there are a very large number of individuals exposed to Biotin on a daily basis, and there is not a parallel appearance of irritation, sensitization, or other adverse reactions. Based on these available data, it was concluded that Biotin is safe as used in cosmetic formulations.

INTRODUCTION

Biotin, vitamin B_7 (Lewis 1993) or vitamin H (Wenninger, Canterbery, and McEwen 2000), is a water-soluble vitamin (Mock 1991) that functions as a hair-conditioning agent and a skin-conditioning agent—miscellaneous in cosmetic products (Wenninger, Canterbery, and McEwen 2000).

CHEMISTRY

Definition and Structure

Biotin (CAS No. 58-85-5) is the organic compound that conforms generally to the formula (Wenninger, Canterbery, and McEwen 2000) shown in Figure 1.

Biotin is also known as:

- (+)-Biotin; *d*-Biotin; *d*-(+)-Biotin (Lewis 1993)
- [3aS-(3aα,4b,6aα)]-Hexahydro-2-Oxo-1H-Thieno[3,4-*d*]Imidazole-4-Pentanoic Acid (Wenninger, Canterbery, and McEwen 2000; Budavari 1989)
- 1H-Thieno[3,4-*d*]Imidazole-4-Pentanoic Acid, Hexahydro-2-Oxo-, [3aS-(3aα,4b,6aα)]-; (Wenninger, Canterbery, and McEwen 2000)
- 1H-Thieno[3,4-*d*]Imidazole-4-Pentanoic Acid, Hexahydro-2-Oxo-, [3aS-(3aα,4β,6aα)]-; (3aS,4S,6aR)-Hexahydro-2-Oxo-1H-Thieno[3,4-d]Imidazole-4- Valeric Acid (U.S. Pharmacopeial Convention, Inc. 1995)
- Hexahydro-2-Oxo-1H-Thieno-[3,4-d]Imidazole-4-Pentanoic Acid (Bonjour 1991; Sax 1979)
- cis-Hexahydro-2-Oxo-1H-Thieno[3,4]Imidazole-4-Valeric Acid; cis-Tetrahydro-2-Oxothieno[3,4-*d*]-Imidazoline-4-Valeric Acid (Budavari 1989; Informatics, Inc. 1974)
- cis-Hexahydro-2-Oxothieno[3,4-*d*]Imidazole-4-Valeric Acid (Gennaro 1990)
- (+)-Cis-Hexahydro-2-Keto-1H-Thieno-(3,4)-Imidazole-4-Valeric Acid (Life Science Research Office [LSRO] 1978)
- 2′-Keto-3,4-Imidazolido-2-Tetrahydrothiophene-δ-n-Valeric Acid (Taylor 1988)
- 2-Keto-3,4-Imidazolido-2-Tetrahydrothiophene-n-Valeric Acid (Grant 1972)
- Vitamin B_7 (Lewis 1993)
- Vitamin H; Coenzyme R (Wenninger, Canterbery, and McEwen 2000; Lewis 1993; Budavari 1989; Informatics, Inc. 1974)
- Factor S; Factor S (Vitamin) (Lewis 1993)
- Bioepiderm; and Bios II (Lewis 1993; Budavari 1989)

Physical and Chemical Properties

The physical and chemical properties of Biotin are described in Table 1.

Manufacture and Production

dl-Biotin has been synthesized from 4-benzamido-3-ketotetrahydrothiophene and methyl γ-formylbutyrate (Harris et al. 1945) and from 3,4-diamino-2-carbomethoxythiophene (Rossy et al. 1981).

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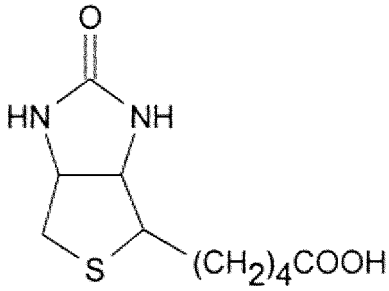

FIGURE 1
Formula for Biotin.

Natural Occurrence

Biotin occurs naturally as the D-isomer and is present in minute amounts in every living cell (Informatics, Inc. 1974). Biotin occurs in animal and plant tissues primarily in combined forms that are liberated by enzymatic hydrolysis during digestion (Gennaro 1990). Food sources of Biotin include organ meats, egg yolk, milk, fish, and nuts (Gilman 1990).

Analytical Methods

Gennaro (1990) stated that microbiological methods are the only feasible methods for the quantitative assay of Biotin because they can detect low concentrations. After simple aqueous or acid extraction combined with heating, a microbiological assay using growth of test organisms as the criterion is usually carried out. The microbiological assay using *Lactobacillus plantarum* is the most common analytical assay for Biotin (Hudson, Subramanian, and Allen 1984). However, microbiological assays detect free but not bound Biotin, and oleic acid can partially substitute for Biotin in certain microorganisms (LSRO 1978).

Biotin has been determined using a radiometric-microbiological assay, liquid chromatography with fluorimetric detection, chemiluminescence energy transfer, polarographic methods (Bonjour 1991), a high-performance liquid chromatography/avidin-binding assay (Zempleni et al. 1996), fluorometic methods (Lin and Kirsch 1979), an isotope dilution assay using ^{3}H-Biotin (Dakshinamurti and Allan 1979), an isotope dilution assay using ^{14}C-Biotin (Hood 1979), enzymatic methods (Haarasilta 1978), and colorimetric procedures (Plinton et al. 1969). Thin-layer chromatography has been used to determine Biotin in the presence of water-soluble vitamins (Gröningsson and Jansson 1979). Liquid chromatography has been used to determine Biotin in pharmaceutical preparations, and it was quantitated using low ultraviolet (UV) wavelength detection (Hudson, Subramanian, and Allen 1984). A gas chromatography method is applicable for the detection and determination of *d*-Biotin in pharmaceutical injectable preparations and agricultural premixes (Viswanthan et al. 1970). Biotin in physiological fluids can be determined using a protein-binding assay (Horsburgh and Gompertz 1978).

