Final Report on the Safety Assessment of Human Placental Protein, Hydrolyzed Human Placental Protein, Human Placental Enzymes, Human Placental Lipids, Human Umbilical Extract, Placental Protein, Hydrolyzed Placental Protein, Placental Enzymes, Placental Lipids, and Umbilical Extract[1]

Various proteins, lipids, or other extracts from human or other animal placentas are described as cosmetic ingredients. Human Placental Protein comprises protein derived from human placentas. Placental Protein is derived from animal placentas. Similarly, Human Placental Lipids and Placental Lipids are the lipid fractions from the same source materials. Hydrolyzed Human Placental Protein and Hydrolyzed Placental Protein are produced from the respective protein extracts by acid, enzyme, or other hydrolysis methods. Human Placental Enzymes and Placental Enzymes are enzymes obtained by aqueous extraction of human or other animal placental material. Human Umbilical Extract and Umbilical Extract are unspecified extracts of material from human or other animal umbilical cords. Different materials called Human Placental Extracts and Placental Extracts, assumed to contain estrogenic hormones or other biologically active substances, are not recognized as cosmetic ingredients, even though the use of these ingredients in cosmetics have been reported to the Food and Drug Administration (FDA). Human-derived ingredients are prohibited from use under the provisions of the European Union cosmetics directive based on concerns about transmission of human spongiform encephalopathies and viral diseases, for example, human immunodeficiency virus (HIV). Umbilical Extract has precedent for unrestricted use in Japan, except for certain products. Most of these ingredients are described as hair-conditioning agents and miscellaneous skin-conditioning agents, although the umbilical extracts function as biological additives in cosmetics. Of the human-derived ingredients, only Human Placental Protein is currently reported to be used. Animal-derived placental proteins, hydrolyzed proteins, lipids, and enzymes were all currently reported to be used. No current uses of the umbilical extracts were reported. Most of the available data relates to placental derivatives that appear to have estrogenic or other biological activity. The one clinical study that appears to utilize proteinaceous material only reported no irritant reaction. Clearly, the available data are insufficient to support safety of these ingredients in cosmetics. The additional data needed include (1) skin sensitization at concentration of use; (2) gross pathology and histopathology in skin and other major organ systems associated with repeated exposures, and dermal reproductive and developmental toxicity data; (3) photosensitization; (4) one genotoxicity assay in a mammalian system; if positive, then a 2-year dermal carcinogenicity study using National Toxicology Program (NTP) methods may be needed; (5) ocular toxicity, if available. Any studies should be done on all ingredients unless chemical analysis data show similarity among ingredients. Because there is confusion and concern about the use of substances with estrogenic or other biological activity in cosmetic formulations, it was concluded that none of these ingredients used in cosmetics should deliver any metabolic/endocrine activity. In addition, any current use of these ingredients should be free of detectable pathogenic viruses or infectious agents.

INTRODUCTION

This report is a compilation of data concerning Human Placental Protein, Hydrolyzed Human Placental Protein, Human Placental Enzymes, Human Placental Lipids, Human Umbilical Extract, Placental Protein, Hydrolyzed Placental Protein, Placental Enzymes, Placental Lipids, and Umbilical Extract. Ingredients designated "human," for example Human Placental Protein, are derived from human sources. Ingredients not designated "human," for example Placental Protein, are derived from bovine and other animal sources.

The "Cosmetic Product-Related Regulatory Requirements and Health Hazard Issues" section of *FDA's Cosmetic Handbook* has stated the following regarding placental-derived ingredients (both human and animal):

> Products containing Estrogenic Hormones, Placental Extract or Vitamins
>
> In addition to being considered misbranded drugs, products claiming to contain placental extract may also be deemed to be misbranded

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> cosmetics if the extract has been prepared from placentas from which the hormones and other biologically active substances have been removed and the extracted substance consists principally of protein. The FDA recommends that this substance be identified by a name other than "placental extract" which describes its composition more accurately because consumers associate the name "placental extract" with a therapeutic use or some biological activity (FDA 1994).

Human placental extract and placental extract are not recognized as cosmetic ingredients in the Cosmetic, Toiletry, and Fragrance Association (CTFA) *International Cosmetic Ingredient Dictionary and Handbook* (Wenninger and McEwen 1997). However, in January 1998 "human placental extract" and "placental extract" were reported to be used in 14 and 31 cosmetic formulations, respectively (see Use section).

The Cosmetic Ingredient Review (CIR) Expert Panel expects that cosmetic grade Human Placental Protein, Hydrolyzed Human Placental Protein, Human Placental Enzymes, Human Placental Lipids, Human Umbilical Extract, Placental Protein, Hydrolyzed Placental Protein, Placental Enzymes, Placental Lipids, and Umbilical Extract do not contain hormones or other biologically active components. Similarly, the Panel expects that ingredients identified as human placental "extract" or placental "extract" are also free of biological activity.

The published literature contains numerous articles concerning human placental extract. Three preparation techniques are noted and are detailed in the Chemistry—Method of Production section of this report. Because limited information was found regarding the cosmetic ingredients, articles concerning presumed biologically active human placental extract and placental extract are contained in this report. How the composition of these tested extracts compares to those reported to be used in cosmetic formulations is not known.

CHEMISTRY

Definition

No definitions were found for cosmetic-grade human placental extract or placental extract.

Human Placental Protein

This ingredient is the protein derived from human placenta obtained from normal afterbirth (Wenninger and McEwen 1997). Younoszai and Haworth (1969) reported that term placentas are comprised of 12.0% ± 0.12% protein by wet weight, or 78.5% ± 0.85% by dry weight.

Hydrolyzed Human Placental Protein

This ingredient is the hydrolysate of Human Placental Protein (*q.v.*) derived by acid, enzyme, or other method of hydrolysis (Wenninger and McEwen 1997).

The CAS number 73049-73-7 refers to several hydrolyzed proteins in the CTFA *International Cosmetic Ingredient Handbook*. Further, the published literature identifies the CAS number as that of tryptone, which is defined as "a peptone produced by proteolytic digestion with trypsin" (Taylor 1988). Because it is not specific to Hydrolyzed Human Placental Protein, this CAS number was not used to obtain published articles.