Impurities

One company requires that Biotin contain <0.25% diamino derivative, biotinylbiotin, and monobenzyl biotin (Cosmetic, Toiletry, and Fragrance Association [CTFA], personal communication). Biotin, which is to be not less than 97.5% and more than 100.5% by assay, may contain <3 ppm arsenic and <10 ppm heavy metals (as Pb) (CTFA 1999a); Schweizerhall, Inc. (1998) reports that Biotin contains <10 mg/kg heavy metals (as Pb). According to the *Japanese Standards of Cosmetic Ingredients*, Biotin is to contain <0.047% chloride, <0.032% sulfate, <20 ppm heavy metals, and <4 ppm arsenic.

Ultraviolet Absorbance

Biotin had low nonspecific absorption in the UVA and UVB range and into the visible spectrum (CTFA 1998). Biotin is gradually destroyed by UV radiation (Bonjour 1991).

USE

Cosmetic

Biotin functions as a hair-conditioning agent and a skin-conditioning agent—miscellaneous (Wenninger, Canterbery, and McEwen 2000). The product formulation data submitted to the Food and Drug Administration (FDA) in 1998 stated that Biotin was contained in a total of 71 cosmetic product formulations (FDA 1998). Table 2 presents the distribution of Biotin uses as a function of the type of cosmetic product. For example, of 200 total bubble bath preparations reported to be on the market, only 1 was reported to contain Biotin.

Concentration of use values are no longer reported to the FDA by the cosmetic industry (FDA 1992). Information received from industry that describes the current concentrations of use as a function of type of cosmetic product is included in Table 2. Industry reported concentrations of use in mascara and nail polish and enamel products for which FDA had not received reports of use (CTFA 1999b).

International

Biotin is listed in the *Japanese Comprehensive Licensing Standards of Cosmetics by Category* (*CLS*) (Rempe and Santucci 1997). Biotin, which conforms to the specifications of the *Japanese Standards of Cosmetic Ingredients*, has precedent for use without restriction in all *CLS* categories, except eyeliner, lip, and oral preparations, for which there is no precedent for use. Biotin 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 1995).

TABLE 1
Physical and chemical properties of Biotin

Property	Description	Reference
Physical characteristics	White to off-white fine crystalline powder	CTFA 1999a
	Practically white, crystalline powder	National Academy of Sciences (NAS) 1996
	Needles obtained with water as the solvent	Lide 1993
	Colorless, crystalline, monocarboxylic acid	Gennaro 1990
	Practically white, crystalline powder in the form of fine, long needles	Informatics, Inc. 1974
	Fine long needles (from liver or milk)	Budavari 1989
Molecular formula	$C_{10}H_{16}N_2O_3S$	Wenninger, Canterbery, and McEwen 2000
Molecular weight	244.31	NAS 1996; Lide 1993
Melting point	Decomposes, 229–232°C	CTFA 1999a; NAS 1996
	Decomposes, 232°C	Lide 1993
Biotin from liver or milk	232–233°C	Budavari 1989
pH of 0.01% aqueous solution	4.5	Budavari 1989
Isoelectric point	pH 3.5	Budavari 1989
Biotin from liver or milk	pH 3.5	Informatics, Inc. 1974
Solubility	Soluble in hot water and dilute alkali, practically insoluble in water and alcohol, and insoluble in other common organic solvents	CTFA 1999a
	More soluble in hot water and dilute alkali, insoluble in other common organic solvents	NAS 1996
	Soluble in hot water and dilute alkali, sparingly soluble in dilute acid, cold water, and alcohol, and insoluble in most organic solvents	Bonjour 1991
	Slightly soluble in water or alcohol	Gennaro 1990
	Soluble in hot water and dilute ethanol, sparingly soluble in cold water, insoluble in chloroform, benzene and ether	LSRO 1978
Specific rotation	+89° to +93° (2%, NaOH 0.1 mol/l)	CTFA 1999a
$[\alpha]^{25^\circ}_D$	+89 to +93°	NAS 1996; Informatics, Inc. 1974
$[\alpha]^{21^\circ}_D$	+91° (1 g dissolved in 0.1 N NaOH)	Budavari 1989
$[\alpha]^{22^\circ}_D$	92° (0.1 N NaOH, 1%)	Bonjour 1991; Grasselli 1973
$[\alpha]^{25^\circ}_D$	+91° (from liver or milk)	Informatics, Inc. 1974
Stability	Dry crystalline D-Biotin is fairly stable to air, daylight, and heat, but is gradually destroyed by UV; aqueous solutions are relatively stable if weakly acidic or alkaline, but the biological activity is destroyed by heating in strongly acidic or alkaline solutions	Bonjour 1991
	Water solutions are stable at 100°C; the dry substance is thermo- and photostable; unstable in strong acids and alkaline solutions and in oxidizing agents	Gennaro 1990
	Pure compound is stable to air and temperature, moderately acid and neutral solutions are stable for several months, alkaline solutions are less stable, but appear reasonably stable up to pH 9	Informatics, Inc. 1974
Reactivity	Emits toxic fumes of NO_x and SO_x when heated to decomposition	Lewis 1993
	Incompatible with nitrous acid, oxidizing agents, formaldehyde, strong acid, or alkali	Budavari 1989; Informatics, Inc. 1974

TABLE 2
Product formulation data on Biotin

Product category (number of formulations in the category) (FDA 1998)	Number of formulations containing Biotin (FDA 1998)	Current concentration of use (CTFA 1999b)
Bubble baths (200)	1	—
Mascara (167)	—	0.0001%
Other eye makeup preparations (120)	2	0.01%
Hair conditioners (636)	20	0.0001%–0.01%
Shampoos—noncoloring (860)	11	0.0001%–0.001%
Tonics, dressings, and other hair-grooming aids (549)	7	0.001%
Other hair preparations (276)	2	—
Foundations (287)	2	0.0001%–0.001%
Nail polish and enamel (80)	—	—
Bath soaps and detergents (385)	2	—
Cleansing preparations (653)	1	—
Face and neck preparations—excluding shaving (263)	1	0.002%–0.6%
Body and hand preparations—excluding shaving (796)	1	—
Moisturizing preparations (769)	5	0.005%
Night preparations (188)	4	—
Paste masks/mud packs (255)	2	—
Skin fresheners (184)	2	—
Other skin care preparations (692)	6	—
Indoor tanning preparations (62)	1	—
Other suntan preparations (38)	1	—
1998 total for Biotin	**71**	

Noncosmetic

Biotin is generally recognized as safe as a dietary supplement, as a nutrient, and in animal feed (FDA 1997). Its functional use in food is as a nutrient and dietary supplement (NAS 1996). The adult daily requirement has been assigned a provisional value of 30 to 100 μg Biotin by the Committee on Dietary Allowances, and the average daily diet provides 100 to 300 μg (Gilman 1990).

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, and Excretion

A group of 11 male humans were given twice daily for 90 days a vitamin supplement that contained 396 μg Biotin (as analyzed) (Singh, Moses, and Deuster 1992). A second group was given a placebo and served as the control. For 4 days prior to dosing and at weeks 6 and 13, baseline dietary records, fasting blood samples, and 24 hour urine samples were collected. This information and these values were also collected from nine test and eight control subjects 13.5 weeks after dose termination. In test subjects, a significant increase in the Biotin concentration in the blood and urine was observed after 6 and 13 weeks of dosing. These values returned to baseline values when measured 13.5 weeks after dose termination.

Male Sprague-Dawley rats that had been fed a Biotin-sufficient diet containing 1640 pmol/g Biotin for at least 1 week were used to study Biotin excretion (Wang, Patel, and Mock 1996). In a dose-range study, rats were dosed with 6.6 to 1025 pmol/g D-carbonyl-^{14}C-Biotin. Urine was collected after 3, 6, 9, and 24 hours, and daily for 5 days. At all doses, at least 50% of the radioactivity was excreted in the urine within 72 hours. At the 1025-pmol/g dose, approximately 80% of the radioactivity was excreted within 24 hours. Based on the results of the dose-range study, 57 pmol/g was used in the primary study.

In the primary study using six animals, >98% of the total radioactivity was excreted in the urine; Biotin, bisnorbiotin, and biotin sulfoxide accounted for >90% of the total urinary radioactivity. After 24 hours, unchanged Biotin accounted for 51% of the radioactivity, bisnorbiotin accounted for 29%, and biotin sulfoxide for 10%. On day 2, bisnorbiotin was the principal metabolite; the metabolite profiles were then relatively stable. Of the radioactivity extracted from the feces, 93% was unchanged Biotin, 6% was biotin sulfoxide, and 1% was bisnorbiotin. The researchers compared the metabolic profile of the rats with that obtained using 10 human subjects. During the first 24 hours, the human and rat profiles were similar; however, significant differences were observed between days 2 to 5.

Rats were dosed by intraperitoneal (IP) injection with 4 to 5 ml solution containing 13.5 μg ureido carbonyl-labeled ^{14}C-Biotin (0.063 μC) (Fraenkel-Conrat and Fraenkel-Conrat 1952). Urine, feces, and expired carbon dioxide were collected. Approximately 85% of the radioactivity was excreted in the urine within 24 hours, and 87% was excreted over 3 days. Approximately 7% of the radioactivity was recovered in the feces over 3 days. Approximately 5% to 8% of the radioactivity remained in the liver after 3 to 6 days, considerably less was found in the kidneys and spleen, and no radioactivity was detected in the adrenal glands, mesentery, lungs, blood, thyroid gland, or skeletal muscle.

Groups of six male Sprague-Dawley rats that had been fed Biotin-sufficient or Biotin-free diets were dosed by IP injection with 0.5 μg, 4 μg, or 1 mg/100 g body weight ureido carbonyl-labeled ^{14}C-Biotin (Lee, Wright, and McCormick 1972). Urine, feces, and expired carbon dioxide were collected. For the rats fed the Biotin-sufficient diet and then dosed with 1 mg Biotin, >90% of the dose was excreted in the urine within 12 hours. Significant radioactivity was not expired in carbon dioxide, and only a trace was excreted in the feces. The initial excretion rate decreased with decreasing doses of Biotin; this decrease was more marked with Biotin-deficient rats. For the animals fed a Biotin-sufficient diet and dosed with 0.5 or 4 μg Biotin, 47% of the dose was excreted after 12 hours and 83% was excreted after 8 hours, respectively; for the animals fed a Biotin-free diet and dosed with 0.5 μg, 29% of the dose was excreted 12 hours after dosing.

Groups of five male Sprague-Dawley rats that had been fed Biotin-sufficient or Biotin-deficient diets were dosed by IP injection with 0.5 mg/100 g body weight ureido carbonyl-labeled ^{14}C-Biotin (Lee et al. 1973). The excretion rate was similar for both groups. Approximately 95% of the dose was excreted in the urine within 24 hours, and 84% was excreted within the first 3 hours. Significant radioactivity was not found in the feces or expired carbon dioxide.

Fasted rats and chicks fed a Biotin-deficient diet for 6 and 5 weeks, respectively, were dosed intramuscularly with 1 μc (10 μg) Biotin-C^{14}OOH/100 g body weight (Dakshinamurti and Mistry 1963). Approximately 81% of the dose was recovered for rats after 4 hours, with approximately 30%, 16%, and 20% of the dose recovered in the excreta, liver, and injected muscle, respectively. Approximately 82% of the dose was recovered for chicks after 3 hours, with approximately 31%, 17%, and 14% of the dose recovered in the excreta, liver, and kidneys, respectively.

The uptake of *d*-[8,9-^{3}H(N)] Biotin by isolated hepatocytes from male Sprague-Dawley rats fed chow or a Biotin-deficient diet was studied (Rose et al. 1986). Uptake of Biotin was less by cells from rats fed a Biotin-deficient diet. Biotin uptake was markedly sensitive to temperature. ^{3}H-Biotin was Na^{+} dependent and facilitated by an acid-anion carrier.

Rose (1996) stated that, in both humans and rats, total excretion of Biotin is greater than dietary intake and postulated that Biotin synthesis occurs by microorganisms in the colon.

Biochemistry

Intestinal synthesis is an important factor in the supply of Biotin to the body (Gennaro 1990). Biotin-producing microorganisms exist in the intestinal tract (Bonjour 1991).