Human Placental Enzymes

These ingredients are the enzymes derived from human placentas obtained from normal afterbirth (Wenninger and McEwen 1997).

Human Placental Lipids

These ingredients are the lipids derived from human placentas obtained from normal afterbirth (Wenninger and McEwen 1997).

Younoszai and Haworth (1969) reported that term placentas are comprised of ~0.4% lipids by wet weight, or ~2.9% by dry weight. The English abstract of a French article described the lipid content of blood-free placentas to have the following composition: 62% phospholipids, 13% to 18% free fatty acids, and 16% to 18% nonesterified cholesterol. Arachidonic acid accounted for 19.5% of total fatty acids (free and esterified). The 62% phospholipid content was itself composed of 40% diacyl phosphatidylcholine, 25% sphingomyelin, 10% ethanolamine plasmalogen, 7% diacyl phosphatidylethanolamine, 4% phosphatidylserine, 3% phosphatidylinositol, and 9% lysophospholipids. The investigators reported that the industrial process used to extract blood from the placenta did not induce either fatty acid oxidation or phospholipid hydrolysis (Chirouze, Entressangeles, and Helme 1987).

Human Umbilical Extract

This ingredient is an extract of human umbilical cord (Wenninger and McEwen 1997).

Placental Protein

Placental Protein is a mixture of proteins derived from animal placentas (Wenninger and McEwen 1997).

Hydrolyzed Placental Protein

This ingredient is the hydrolysate of Placental Protein (*q.v.*) derived by acid, enzyme, or other method of hydrolysis (Wenninger and McEwen 1997). Like its human-derived counterpart, Hydrolyzed Placental Protein is also identified by the CAS number 73049-73-7.

Placental Enzymes

Placental Enzymes is a mixture of enzymes obtained from an aqueous extraction of animal placentas (Wenninger and McEwen 1997).

Placental Lipids

Placental Lipids is a mixture of lipids derived from animal placentas (Wenninger and McEwen 1997).

Umbilical Extract

Umbilical Extract is an extract of animal umbilical cords (Wenninger and McEwen 1997).

Method of Preparation

Human Placental Extract

Two placental extracts (one human the other bovine) were described as used in "dermocosmetology." These extracts were obtained using the Filatov technique (not explained) under conditions "favoring the development of biogenic stimulins." The extracts were purified by filtration and were sterilized by autoclaving at 120°C. They were supplied as pale yellow liquids with a characteristic odor (CTFA 1998).

Several studies cited in this report tested human placental extract that was prepared in one of two ways. Fresh human placentas from normal deliveries were collected, washed to remove blood, homogenized with buffer, filtered until clear, and the preservative, benzyl alcohol added. Each milliliter of extract was derived from 0.1 g of fresh human placenta. Known constituents of the extract include human placental lactogen (HPL), corticotropin-releasing factor (CRF), fibrin-stabilizing factor (FSF), and lactoferrin (Banerjee, Bishayee, and Chatterjee 1993). Studies cited in this report that used this extraction method identified the human placental extract as HPE.

Other studies tested a human placental extract fraction (EAP). Placentas were collected at delivery and immediately frozen at −20°C. Pools of 500 to 600 placentas were mechanically ground, and then stirred until thawed in an 8% (*v*/*v*) ethanol/water solution. Placental blood was separated from the tissue by means of a press. The tissue pulp was extracted with acid and the extract was neutralized and precipitated with 15% ethanol. The supernatant was recovered by centrifugation, concentrated by ultrafiltration, and diafiltered against 0.9% NaCl solution with 10,000-Da cut-off membranes. One liter of the fraction "corresponded to" ~21 kg of placental tissue pulp (Klein, Chiodino, and Yamasaki 1991).

Contaminants

Human Placental Extract

One source reported that two Filatov-type placental extracts (human and bovine) used for "dermocosmetology" were devoid of estrogenic activity. The extracts were subcutaneously administered (20 ml/kg) to 11 female Sprague-Dawley rats (3 weeks old). Five hours after dosing, the rats were killed and the uterus was removed, weighed, dehydrated, and reweighed. The difference between fresh and dry weight was used to calculate the water content of the uterus. An increase in this parameter was evidence of estrogenic activity. No significant increase was noted compared to nontreated controls (CTFA 1998).

Beyssac, Martini, and Cotte (1986) detected estriol at a calculated maximum of 100 μg/l in various human placental extract preparations that were defined as "used in the cosmetics industry." In addition, a survey concerning use of hormone/placenta-containing hair preparations by children measured an estriol content of 1.9% (*w*/*w*) in one placenta-containing hair preparation (not distinguished as human or animal in origin). The investigator of the survey suggested that use of these products by children could cause sexual maturation at an earlier age (Tiwary 1997).

USE

Cosmetic

The human and animal placental-derived protein, hydrolyzed protein, enzyme, and lipid ingredients all function in cosmetic formulations as hair-conditioning agents and skin-conditioning agents—miscellaneous. Human Umbilical Extract and Umbilical Extract are used as biological additives (Wenninger and McEwen 1997).

As of January 1998, Human Placental Protein was reported used in 30 cosmetic formulations. Two uses of "human placental extract, liquid" and 12 uses of "human placental extract, lyophilized" were reported. Placental Protein (identified as animal or bovine) was used in five formulations; Hydrolyzed Placental Protein was used in seven formulations; Placental Enzymes was used in seven formulations; and Placental Lipids, bovine was used in one formulation. "Placental extract" was used in 31 formulations (FDA 1998) (Table 1). Where available, current concentration of use data (CTFA 1999) are also shown in Table 1. Historical concentration of use data are also given in Table 1.

One source recommended use of human and bovine placental extract at concentrations between 5% and 20% (CTFA 1998).

The European Community Directive prohibits the use of Human Placental Protein, Hydrolyzed Human Placental Protein, Human Placental Enzymes, Human Placental Lipids, and Human Umbilical Extract in cosmetics. The European Union designation, "cells, tissues or products of human origin," is listed in Annex II—"List Of Substances Which Must Not Form Part Of The Composition Of Cosmetic Products." The preamble to the 18th Commission Directive stated (Cosmetics Directive of the European Union 1995):

> Whereas cells, tissues or products of human origin are liable to transmit the Creutzfelt-Jakob disease, human spongiform encephalopathy, and certain virus diseases; whereas it is therefore necessary, given the current state of scientific knowledge, to prohibit their use in cosmetic products.