Biotin functions in carbon dioxide fixation reactions in intermediate metabolism, transferring the carboxyl group to acceptor molecules (Gennaro 1990). It acts similarly in decarboxylation reactions. Biotin is essential in human metabolism for its part in the previously described enzymatic steps, in catalyzing deamination of amino acids, and in oleic acid synthesis. Biotin is a cofactor for the enzymatic carboxylation of pyruvate, acetyl coenzyme A (CoA), propionyl CoA, and β-methylcrotonyl CoA, and, therefore, plays an important role in carbohydrate and fat metabolism (Gilman 1990).

The effect of Biotin on free-radical generation was examined in a spectrophotometric assay of cytochrome *c* reduction and determination of the 2-methyl-6-phenyl-3,7-dihydroimidazo[1,3 -a]-pyrazin-3-one (CLA)-dependent chemiluminescence response of human neutrophils or a hypoxanthine-xanthine oxidase (XOD) system (Sekiguchi and Nagamine 1994). In the cytochrome *c* reduction assay, two Biotin and two non-Biotin groups were used, and one of each was either stimulated or not stimulated with *N*-formylmethionylleucylphenylalanine (f-MLP). Superoxide anion (O_2^-) generation from neutrophils stimulated by f-MLP was reduced significantly by the addition of Biotin. Also, Biotin significantly reduced the generation of free-radical species (including O_2^-) in a concentration-dependent manner in the CLA-dependent chemiluminescence test of neutrophils stimulated by f-MLP. The IC_{50} was 1.12×10^{-7} moles. Using the hypoxanthine-XOD system, Biotin did not have an inhibitory effect on oxidative metabolism by directly scavenging superoxide anion.

The mechanism of Biotin uptake was examined using cultured Hep G_2 cells (Said, Ma, and Kamanna 1994). Biotin uptake was mediated through a Na^{+}-dependent, carrier-mediated mechanism; it was dependent on incubation temperature and intracellular energy.

BIOTIN DEFICIENCY

Dietary Impact

Male Long-Evans rats were fed a Biotin-deficient diet, a diet supplemented with 0.2 mg/kg Biotin, or the diet supplemented with 0.2 mg/kg Biotin and 5% cellulose for 3 weeks (Rader et al. 1986). Rats were weighed daily, feed consumption was measured weekly, and the animals were killed on day 20 or 21. The animals fed the Biotin-deficient diet had significantly decreased growth and their concentrations of some trace minerals were significantly increased as compared to animals of the other two groups.

Male inbred-BHE kine 3 rats were fed a semipurified diet with or without 5 mg/kg Biotin and controls were fed chow for 6 weeks (Marshall et al. 1972). Feed consumption and body

weights were significantly decreased and all relative organ-to-body weights, except relative liver weights, were increased for the animals fed the semipurified diet without Biotin when compared to those fed the Biotin-supplemented diet and those fed chow. Animals fed the Biotin-supplemented diet had decreased serum cholesterol concentrations compared to the group fed the Biotin-deficient diet.

Rats were fed a diet containing crystalline egg albumin with or without 25 μg Biotin or a diet with casein instead of egg white for 6 weeks (Tuszyńska 1970a). After 6 weeks, the rats fed the diet without Biotin had poor growth, loss of body fat, "bald patches" on the back, neck, and head with "dull and sparse hair" elsewhere, purulent conjunctivitis, "circles around the eyes," and a "kangaroo-like posture." Half of the animals died when the Biotin-deficient diet was continued for an additional 2 weeks. Biotin supplementation reversed the adverse effects. Tuszyńska (1970b) administered 500 μg Biotin orally and dermally to Biotin-deficient rats every other day for 2 weeks, giving a total dose of 4 mg. Oral administration of Biotin produced faster improvement and cessation of the Biotin-deficient signs. Three times as much Biotin was needed when applied to the skin to exert the same affect as Biotin given orally.

Specific-pathogen-free Sprague-Dawley rats were fed for 7 weeks a Biotin-deficient basal diet that contained 30% egg white solids; some of the animals were given 150 μg Biotin orally once weekly (Britton 1980). Animals fed a Biotin-deficient diet and not given Biotin had significantly decreased body weight gains, feed consumption, and feed efficiency ratios compared to those given Biotin. Seven of eight animals fed the Biotin-deficient diet had severe hair loss. Blood lipids and plasma cholesterol were greater for animals given Biotin, whereas lactate concentrations were greater for the rats fed a Biotin-deficient diet.

Immunological Effects

Female Lewis rats were fed a Biotin-deficient diet, a Biotin-adequate diet, or commercial feed; the animals fed the Biotin-deficient or -adequate diets were pair-fed to ensure they were given the same amount of feed (by weight), whereas the commercial feed was given ad libitum (Rabin 1983). Some of the animals were immunized with purified guinea pig myelin basic protein (MBP) followed by evaluation for experimental allergic encephalomyelitis (EAE); some of the animals were given IP injections of 1 ml of a 2% suspension of sheep erythrocytes, and the hemagglutination antibody and plaque-forming cell responses were determined after 4 days. Groups of 14 animals were not immunized and were fed their respective diets for 20 weeks.

The body weights and thymus gland weights of the animals fed the Biotin-deficient diet were significantly decreased after 20 weeks, and four of the animals had hair loss around the nose while five had eczematous-appearing skin with diffuse hair loss. The 14 animals in each of the other groups appeared normal. All of the animals fed the Biotin-adequate diet or commercial feed and immunized with MBP at 10 weeks had marked clinical changes, including hindquarter weakness or paralysis, and the spinal cords had extensive perivascular lymphocytic infiltration. Animals fed the Biotin-deficient diet did not have signs of EAE and the spinal cords had only a few perivascular lymphocytic infiltrates. Transfer of lymphocytes from animals fed a Biotin-adequate diet and immunized to MBP determined that the afferent limb of immune response is altered by Biotin deficiency. In examining the immune response in animals injected with sheep erythrocytes, it was found that the total number of plaque-forming cells in the spleen and the number per million lymphocytes were significantly decreased for animals fed the Biotin-deficient diet compared to the other animals.

Teratogenic Effects

To determine the teratogenic effects of Biotin deficiency on different species and strains of animals, groups of 11 Jcl:ICR mice, 14 C57BL/6N/Jcl mice, 11 A/Jax scl mice, 10 Jcl:Wistar rats, and 11 Std:Syrian hamsters were fed a Biotin-deficient diet starting on day 0 of gestation (Watanabe and Endo 1989). Groups of 10 to 15 animals per species and strain were fed a control diet that contained 5.0 mg/kg Biotin. Maternal weight gain and general appearance of the animals were observed every other day. The mice, rats, and hamsters were killed on days 18, 20, and 15 of gestation, respectively, and the live and dead fetuses and number of resorptions were counted. The hepatic Biotin concentration was determined in some of the dams and their fetuses.

Maternal body weight gains were decreased 60% to 90% for the ICR and C57BL mice, the rats, and the hamsters fed the Biotin-deficient diet as compared to those animals of the control groups, and daily feed consumption was decreased during the second half of gestation. Significant differences in maternal body weights and feed consumption were not observed for A/Jax mice, and these dams did not have any signs of Biotin deficiency. The frequency of dams with implants did not differ between the Biotin-deficient and the control groups, but the number of dams with live fetuses/number of dams mated was decreased for the C57BL mice and the hamsters fed the Biotin-deficient diets. For all groups except the A/Jax mice, fetal body weights were significantly decreased as compared to the respective control groups. Malformed fetuses were found in all ICR and C57BL mice fed the Biotin-deficient diet, and greater than 90% of the live fetuses were grossly malformed. The most common malformations were cleft palate, micrognathia, and micromelia in both strains; open eyelid was also observed for a number of C57BL mouse fetuses. In the Biotin-deficient A/Jax mouse group, cleft palate and micrognathia occurred at an incidence of 14%, with 31% of the fetuses having malformations. No malformations were observed in the fetuses of rats fed the Biotin-deficient diet. In hamsters, teratogenicity was "rather equivocal" because embryonic lethality was much greater for the animals fed the Biotin-deficient diet, and an insufficient number of fetuses were examined. A large difference in hepatic Biotin concentrations for the control animals was not seen among the species, but the

concentrations of the dams fed the Biotin-deficient diets were about half that of the control animals. A large difference was observed in hepatic Biotin concentrations for the fetuses of the control ICR mice, rats, and hamsters, but the concentrations were similar for fetuses of the Biotin-deficient dams.

Clinical Effect

Biotin deficiency, which can occur by the feeding of uncooked egg whites or by the omission of Biotin from the diet, can cause alopecia and a characteristic scaly, erythematous dermatitis around body orifices in infants, children, and adults (Mock 1991). For adults, prolonged Biotin deficiency can result in depression, lethargy, hallucinations, and paresthesias of the extremities.

ANIMAL TOXICOLOGY

Acute Toxicity

Oral

The oral LD_{50} of Biotin for mice was reported as >10 g/kg (Informatics, Inc. 1974), and the oral LD_{50} for rats was reported as >1.45 mmol/kg (Bonjour 1991), but no details of study procedures were given.

Parenteral

Crittenden (1948) reported that single intravenous (IV) injections to mice of 1 g/kg *dl*-Biotin were tolerated without signs of toxic effects, but details were not provided. Likewise without giving details, Bonjour (1991) reported that the IV LD_{50} of Biotin for mice was >4.1 mmol/kg, and the IP LD_{50} values for rats and cats were >0.12 and >0.001 mmol/kg, respectively.

Short-Term Toxicity

Oral

Groups of 15 and 10 mice approximately 7 and 14 weeks of age, respectively, were dosed orally with 1 mg of *dl*-Biotin or *d*-Biotin in 0.2 ml saline for 60 days; 10 7-week-old and 10 14-week-old mice, the control group, were given 0.2 ml of saline (Crittenden 1948). Signs of toxicity attributable to Biotin were not observed, and no significant differences in mortality were observed among the groups.

Five male rats were dosed orally with 50 mg of *d*-Biotin for 10 days (Crittenden 1948). Signs of toxicity were not observed, and changes in the number of erythrocytes or leukocytes or in the amount of hemoglobin were not seen. Gross lesions were not observed at necropsy.

Bonjour (1991) reported that the short-term LD_{50} was >1450 μmol/kg in a study in which rats were dosed orally with Biotin for 10 days. No further study details were provided.

Groups of Mongolian gerbils (number per group not stated) fed a normal or hypercholesterolemic diet were dosed orally with 1 mg/kg Biotin for 3 or 6 weeks (Informatics, Inc. 1974). At study termination, body weights, adrenal gland weight, liver weight, serum lipids, adrenal gland and hepatic cholesterol, and fecal lipid excretion were determined. Consistently significant changes in serum free fatty acids, triglycerides, or cholesterol were not observed, and little change in adrenal glands or hepatic cholesterol was observed. Body weights of the animals fed normal diet and dosed for 6 weeks were significantly decreased as compared to control values.

Parenteral

Groups of Mongolian gerbils (number per group not stated) fed a basal or hypercholesterolemic diet were dosed subcutaneously (SC) with 1 mg/kg Biotin for 3 or 6 weeks (Informatics, Inc. 1974). At study termination, body weights, adrenal gland weight, liver weight, serum lipids, adrenal gland and hepatic cholesterol, and fecal lipid excretion were determined. Consistently significant changes in serum free fatty acids, triglycerides, or cholesterol were not observed, and little change in adrenal gland or hepatic cholesterol was observed. For animals fed a basal diet, body weights of those dosed for 6 weeks were significantly decreased and liver weights of those dosed for 3 weeks were significantly increased as compared to control values.

Bonjour (1991) reported that the short-term LD_{50} values were >0.41 and >6.15 μmol/kg, respectively, in rabbits and dogs dosed IV with Biotin for 30 and 10 days, respectively, but further details were not provided.

Four dogs were dosed with 10 mg of *d*-Biotin by IV injection for 10 days (Crittenden 1948). Changes in physical, urinary, or blood parameters were not observed. One animal was killed, and gross lesions were not observed.

Subchronic Toxicity

Oral

Groups of five male rats were dosed orally with 5 mg of *dl*-Biotin or *d*-Biotin for 120 days; the control group was given saline (Crittenden 1948). The average body weight of the control group was greater than that of the test groups at study termination. The animals were clinically normal throughout the study. Significant changes in the number of blood cells, the amount of hemoglobin, or in hepatic or renal function were not observed.