Umbilical Extract, identified as umbilical cord extract, is listed in the Japanese *Comprehensive Licensing Standards of Cosmetics by Category* (CLS) (Rempe and Santucci 1997). Umbilical Extract, which conforms to the specification of the *Japanese Cosmetic Ingredients Codex* (JCIC), has precedent for use without restriction in various CLS categories except eyeliner, lip, oral, or bath preparations for which there are no precedent for use.

GENERAL BIOLOGY

Anti-Inflammatory Activity

Human Placental Extract (presumed active, see Introduction)

Banerjee et al. (1990) reported a reduction in carrageenin-induced inflammation in rats that had been given an

TABLE 1
Frequency and concentration of use (FDA 1998)

Product category (no. formulations in category) (FDA 1998)	No. containing ingredient (FDA 1998)	Current concentration of use (CTFA 1999)	Historical concentration of use (FDA 1984)
Human Placental Protein			
Hair conditioners (636)	3	—	—
Shampoos—noncoloring (860)	5	—	—
Tonics, dressings, and other hair-grooming aids (549)	4	—	—
Other hair preparations (276)	1	—	—
Hair rinses—coloring (33)	1	—	—
Douches (5)	1	—	—
Face and neck skin care—excluding shaving (263)	2	—	—
Body and hand skin care—excluding shaving (796)	2	—	—
Moisturizing (769)	4	—	—
Other skin care preparations (692)	7	—	—
1998 total for Human Placental Protein	**30**		
Human Placental Extract			
Aftershave lotion[a] (216)	1	—	—
Face and neck skin care—excluding shaving[a] (263)	1	—	—
Hair conditioners[b] (636)	1	—	0%–0.1%
Rinses—noncoloring[b] (40)	1	—	0%–0.1%
Shampoos—noncoloring[b] (860)	2	—	0%–0.1%
Tonics, dressings, and other hair-grooming aids[b] (549)	2	—	0%–5%
Body and hand skin care—excluding shaving[b] (796)	1	—	0.1%–5%
Moisturizing[b] (769)	1	—	0%–0.1%
Night skin care[b] (188)	1	—	0.1%–5%
Other skin care preparations[b] (692)	3	—	—
1998 total for Human Placental Extract	**12**		
Placental Protein (animal or bovine)			
Makeup bases (132)	1	—	—
Face and neck skin care—excluding shaving (796)	3	0.2%	—
Moisturizing (769)	—	0.2%	—
Night skin care (188)	—	0.2%	—
Skin fresheners (184)	1	—	—
1998 total for Placental Protein	**5**		
Hydrolyzed Placental Protein			
Face and neck skin care—excluding shaving (263)	1	—	—
Body and hand skin care—excluding shaving (796)	1	—	—
Moisturizing (769)	2	—	—
Night skin care (188)	2	—	—
Other skin care preparations (692)	1	—	—
1998 total for Hydrolyzed Placental Protein	**7**		
Placental Enzymes			
Shampoos—noncoloring (860)	1	—	—
Other hair preparations (276)	1	—	—
Face and neck skin care—excluding shaving (263)	1	5%	—
Moisturizing (769)	2	—	—
Other skin care preparations (692)	2	—	—
1998 total for Placenta Enzymes	**7**		

(Continued on next page)

TABLE 1
Frequency and concentration of use (FDA 1998) *(Continued)*

Product category (no. formulations in category) (FDA 1998)	No. containing ingredient (FDA 1998)	Current concentration of use (CTFA 1999)	Historical concentration of use (FDA 1984)
Placental Lipids (bovine)			
Face and neck skin care—excluding shaving (263)	1	4%	—
1998 total for Placental Lipids	**1**		
Placental Extract			
Permanent waves (192)	1	—	—
Shampoos—noncoloring (860)	1	—	0%–0.1%
Tonics, dressings, and other hair-grooming aids (549)	1	—	—
Hair shampoos—coloring (24)	1	—	—
Body and hand skin care—excluding shaving (796)	8	—	0%–>50%
Moisturizing (769)	11	—	0%–5%
Night skin care (188)	2	—	0.1%–5%
Paste masks—mud packs (255)	1	—	1%–5%
Other skin care preparations (692)	5	—	0.1%–1%
1998 total for Placental Extract	**31**		

[a] Ingredient used in liquid form in these product types.
[b] Ingredient used in lyophilized form in these product types.

intraperitoneal (IP) dose of HPE. Maximal suppression was noted with a dose of 0.3 ml/100 g body weight, given $^1/_2$ hour before or after carrageenin administration.

A dose-dependent inhibition of increased hepatic succinic dehydrogenase (SDH) activity (in reponse to carrageenin-induced edema) was noted in rats that had been pretreated with a subcutaneous (SC) dose of 1 to 5 ml/kg of HPE. The extract had little or no effect on the hepatic SDH activity of normal rats (Banerjee et al. 1994b).

A study that investigated the biochemical mechanism for the anti-inflammatory action reported a significant reduction in glucose-6-phosphate dehydrogenase activity in the liver, kidneys, and brain of rats following IP administration of a commercial HPE (0.4 ml/100 g body weight). The investigators cautioned that this enzyme was key in the production of NADPH and that inhibition can result in decreased amounts of reduced glutathione that was involved in free-radical scavenging. Enzyme inhibition also could alter steroid synthesis (Banerjee et al. 1992).

Clinical/Therapeutic Application

Vitiligo—Human Placental Extract (presumed active, see Introduction)

Several studies have reported the use of HPE in treatment of vitiligo (skin disorder marked by loss of pigmentation). "Remarkable improvement" in 20.6% and "moderate improvement" in 50% of 34 patients with vitiligo was noted after topical treatment (Sharma et al. 1988). Another study tested the repigmentation claims of a commercially available topical HPE. Following manufacturer's instructions, the extract was applied to affected areas three times a day, and the treated area was exposed to infrared light following the third application. Of 16 patients with vitiligo, 69% had no significant repigmentation, 19% had scattered repigmentation, and 12% had obvious repigmentation of some lesions. Five had complete repigmentation of some lesions within 3 to 16 months, and two (one a child, and the other a man who had been recently diagnosed) had an "obvious reduction in vitiliginous areas" (Suite and Quamina 1991).