Bonjour (1991) reported that the subchronic LD_{50} values were >0.82 and >0.41 μmol/kg, respectively, in rabbits and piglets dosed orally with Biotin for 102 and 122 days, respectively, but further details were not provided.

Parenteral

Bonjour (1991) reported that the subchronic LD_{50} was >0.82 μmol/kg in rabbits dosed SC with Biotin for 102 days, but further study details were not provided.

Chronic Parenteral Toxicity

Bonjour (1991) reported that the chronic LD_{50} was >0.41 μmol/kg in rabbits dosed SC with Biotin for 180 days, but details of the study were not provided.

Dermal Irritation

Intradermal injection of 0.1 cc Biotin into the abdominal skin of guinea pigs did not produce irritation at the site of the injection (Crittenden 1948). Intramuscular injection of 0.5 or 1.0 ml of 0.1% *d*-Biotin into rabbits did not produce irritation.

Dermal Sensitization

Published data on the sensitization potential of Biotin using animals were not found.

Ocular Irritation

Application of 0.1% *d*-Biotin, pH 7.3, to the eye of rabbits was reported to produce only slight and transient irritation, but details of the study were not provided (Crittenden 1948).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Three groups of six female Holtzman rats, after having a normal estrus for three cycles, were dosed SC with 50 mg/kg Biotin in 0.2 ml of 0.1 N NaOH in morning and evening injections during the diestrus stage (Paul, Duttagupta, and Agarwal 1973a). The animals were killed 7, 14, or 21 days after dosing. A control group of six rats was given SC an equal volume of 0.1 N NaOH during the diestrus stage and killed after 7 days. The estrus cycle was studied daily, and the leukocyte concentration of vaginal smears was determined. The ovaries were examined microscopically and the hepatic and uterine glycogen concentrations were estimated. The Biotin-treated animals had irregular estrus cycles, and the number of vaginal leukocytes progressively increased until day 14, and then declined sharply. Biotin did not significantly alter ovarian, uterine, or liver weights, and body weight gain was normal. At microscopic examination of the ovaries, it was observed that the process of formation of the corpora lutea was enhanced, but several of the corpora lutea were atrophic. Hepatic glycogen, concentration and total, was significantly reduced after 14 days and returned to control amounts after 21 days. Uterine glycogen, concentration and total, was slightly reduced at 21 days after Biotin treatment.

Groups of 12 female Holtzman rats were dosed with 50 mg/kg Biotin dissolved in 0.5 ml of 0.1 N NaOH in two SC injections (Paul, Duttagupta, and Agarwal 1973b). The rats were then mated with untreated males 7, 14, or 21 days after dosing. An untreated group of 12 rats was the negative-control group. Six rats of each group were killed on day 14 of gestation, and the remaining six were killed on day 21 of gestation. A group of six rats was treated with Biotin and mated, and the animals in this group were given 1 μg 17β-estradiol in 0.1 ml olive oil on days 6 to 21 of gestation. Another group of six rats was dosed SC with 100 mg/kg Biotin in 1 ml of 0.1 N NaOH in four injections for 2 days. The animals of these two groups were killed on day 21 of gestation.

Of the animals killed on day 14 of gestation, six, four, and three mated 7, 14, and 21 days after dosing, respectively, had fetuses. Of the animals killed on day 21 of gestation, two, three, and one mated 7, 14, and 21 days after dosing, respectively, had fetuses. All negative-control animals had fetuses at both 14 and 21 days of gestation, and all of the animals given 17β-estradiol had fetuses. None of the animals dosed with 100 mg/kg Biotin mated in the 2 months following dosing. The fetal and placental weights of the existing neonates of animals dosed with 50 mg/kg Biotin were decreased as compared to negative control values. The fetal and placental weights of neonates of animals given 17β-estradiol were comparable to those of the negative controls.

SPF outbred rats (Ibm:RORO$_f$) were dosed SC with 5 or 50 mg/kg *d*-Biotin in 0.1 N NaOH, pH 12, given in two doses 5 hours apart on the day of vaginal estrus (Mittelholzer 1976). One control group of eight animals was given SC 0.1 N NaOH, and an untreated control group of seven animals was not dosed. Groups of eight animals were mated 7, 14, and 21 days after dosing and killed and examined on day 21 of gestation.

Significant differences were not observed in the number of implantations per gravid animal, the number of resorption sites, fetal weight, placental weight, ovary weight, or in the number of animals with irregular cycles or without evidence of mating between treated animals and animals of the control groups. The only difference observed was in the number of gravid animals. For the animals mated 7 days after dosing, 6/7, 8/8, and 7/8 animals of the 5-mg/kg, 50-mg/kg, or untreated-control groups, respectively, mated and 88%, 71%, and 67%, respectively, of the animals that mated had fetuses on day 21 of gestation. Of the animals mated 14 or 21 days after dosing, all of the animals of each group mated, and 63%, 88%, and 100% of the animals in the 5-mg/kg, 50-mg/kg, or untreated-control groups, respectively, had fetuses on day 21 of gestation. Lesions were not observed in the ovaries at microscopic examination.

Groups of Holtzman rats were mated and the gravid females were dosed SC with 100 mg D(+)-Biotin in 0.2 ml of 0.1 N NaOH/kg body weight on days 0 and 1 of gestation (Paul and Duttagupta 1975). Nine animals were dosed with Biotin only, seven were given Biotin and 0.1 μg 17β-estradiol in 0.05 ml olive oil SC on days 5 to 20 of gestation, and seven were given Biotin and 4 mg progesterone in 0.2 ml olive oil SC on days 5 to 20 of gestation. Nine gravid animals were untreated and used as a negative-control group. Three groups of six nongravid animals were dosed in the same manner as the gravid animals and used as nonpregnant treated controls. The animals were killed and examined on day 21 of gestation.

Complete resorption of the fetuses occurred in eight of the nine rats dosed with Biotin only; dosing with estrogen or progesterone prevented the resorptions. Fetal and placental weights from animals dosed with Biotin and estrogen or progesterone were decreased as compared to controls, but the decrease was not statistically significant. Biotin caused a decrease in body weights of gravid and nongravid animals; body weights of gravid animals given Biotin and progesterone were similar to gravid untreated control, whereas body weights of gravid animals given Biotin and estrogen were increased. The uterine weights of

gravid animals given Biotin and estrogen were similar to that of gravid untreated controls, whereas the uterine weights of animals dosed with Biotin and progesterone were statistically significantly decreased.

The hepatic glycogen concentration of the gravid animals of all treatment groups and of the nongravid animals given Biotin only was similar to that of gravid controls; a decrease in the hepatic glycogen concentration was observed for nongravid animals dosed with Biotin and estrogen or progesterone. A statistically significant decrease in uterine glycogen concentration was observed in the animals dosed with Biotin that had resorptions. The hepatic RNA concentration was statistically significantly reduced in gravid animals dosed with Biotin, but the uterine concentrations were not affected. Hepatic RNA concentrations of gravid animals dosed with Biotin and estrogen were similar to those of untreated gravid controls, whereas uterine RNA concentrations of animals of this group were greater than control values. A statistically significant increase in uterine DNA concentrations occurred in rats that were dosed with Biotin and had resorptions; uterine DNA concentrations of gravid animals dosed with Biotin and estrogen or progesterone were similar to control values. Hepatic and uterine protein concentrations were statistically significantly decreased in gravid animals dosed with Biotin; uterine protein concentrations of gravid animals dosed with Biotin and estrogen were similar to those of gravid controls. Glucose-6-phosphate dehydrogenase (G-6-PD) activity in the ovaries, adrenal glands, liver, and uterus of gravid animals dosed with Biotin were statistically significantly decreased as compared to gravid untreated controls; dosing with estrogen or progesterone in addition to Biotin significantly increased the enzymic activity as compared to animals given Biotin only, but the values were still lower than those of controls.

In a similar study, groups of Holtzman rats were mated, and gravid females were dosed with 100 mg D(+)-Biotin in 0.2 ml of 0.1 N NaOH/kg body weight on days 13 and 14 of gestation (Paul and Duttagupta 1976). Eleven animals were dosed with Biotin only, seven were given Biotin and 0.1 μg 17β-estradiol in 0.05 ml olive oil SC until day 20 of gestation, and seven were given Biotin and 4 mg progesterone in 0.2 ml olive oil SC until day 20 of gestation. Nine gravid animals were untreated and used as a negative-control group. The animals were killed and examined on day 21 of gestation.

Resorptions occurred in 2 of the 11 animals dosed with Biotin only. The maternal body weights and the fetal, uterine, and placental weights of the remaining nine animals of this group were statistically significantly decreased as compared to controls. The maternal body weights and the fetal, uterine, and placental weights of the animals dosed with Biotin and estrogen and the maternal body weights and uterine weights of the animals dosed with Biotin and progesterone were similar to control values. Hepatic and ovarian weights were similar for animals of the test and control groups.

Dosing with Biotin or Biotin and progesterone resulted in a statistically significant decrease in uterine and placental glycogen concentrations; this decrease was not observed upon dosing with Biotin and estrogen. Hepatic glycogen and blood glucose concentrations were similar for animals of the treated and control groups. Biotin and Biotin plus progesterone caused a statistically significant decrease in the amount of hepatic protein. Hepatic RNA concentrations were statistically significantly increased by Biotin and estrogen. Ovarian, uterine, and hepatic, but not adrenal gland or placental, G-6-PD activity was statistically significantly decreased in animals dosed with Biotin; in animals dosed with Biotin and estrogen, adrenal gland G-6-PD activity was statistically significantly reduced.

ICR mice were dosed with Biotin orally or SC to determine the reproductive and developmental effects of Biotin (Watanabe 1996). Groups of gravid mice were fed 0 or 1000 mg/kg Biotin (0.1%) in commercial feed throughout gestation, dosed SC with 0 or 150 mg/kg Biotin in olive oil, or dosed SC with 0 or 50 mg/kg Biotin in 0.1 N NaOH, pH 11; the animals of the SC groups were dosed with 0.5 ml in three abdominal regions on days 0, 6, and 12 of gestation. An untreated control group was fed commercial diet. The animals were killed and examined on day 17 of gestation. The Biotin concentration of maternal and fetal organs was determined microbiologically, and the biotinidase activity of mice in the dietary group was measured on day 17 of gestation. All animals were observed daily for signs of toxicity and any deaths were recorded.

No signs of toxicity, behavioral changes, and mortality were observed. The number of resorbed or dead fetuses was slightly increased in the group dosed with Biotin in olive oil as compared to the group dosed with Biotin in NaOH; however, in none of the test groups was the increase statistically significant when compared to the untreated control group. Maternal body weight gain of all groups was statistically significantly decreased as compared to the untreated controls, but this difference was not attributed to Biotin. Fetal parameters were similar for all test groups, and a significant difference in external malformations was not observed between the Biotin-treated and untreated control groups. No microscopic differences in the maternal liver, placenta, or ovaries were observed. In the treated groups, Biotin accumulation was observed in maternal and fetal organs. A statistically significant increase in biotinidase activity was observed in the maternal serum and the placenta of rats fed Biotin as compared to controls, but no changes were observed in serum estradiol-E2 content between these groups. The researcher concluded that Biotin "did not disturb normal reproductive functions or embryonic development" in mice.

GENOTOXICITY

The mutagenic potential of $\leq$10,000 μg/plate *d*-Biotin in dimethyl sulfoxide was determined with and without metabolic activation in an Ames *Salmonella*/microsome assay using *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 and in *Escherichia coli* WP2 (uvrA) (SRI International 1979). Negative and positive controls were used,

and each test was performed in duplicate. *d*-Biotin was not mutagenic or toxic. A precipitate was observed on all plates at a concentration of 10,000 μg.

In a Tradescantia-Micronucleus test, the mutagenic potential of 0.01 to 0.5 mM Biotin was determined with treatment made by absorption through the stem (Ma et al. 1984). Biotin was mutagenic, with a minimum effective dose of 0.1 mM.

The mutagenic potential of Biotin was also determined in the RK bacterial test using *E. coli* strain CHY832 (Hayes et al. 1984). Biotin, 1.0 mg/ml and pH 7.4, was not mutagenic.