Pal et al. (1995) conducted a guinea pig study to determine the chemical agent responsible for repigmentation. Human placentas were chopped, blended, extracted with ethanol, and then filtered. The 60% hydroalcoholic extract was topically applied around the nipples covering the areola zones of immature male white guinea pigs daily for 60 days followed by 15 minutes of infrared exposure. Clear pigmentation and hypertrophy was noted to varying degrees. The extract was chemically analyzed and glycosphingolipids, known modulators of B and T cells, were considered to induce melanocytes resulting in skin pigmentation.

Chorioretinal Dystrophy—Human Placental Extract (presumed active, see Introduction)

Thirty-four panelists with chorioretinal dystrophy (myopic or senile) of different degrees of anatomofunctional alteration received daily intramuscular doses of 3 ml human placental extract (equivalent to 1.80 g of fresh organ) for 20 days. The preparation method of the extract was not reported. Varying improvement was noted in visual acuity, the luminous sense, the visual field and the electrophysiological activity of the retina.

The investigators considered the "efficacy" of the extract was due to the "high proteic value, to polypeptide with a low molecular weight and to free amino-acids, particularly alanine, leucine, lysine, (and) valine" (Girotto and Malvinerni 1982).

Neurological Activity

Human Placental Extract (presumed active, see Introduction)

In response to claims that HPE increased the grasping, learning, and retention capacity of children of slow-learners, Banerjee, Bishayee, and Chatterjee (1995) investigated the effect of the extract on rat monoaminergic neurotransmitters and brain monoamine oxidase (MAO) activity. Subchronic IP administration (once daily for 5, 10, 15, or 20 days) of human placental extract (2 to 4 ml/kg/day) increased brain concentration of monoamines and decreased MAO activity in rats.

Cellular Effects

Human Placental Extract (presumed active, see Introduction)

Ikawa, Aida, and Saito (1975) reported that addition of 2% to 3% of a commercial human placental extract preparation to the clonal culture lines of Friend leukemia cells resulted in hemoglobin production after 4 to 6 days.

O'Keefe and Chiu (1988) reported that incubation with placental extract (100 to 200 μg) resulted in a 50-fold increase of thymidine incorporation by keratinocytes.

Kimoto et al. (1987) reported that a placental extract with demonstrated antimutagenic activity in the Ames assay "greatly diminished" adriamycin-mediated toxicity. The placental extract was prepared by homogenization of full-term human placentas, incubation with pronase, centrifugation, lyophilizing the supernatant, dissolving the resulting powder, and eluting fractions via a Sephadex column. Fractions were tested in the Ames assay. The fraction demonstrating antimutagenicity was used in in vitro studies with Adriamycin. These in vitro studies noted that the fraction diminished superoxide production by Adriamycin-incubated liver microsomes and reduced the effects of aeration on Adriamycin semiquinone radical generation.

ANIMAL TOXICOLOGY

Short-Term Oral Toxicity

Dried Human Placenta (presumed active, see Introduction)

A group of 10 male weanling rats were fed a diet in which the 10% protein allotment consisted of human placenta that had been dried and powdered. The control group was fed casein. Diets also had 15% fat and were complete with regard to vitamins and minerals. Rats were killed after either 4 or 8 weeks of feeding, and the liver and testes were removed. No changes were noted in the testes. Fatty changes in the liver of placenta-fed rats was noted at microscopic examination. The lesions consisted of diffuse cytoplasmic vacuolation of hepatocytes; the lesion was severe in cells of the periportal and adjacent mid-zones and mild in cells of the central zone. A "striking amount of stainable lipids" was noted when stained with oil red 0. The lesions were similar in rats killed at either 4 or 8 weeks of feeding. Mild-to-moderate vacuolation was also noted in controls rats, but little-to-no stainable fat was detected (Bamji and Krishnamurthi 1970).

In order to elucidate the cause of the lesions, a second study was conducted in which six male rats were fed a 20% placenta-protein diet. The diet contained 0.4 g cholesterol, 0.02 mg estradiol, and 0.5 mg progesterone/100 g diet and controls were fed a casein diet containing a comparable amount of one or more of these hormones. Animals were killed after 4 weeks, and the liver and testes were removed for microscopic examination. The liver was also analyzed for lipids and proteins. No changes were noted in the testes. Livers of test animals were mottled in appearance with no significant change in weight. Total lipids, triglycerides, and total cholesterol concentrations were markedly greater in the liver of rats fed the placenta diet compared to the control group. No changes in phospholipid and protein concentration were noted. Similar changes in hepatic lipids were noted in rats that were fed casein supplemented with cholesterol, but not in those that were fed casein supplemented with estrogen and/or progesterone. At microscopic examination, the livers of rats fed the placenta diet and those fed casein supplemented with cholesterol (with or without hormones) had mild cytoplasmic vacuolation of cells of the periportal and adjacent midzone lobules. In oil red 0–stained sections mild accumulation of fat as multiple small and fine round globules was observed in the cytoplasm without nuclear displacement (Bamji and Krishnamurthi 1970).

In an earlier study, 20 rats were fed ad libitum for 112 days 5.0% acetone-dried human placental powder or 0.30% Human Placental Lipids (identified as placental lipid extract). Hepatic malic oxidase (MO) activity ($p < .05$) and testicular oxygen uptake ($p < .01$) were significantly increased. Hepatic succinoxidase (SO) activity was comparable between treated and control rats. In the second part of the study, immature male rats were fed raw human placenta for 83 days. Significant increases in MO ($p < .05$) and SO ($p < .01$) activities, increased testicular oxygen uptake, and atrophy of the testes were noted. The atrophy was attributed to the sex hormones contained in the placenta, but the investigators did not consider hormones to have induced the increased oxygen uptake (Gershbein and Malik 1967).