CARCINOGENICITY

Published data on the carcinogenic potential of Biotin were not found.

CLINICAL ASSESSMENT OF SAFETY

Urticarial Reaction—Case Report

A laboratory worker who reacted when mixing a "dry dusty diet" was patch tested with four substances: rice and 3% "butter yellow" in olive oil; rice and a vitamin B complex including thiamine chloride, choline chloride, vitamin B_2, riboflavin, pyridoxine, nicotinic acid, pantothenic acid, and Biotin; crystalline nicotinic acid; and Biotin concentrate (Keller 1942). Rice and butter yellow produced no reaction, the vitamin B complex produced erythema and central edema that lasted for 10 hours, the crystalline nicotinic acid produced erythema without edema that subsided after 1 hour, and the Biotin concentrate produced "a central, firm, raised, pale area with a surrounding zone of spreading erythema" within 2 hours.

Clinical Studies

Biotin has been administered orally, intramuscularly, and intravenously to children, patients, and normal subjects without adverse effects. These clinical studies are described briefly in Table 3.

SUMMARY

Biotin, also known as vitamin B_7 or vitamin H, is a water-soluble vitamin used as a hair conditioner and as a skin-conditioning agent in cosmetics. In 1998, it was reported to the FDA that Biotin was used in 75 cosmetic formulations at concentrations of $\leq$0.6%.

Biotin occurs naturally as the D-isomer and has been synthesized from 4-benzamido-3-ketotetrahydrothiophen e and methyl

TABLE 3
Clinical use of Biotin

Subject	Dose	Reference
5-month-old infant with metabolic acidosis and ketosis	10 mg/kg/day orally for 3 weeks	Informatics, Inc. 1974
28 infants with Leiner's disease or other forms of dermatitis	20 mg orally or 0.1 mg/day intramuscularly (IM) for 3–5 weeks	Informatics, Inc. 1974
30 infants with dermatitis seborrhoides	5 mg/day IM for 6–10 days, then orally until day 10 or 15	Informatics, Inc. 1974
2-year-old with propionicacidemia and secondary ketotic hyperglycemia	5 mg twice daily orally for 5 days	Informatics, Inc. 1974
Alopecic children	0.25 mg orally 2–3 times/day for 3 months	Informatics, Inc. 1974
8 patients with seborrhea or other forms of dermatitis	250 μg/day for 4–8 weeks	Informatics, Inc. 1974
211 patients with erythroderma and 192 with seborrheic dermatitis	1–4 tablets orally or 1–2 ampuls IM—containing 5 mg for 1–3 weeks	Informatics, Inc. 1974
5 patients with onychodystrophy	120 mg/day orally for 40 days	Informatics, Inc. 1974
Normal subjects and patients with atherosclerosis	1, 3, or 5 mg every 24 h, internally, over 7 and 14 days	Informatics, Inc. 1974
12 normal subjects and 140 patients with atherosclerosis and hypertensive disease	1, 3, or 5 mg/day for 7 or 14 days (route not specified)	Informatics, Inc. 1974
Women with diffuse alopecia	10 mg/day for 28 days (route not specified)	Informatics, Inc. 1974
9 infants with seborrheic dermatitis and 2 infants with Leiner's disease	Total dosage ranged from 3 mg over 2 weeks to 10 mg injected over 4 days	Informatics, Inc. 1974
4-month-old infant	40 mg injected over 2 months, 20 mg of which were given in 4 days	Informatics, Inc. 1974
11 hemodialysis patients	50 mg intravenously for 2 months	Koutsikos et al. 1996

γ-formylbutyrate and from 3,4-diamino-2-carbomethoxythiopene. Biotin had low nonspecific absorption in the UVA and UVB range and into the visible spectrum.

Upon oral and IP administration, Biotin is excreted primarily in the urine.

The oral LD_{50} values of Biotin for mice and rats were >10 g/kg and >1.45 mmol/kg, respectively. The IV LD_{50} for mice and the IP LD_{50} values for rats and cats were >4.1, >0.12, and >0.001 mmol/kg, respectively. Biotin was not toxic in oral short-term or subchronic toxicity studies.

Intradermal injection of 0.1 cc Biotin into guinea pigs did not produce skin irritation. Biotin, 0.1% and pH 7.3, produced slight and transient ocular irritation in rabbits.

In a number of reproductive studies using rats, the number of resorptions in animals dosed with Biotin by SC injection was increased as compared to controls, and fetal, uterine, and placental weights were decreased. Dosing with estrogen generally prevented the resorptions. In a reproductive study in which mice were dosed with Biotin orally and by SC injection, significant differences were not observed between treated and control groups.

Biotin was not mutagenic in an Ames test or an RK bacterial test, but was mutagenic in a Tradescantia-Micronucleus test.

In a case study, Biotin produced an urticarial reaction, but other clinical studies produced no adverse reactions.

DISCUSSION

The Expert Panel recognized that data on the irritation and sensitization potential of Biotin were absent from the report, but the Panel noted that a large number of people are exposed to Biotin daily. The Expert Panel was of the opinion that if Biotin had a strong potential for irritation or sensitization, case reports would be available in the published literature. The lack of such case reports was an indicator to the Expert Panel that Biotin did not have a strong potential for skin irritation or sensitization. UV absorption data demonstrated that phototoxicity would not be a concern.

Several of the available studies that assessed reproductive and developmental toxicity suggested effects of Biotin and one genotoxicity study was positive, but other studies suggested no effects. Assuming that a product containing 0.6% Biotin was applied at a rate of 15 g/day, and approximately 10% of the Biotin was absorbed, it was postulated that the daily exposure to Biotin would be approximately twelve-fold the total potential dietary intake. Based on the low toxic potential of Biotin, however, and on Biotin's rapid metabolism and excretion, such that accumulation would not be a problem, the Expert Panel was of the opinion that no toxic effects would be expected.

CONCLUSION

On the basis of the animal and clinical data included in this report, the Cosmetic Ingredient Review Expert Panel concludes that Biotin is safe as used in cosmetic formulations.

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[2]Available for review: Director, Cosmetic Ingredient Review, 1101 17th Street, N.W., Suite 310, Washington, DC 20036, USA.