Parenteral Toxicity—Acute

Human Placental Protein

Some published literature refers to human placental protein(s) that have hormone-like effects. These proteins are different from the cosmetic ingredient. Florini et al. (1966) reported a "human placental protein" that when injected into hypophysectomized rats and mice, had anabolic effects such as those noted after dosing with human growth hormone. The protein also reacted to antisera for human growth hormone.

Using the extraction technique of Florini et al. (1966), Riggi et al. (1966) reported that intramuscular (IM) administration of "purified human placental protein" into fasted rabbits (25 or 50 mg/kg), and monkeys (50 or 100 mg/kg) produced significant increases in plasma free fatty acid concentrations. Plasma lactescence associated with hypertriglyceridemia and hyperglycemia developed in rabbits following daily SC dosing with 75 mg/kg for 25 days. In mice, hepatic lipidosis developed after injections with 16 mg of Human Placental Protein daily for 7 days. All of the described effects were similar to those observed following porcine growth hormone administration.

Human Placental Extract (presumed active, see Introduction)

Banerjee, Bishayee, and Chatterjee (1993) reported that a single IP dose of HPE (4 ml/kg) to rats caused a significant enhancement of lipid peroxidation with a decline in both hepatic and blood glutathione (GSH) concentrations. A dose-dependent increase in glutathione *S*-transferase (GST) activity and dose-dependent inhibition of catalase, glutathione peroxidase, and glutathione reductase activities were noted. The extract was considered hepatotoxic because it increased serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, serum lactate dehydrogenase activities, and blood methemoglobin concentration. The magnitude of the increase of serum enzymes was "much less" than that induced by carbon tetrachloride.

In a subsequent study by Bishayee, Banerjee, and Chatterjee (1995), rats were given a single IP injection of HPE (4 ml/kg) and some were killed at 2, 6, 24, 45, 72, and 96 hours post treatment. Livers were removed, homogenized, and centrifuged, and the fractions were analyzed for cytochrome and enzymic activity. The vehicle control received 1.5% (*v*/*v*) Benzyl Alcohol in buffer. Maximal induction of hepatic microsomal cytochrome P-450 and cytochrome b_5 activities was noted beginning at 24 hours post dosing. Cytosolic GST activity was also significantly increased beginning at 48 hours post dosing. A reduction in microsomal UDP-glucuronyltransferase activity was also observed. All activity returned to zero-time values 96 hours after treatment.

Parenteral Toxicity—Short-Term

Human Placental Extract (presumed active, see Introduction)

Dose-dependent increases in hepatic cytochrome parameters and GST activity were noted in rats following repeated IP dosing (30 days) with 1, 2, or 4 ml/kg HPE. The cytochrome changes were significant with the 2 ml/kg dose ($p < .05$); more pronounced increases were noted in the 4 ml/kg dose group where the change in cytochrome P-450 activity was 130% ($p < .01$) and the increase in cytochrome b_5 activity was 88% ($p < .05$) greater than the control. Microsomal NADPH cytochrome c reductase activity was not affected by either acute or repeated treatment. The investigators cautioned that human placental extract had "substantial ability to alter the patterns of drugs metabolizing enzyme systems in mammals," and that prolonged administration could induce some forms of hepatic neoplasms (Bishayee, Banerjee, and Chatterjee 1995).

Similar findings were reported in an earlier study in which HPE (1 to 4 ml/kg) was injected IP into rats for 15 days. Significant increases were noted in the activities of serum glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, lactic dehydrogenase, alkaline phosphatase, glutamic dehydrogenase, and sorbitol dehydrogenase. Activities of other enzymes were also increased. A marked depletion of cytochrome P-450 and reduction of hepatic glycogen and protein concentrations were noted with a concurrent rise in hepatic lipid peroxides. The investigators considered that the active components of the extract, 19-hydroxyprogesterone and corticotropin-releasing factor, were responsible for the alterations (Banerjee et al. 1994a).

Dermal Irritation

Human and Animal Placental Extract (presumed active, see Introduction)

Two Filatov-type placental extracts (human and bovine) used for "dermocosmetology" were each applied (0.5 ml) under gauze to rabbit skin. Both extracts were nonirritating. No further details were given (CTFA 1998).

Ocular Irritation

Human and Animal Placental Extract (presumed active, see Introduction)

Two Filatov-type placental extracts (human and bovine) used for "dermocosmetology" were each instilled (0.5 ml) into one conjunctival sac of six rabbits. The human placental extract was "very slightly irritating" and the bovine placental extract was nonirritating. No further details were given (CTFA 1998).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

In Vitro

Animal Placental Extract (presumed active, see Introduction)

Mammalian embryonic development was studied by Huxham et al. (1982) using a postimplantation rat-embryo culture. Wistar rat conceptuses were explanted on day 9.5 and cultured for 2 days with homogenate preparations from normal rat placenta or decidua. Conceptuses were examined for heart beat, vitteline circulation, yolk-sac diameter, and the achievement of allantoic fusion with the ectoplacental cone. Abnormal embryos (neural-tube defects, severe reduction in embryonic size) were produced with 2.5 to 4 mg/ml of the placental homogenate and 1.2 to 4 mg/ml of the decidual homogenate. Abnormalities were not induced by either solutions of bovine serum albumin or protein preparations of rat lung tissue.

GENOTOXICITY

Human Umbilical Extract

Immunizing mice with an extract of human umbilical cord significantly decreased the number of micronuclei in bone marrow cells for 5 days. The extract was described as having no clastogenic property. The investigators hypothesized that the antimutagenic effect was related to interferon induction by the extract (Mkrtchian and Nersessian 1993).

PROTECTIVE ACTIVITY

Human Placental Extract

Klein et al. (1991) reported that in in vitro studies, EAP inhibited growth of Ha-ras–transformed BALB/c 3Tc cells and human squamous lung carcinoma A-2182 cells. The fraction did not alter anchorage-dependent growth of these cells, but a slight mitogenic activity was noted in nontransformed cells. No significant cytotoxicity was noted. The fraction did contain transforming growth factor β, but the investigators did not consider that the growth factor was solely responsible for the observed growth suppression.

In a subsequent study, Klein, Chiodino, and Yamasaki (1993) reported that EAP suppressed growth of only the most highly tumorigenic cells in soft agar medium; growth of non- and low-tumorigenic counterparts was not affected or was stimulated, respectively, by the extract. Cells of both the colorectal and esophageal cell lines that had the greatest percentage of colonies in soft agar had their colony-forming efficiency decreased by the presence of 100 μg/ml EAP. In contrast, cells that did not give any colonies in soft agar did not grow in either the absence or presence of EAP. Growth of cells with an intermediate colony-forming efficiency was stimulated (by 150% in colorectal cells, and 200% in esophageal cells) in the presence of EAP. Similar findings were noted with murine BALB/c 3T3 1-1 cells that had been transfected or infected with various oncogenes. Further, "EAP did not significantly affect the doubling time of anchorage-dependent cell growth, suggesting that the extract specifically suppresses tumorigenic characteristics of cells such as their ability to grow in soft agar medium." Transforming growth factor β was most effective on less tumorigenic cells.

Human Placental Extract (presumed active, see Introduction)

Komura et al. (1983) conducted a chemical study on the antimutagenic action of human placental extract. A human placenta was washed, diced, and homogenized. The liquid was centrifuged; then the supernatant was boiled and recentrifuged, it was a pale pink liquid. The yield from one placenta was ~300 ml of "active juice." Two placentas were used to prepare HPE_1 and HPE_2. Bacteria (*Eschericheria coli*) were mutated by either radiation or incubation with a chemical mutagen N-methyl-N-nitro-N-nitrosoguanidine (MNNG) and then combined with HPE and plated. A specific activity AD_{50} was defined as the dose that reduced the number of induced mutations by 50% without affecting cellular survival. The specific activities of HPE_1 against ultraviolet (UV)-, γ-ray–, and MNNG-induced mutations were 160, 50, and 200 μl/plate, respectively. The specific activities of HPE_2 against UV- and MNNG-induced mutations were 50 and 80 μl/plate, respectively. Cobalt(II) ions were considered essential for the antimutagenic action. However, the investigators noted studies using other mammal placentas and considered that "low molecular, nonproteic factors" could also have played a role.

Placental Extract (presumed active, see Introduction)

Mochizuki and Kada (1982) investigated the antimutagenic action of extracts prepared from the placentas of a human, monkey, dog, rat, and mouse. Placentas were washed with potassium chloride, homogenized without buffer, and centrifuged. The supernatant was treated with pronase followed by overnight dialysis in distilled water. Each solution was heated and loaded onto ion-exchange resin columns and eluted with water. Fractions were collected and evaporated under vacuum; the residue was dissolved in water and filtered using millipore filters. Bacteria (*E. coli* B/r WP2 trp^-) were mutated by either radiation or incubation with MNNG and then combined with an extract and plated. The extracts were also tested alone and were not mutagenic. The number of mutant colonies induced by UV irradiation, γ-ray, and MNNG were "decreased markedly in the presence of the placental extracts without significant effects on survival." The data were not analyzed for statistical significance.

Human Umbilical Extract

Vaccination of rats and mice with an extract of human umbilical cord resulted in a significant inhibition of growth, decreased tumor incidence, and partial resorption of ascitic fluid of transplantable tumors such as Ehrlich's ascites tumor, sarcoma 37, and Zajdel's hepatoma. The inhibition was not noted when sarcoma 180 was transplanted. Vaccination also interfered with dimethyl benzanthracene and benzo(a)pyrene-induced carcinogenesis by reducing tumor incidence and increasing the latent period and slowing cancer progression (Mkrtchyan et al. 1990).

CLINICAL ASSESSMENT OF SAFETY

Patch Testing

Human Placental Protein

A patch testing reference book by DeGroot (1994) noted that the published literature does not contain recommended test concentrations for Human Placental Protein. As a guide to the clinician, DeGroot reported the findings of an unpublished (and at the time, ongoing) study by members of the Dutch Contact Dermatitis Group. No irritant reaction was noted in 1 to 20 patients (exact number tested with ingredient not specified) suffering from or suspected to suffer from cosmetic product contact allergy after being patch tested with 30% Human Placental Protein aqua.

Animal Placental Extract (presumed active, see Introduction)

von den Driesch et al. (1993) reported contact dermatitis of the hand in a cosmetician up to 3 hours after external application of calf placenta extracts. She had previously worked as a hairdresser but developed a delayed-type allergy to *p*-phenylenediamine. The placenta extract contained mesodermin, collagen, and hyaluronic acid. The cosmetician and 10 healthy volunteers were tested with the extract and the solvent via the prick and scratch-chamber method. The cosmetician reacted in both tests. In the scratch-chamber test, the dissolved extract produced an eczematous reaction at day 1 and the undissolved extract caused a reaction after 2 days; the dissolved antigens were considered to have had greater penetration.

SUMMARY

The ingredients Human Placental Protein, Hydrolyzed Human Placental Protein, Human Placental Enzymes, and Human Placental Lipids are derived from human placentas obtained from normal afterbirth. Placental Protein, Hydrolyzed Placental Protein, Placental Enzymes, and Placental Lipids are mixtures derived from animal placentas. Human Umbilical Extract and Umbilical Extract are obtained from human and animal umbilical cords, respectively.

The human and animal placental-derived ingredients function in cosmetic formulations as hair- and skin-conditioning agents. The umbilical cord extracts are reported to function as biological additives.

As of January 1998, Human Placental Protein was reported to be used in 30 cosmetic formulations, Placental Protein (identified as animal or bovine) was used in five formulations, Hydrolyzed Placental Protein was used in seven formulations, Placental Enzymes was used in seven formulations, and Placental Lipids, bovine was used in one formulation. In addition, human placental "extract" and placental "extract" were reported to be used in 14 and 31 cosmetic formulations, respectively. These two extracts are not recognized as cosmetic ingredients. Virtually all of the available safety test data related to extracts. These extracts are presumed to be biologically active, for example containing hormones.

Animal and clinical studies testing biologically active human placental "extract" have reported anti-inflammatory activity, improvement in the treatment of vitiligo and chorioretinal dystrophy, and neurological and cellular effects.

Oral- and parenteral-dose rat studies tested biologically active extracts and protein preparations and found changes in hepatic enzyme activities. Placental "extracts" (both human and animal) were negative in dermal and ocular irritation studies using rabbits. In in vitro studies, placental "extracts" demonstrated antimutagenic action in bacteria and had anticarcinogenic activity against some tumor cell lines.

Human umbilical extract was negative for mutagenicity in a micronuclei assay, and inhibited growth of tumors transplanted in rats and mice.

Some studies stated that protein was tested. However, the study design appeared to test the effects of hormonal activity. The one exception is a clinical study that reported no irritant reaction to Human Placental Protein following patch testing of patients with cosmetic product contact allergy.

DISCUSSION

The CIR Expert Panel faced many issues with this group of ingredients. First was the reported use of substances identified as "human placental extract" and "placental extract." These names are not recognized in the CTFA *International Cosmetic Ingredient Dictionary and Handbook*. Further, FDA warned that cosmetics claiming to contain these ingredients may be misbranded and recommended using nomenclature other than "extract." The CIR Expert Panel advised industry that cosmetic formulations should not be identified as containing "human placental extract" or "placental extract" so as to comply with FDA guidelines.

The Expert Panel expected that the CTFA-recognized ingredients—Human Placental Protein, Hydrolyzed Human Placental Protein, Human Placental Enzymes, Human Placental Lipids, Human Umbilical Extract, Placental Protein, Hydrolyzed Placental Protein, Placental Enzymes, Placental Lipids, and Umbilical Extract—will not deliver any metabolic/endocrine activity (e.g., hormones, growth factors).

The Panel was also concerned with the dangers inherent in using human or animal-derived ingredients, namely the transmission of infectious agents. The CIR Expert Panel stressed that these ingredients must be free of detectible pathogenic viruses or infectious agents (e.g., HIV, Bovine Spongiform Encephalopathy (BSE), or Creutzfeld-Jakob disease prions). Suppliers and users of these ingredients must accept responsibility for assuring that these ingredients are risk-free. Tests to assure the absence of a pathogenic agent in the ingredients, or controls to assure derivation from pathogen-free sources are two approaches that should be considered.

With the above conditions met, the CIR Expert Panel noted that additional data still were needed to assess the safety of the cosmetic ingredients. The vast majority of studies cited in this report tested biologically active "extracts" and other preparations. Thus, the Panel was unable to apply results of these studies to the safety assessment of the cosmetic-grade ingredients.

Section 1, paragraph (p) of the CIR Procedures states that "A lack of information about an ingredient shall not be sufficient to justify a determination of safety." In accordance with Section 30(j)(2)(A) of the Procedures, the Expert Panel informed the public of its decision that the data on these ingredients were not sufficient for determination whether the ingredient, under relevant conditions of use, was either safe or unsafe. The Panel released a Notice of Insufficient Data on March 20, 1998, outlining the data needed to assess the safety of these ingredients. Comments concerning a human and bovine placental extract were received during the 90-day public comment period. However, additional data needed* to make a safety assessment are:

1. Skin sensitization at concentration of use
2. Gross pathology and histopathology in skin and other major organ systems associated with repeated exposures, and dermal reproductive and developmental toxicity data
3. Photosensitization
4. One genotoxicity assay in a mammalian system; if positive, then a 2-year dermal carcinogenicity study using NTP methods may be needed
5. Ocular toxicity, if available

(*To be done on all ingredients unless chemical analysis data shows similarity among ingredients.)

CONCLUSION

The CIR Expert Panel concludes that the available data are insufficient to support the safety of Human Placental Protein, Hydrolyzed Human Placental Protein, Human Placental Enzymes, Human Placental Lipids, Human Umbilical Extract, Placental Protein, Hydrolyzed Placental Protein, Placental Enzymes, Placental Lipids, and Umbilical Extract for use in cosmetic products. If these ingredients are used, they should not deliver any metabolic/endocrine activity, and they must be free of detectable pathogenic viruses or infectious agents.

REFERENCES

Bamji, M. S., and D. Krishnamurthi. 1970. Fatty change in the liver of rats fed diets containing dry human placenta. Influence of placental steroids. *Indian J. Med. Res.* 57:158–165.

Banerjee, K. K., D. Bharadwaj, D. P. Ghosh, and R. N. Hati. 1990. Effect of human placental extract "Placentrex" on experimental inflammation. *Indian Med. Gazette* 124:243–244.

Banerjee, K. K., A. Bishayee, S. Banik, and M. Chatterjee. 1992. Acute effect of human placental extract on NADPH producing pathway in rat. *Indian J. Pharmacol*. 24:176–177.

Banerjee, K. K., A. Bishayee, S. Banik, and M. Chatterjee. 1994a. Hepatotoxicity of human placental extract in rats: A biochemical evaluation. *Nihon Univ. J. Med.* 36:197–206.

Banerjee, K. K., A. Bishayee, and M. Chatterjee. 1993. Elevated lipid peroxidation, decreased glutathione levels and changes in glutathione-related enzymes in rats treated with human placental extract. *Acta Med. Okayama* 47:223–227.

Banerjee, K. K., A. Bishayee, and M. Chatterjee. 1994b. Role of human placental extract on succinic dehydrogenase activity in carrageenin-induced edema in rats in vivo and its effect on erythrocyte lysis, platelet aggregation and trypsin activity in vitro. *Indian J. Physiol. Pharmacol*. 38:121–124.

Banerjee, K. K., A. Bishayee, and M. Chatterjee. 1995. Effects of human placental extract on brain monoamines and monoamine oxidase activity in rats. *Tohoku J. Exp. Med.* 176:17–24.

Beyssac, I., M. C. Martini, and J. Cotte. 1986. Oestrogen identification and dosage in filatove human placenta extracts by high performance liquid chromatography. *Int. J. Cosmet. Sci.* 8:175–188.

Bishayee, A., K. K. Banerjee, and M. Chatterjee. 1995. Effects of human placental extract on hepatic drug metabolizing enzyme. *Riv. Eur. Sci. Med. Farmacol*. 17:19–26.

Chirouze, V., B. Entressangles, and J. P. Helme. 1987. Human placental as a source of long-chain polyunsaturated fatty acids. I. Placental lipid composition and potential yields of arachidonic acid. (Translated from the French article.) *Rev. Fr. Corps. Gras.* 34:275–280.

Cosmetics Directive of the European Union. 1995. Updated version—incorporating all amendments until August 1, 1995. Dir 76/768/EEC, Annex VI, part I.

Cosmetic, Toiletry, and Fragrance Association (CTFA). 1988. Placental Protein: Specifications and toxicity data. Unpublished data submitted by CTFA. (32 pages.)[3]

Cosmetic, Toiletry, and Fragrance Association (CTFA). 1998. Specification and summaries of primary dermal and ocular irritation studies on two placental extracts. Unpublished data submitted by CTFA. (30 pages.)[3]

CTFA. 1999. Ingredient use data. Unpublished data submitted by CTFA. (1 page.)[3]

DeGroot, A. C. 1994. *Patch testing: Test concentrations and vehicles for 3700 chemicals*, 2nd ed., 11, 144. Amsterdam: Elsevier.

Florini, J. R., G. Tonelli, C. B. Breuer, J. Coppola, I. Ringler, and P. H. Bell. 1966. Characterization and biological effects of purified placental protein (human). *Endocrinology* 79:692–708.

Food and Drug Administration (FDA). 1984. Cosmetic product formulation and frequency of use data. *FDA database*. Washington, DC: FDA.

FDA. 1994. *FDA's cosmetic handbook*, 12–13. Washington, DC: Center for Food Safety and Applied Nutrition/FDA.

FDA. 1998. Cosmetic Product formulation data. FDA computer printout.

Gershbein, L. L., and D. A. Malik. 1967. Metabolic studies in rats fed sterol-containing diets, raw human placenta, and various hog organs as such and with vitamin supplements. *Int. Z. Vitaminforsch.* 37:57–73.

Girotto, G., and W. Malinverni. 1982. Use of placental extract for the treatment of myopic and senile chorio-retinal dystrophies. *Int. J. Tissue React.* 4:169–172.

Huxham, I. M., M. Gupta, R. Azouble, and F. Beck. 1982. Dose dependent induction of embryonic abnormalities *in vitro* by tissue homogenates of placenta and decidua. *Br. J. Exp. Pathol.* 63:95–102.

Ikawa, Y., M. Aida, and M. Saito. 1975. Hemoglobin production in cultured Friend leukemia cells induced by a human placental extract preparation. *Gann* 66:583–584

Kimoto, E., H. Tanaka, T. Inoue, N. Toh, M. Kojima, and H. Hamada. 1987. Influence of placental extract upon the adriamycin-mediated radical reactions. *Fukuoka Daigaku Rigaku Shuho* 17:65–71.

Klein, J. L., C. Chiodino, and H. Yamasaki. 1993. Growth of only highly tumorigenic cell lines is inhibited by EAP, a human placental fraction. *Cancer Lett.* 70:91–99.

Klein, J. L., E. Hamel, J. L. Tayot, and H. Yamasaki. 1991. Growth suppression of transformed cells by a human placental extract not related to transforming growth factor beta. *J. Cancer Res. Clin. Oncol.* 117:192–196.

Komura, H., H. Minakata, H. Mochizuki, and T. Kada. 1983. Antimutagenicity of human placenta extract: Chemical studies. *Carcinog. Mutagens Environ.* 2:85–90.

Mochizuki, H., and T. Kada. 1982. Antimutagenic action of mammalian placental extracts on mutations induced in *Escherichia coli* by UV radiation, gamma-rays and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. *Mutat. Res.* 95: 457–474.

Mkrtchian, L. N., and A. K. Nersessian. 1993. [Effect of immunization of mice with human umbilical cord extract on micronucleus induction by cyclophosphane.] Title translated from Russian. *Genetika* 29:866–868.

Mkrtchyan, L. N., E. R. Ter-Pogosyan, A. K. Nersesyan, E. V. Petanova, and S. O. Martirosyan. 1990. [The antitumor effect of an extract of human umbilical cord.] Title translated from Russian. *Vopr. Onkol.* 36:956–960.

O'Keefe, E. J., and M. L. Chiu. 1988. Stimulation of thymidine incorporation in keratinocytes by insulin, epidermal growth factor, and placental extract: Comparison with cell number to assess growth. *J. Invest. Dermatol.* 90:2–7.

Pal, P., R. Roy, P. K. Datta, A. K. Dutta, B. Biswas, and R. Bhadra. 1995. Hydroalcoholic human placental extract: Skin pigmenting activity and gross chemical composition. *Int. J. Dermatol.* 34:61–66.

[3]Available for review: Director, Cosmetic Ingredient Review 1101 17th Street, NW Suite 310, Washington, DC 20036-4702, USA.

Rempe, J. M., and L. G. Santucci. 1997. *CTFA list of Japanese cosmetic ingredients*, 3rd ed. Washington, DC: CTFA.

Riggi, S. J., C. R. Boshart, P. H. Bell, and I. Ringler. 1966. Some effects of purified placental protein human on lipid and carbohydrate metabolism. *Endocrinology* 79:709–715.

Sharma, S. K. 1988. Topical human placental extract for the treatment of vitiligo. *Indian J. Dermatol. Veneral Leprol.* 54:199–201.

Suite, M., and D. B. Quamina. 1991. Treatment of vitiligo with topical melagenine—A human placental extract. *J. Am. Acad. Dermatol.* 24(6 Pt 1): 1018–1019.

Taylor, E. J., ed. 1988. *Dorland's illustrated medical dictionary*, 27th ed., 1765. Philadelphia, PA: WB Saunders.

Tiwary, C. M. 1997. A survey of use of hormone/placenta-containing hair preparations by parents and/or children attending pediatric clinics. *Military Med.* 162:252–256.

von den Driesch, P., M. Fartasch, T. L. Diepgen, and K. P. Peters. 1993. Protein contact dermatitis from calf placenta extracts. *Contact Dermatitis* 28:46–47.

Wenninger, J. A., and G. N. McEwen, Jr., eds. 1997. *International cosmetic ingredient dictionary and handbook*, 7th ed., 617–618, 636, 638, 1053, 1444. Washington, DC: CTFA.

Younoszai, M. K., and J. C. Haworth. 1969. Chemical composition of the placenta in normal preterm, term and intrauterine growth-retarded infants. *Am. J. Obstet. Gynecol.* 103:262–264.