Scientific Literature Review

Chlorphenesin as Used in Cosmetics

November 28, 2011

All interested persons are provided 60 days from the above date to comment on this Scientific Literature Review and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Director, Dr. F. Alan Andersen.

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TABLE OF CONTENTS

INTRODUCTION 0
CHEMISTRY 0
Definition and Structure 0
Chemical and Physical Properties 0
Methods of Production 0
Reactivity 0
USE 0
Cosmetic 0
Noncosmetic 1
TOXICOKINETICS 1
Absorption, Distribution, Metabolism, and Excretion 1
Percutaneous Absorption 1
TOXICOLOGY 2
Acute Oral Toxicity 2
Repeated Dose Toxicity 2
Ocular Irritation 3
Skin Irritation 3
Skin Irritation and Sensitization 3
Case Reports 4
Photoallergenicity 5
Immunosuppression 5
REPRODUCTIVE AND DEVELOPMENTAL TOXICITY 5
GENOTOXICITY 6
CARCINOGENICITY 6
Antitumorigenicity 6
Anticarcinogenicity 6
BIOLOGICAL EFFECTS 7
Effect on Second Messenger 7
Effect on Histamine Release 7
DATA NEEDS 7
SUMMARY 8

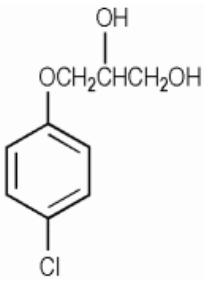
INTRODUCTION

Available data relevant to the safety of Chlorphenesin as used in cosmetics are reviewed in this Scientific Literature Review. As stated in the *International Cosmetic Ingredient Dictionary and Handbook*,[1] this ingredient functions as a biocide in cosmetic products. Chlorphenesin is being reviewed at the request of the Food and Drug Administration (FDA). According to an FDA enforcement report, dated August 6, 2008, Mommy's Bliss Nipple Cream was recalled because it was considered an unapproved new drug product that contained chlorphenesin and phenoxyethanol.[2] In the Agency's warning to consumers, the FDA stated that chlorphenesin relaxes skeletal muscle and can depress the central nervous system (CNS) and cause respiratory depression in infants. The warning also stated that phenoxyethanol, the other ingredient of concern, can depress the CNS and may cause vomiting and diarrhea, which can lead to dehydration in infants. According to FDA, these 2 ingredients may interact with one another to further compound and increase the risk of respiratory depression in nursing infants. It was also noted that chlorphenesin can harm the mother by causing dermatitis.[3]

CHEMISTRY

Definition and Structure

Chlorphenesin (CAS No. 104-29-0) is defined as the organic compound that conforms to the following formula:[1]



Other names for this chemical include: 3-(4-Chlorophenoxy)-1,2-Propanediol; 1,2-Propanediol,3-(4-Chlorophenoxy)-; and p-Chlorophenyl Glyceryl Ether.[1]

Chemical and Physical Properties

A UV spectral analysis of 0.01% aqueous Chlorphenesin solution indicated maximum absorption at 279 nm.[4] Additional properties of Chlorphenesin are found in Table 1.

Methods of Production

Chlorphenesin is prepared by condensing equimolar amounts of p-chlorophenol and glycidol in the presence of a tertiary amine or a quaternary ammonium salt as a catalyst.[5]

Reactivity

The production of formaldehyde from Chlorphenesin by oxidation with periodic acid has been reported.[6]

USE

Cosmetic

Chlorphenesin reportedly functions as a biocide in cosmetic products.[1] According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP) in 2011, Chlorphenesin was being used in cosmetic products.[7] These data are summarized in Table 2. Results from a survey of

ingredient use concentrations provided by the Personal Care Products Council (also included in Table 2) in 2011 indicate that Chlorphenesin was being used at concentrations up to 0.32% (rinse-off products) and up to 0.3 % (leave-on products).[8]

Cosmetic products containing Chlorphenesin may be applied to the skin and hair, or, incidentally, may come in contact with the eyes and mucous membranes. Products containing these ingredients may be applied as frequently as several times per day and may come in contact with the skin or hair for variable periods following application. Daily or occasional use may extend over many years.

Chlorphenesin is used in hair, foot, and suntan sprays, and could possibly be inhaled. In practice, 95% to 99% of the aerosols released from cosmetic sprays have aerodynamic equivalent diameters in the 10 to 110 µm range, with propellant sprays yielding a greater fraction of droplets/particles below this range when compared to pump sprays .[9,10] Therefore, most aerosols incidentally inhaled from these sprays are deposited in the nasopharyngeal region and are not respirable to any appreciable level.[11,12] There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic diameters in the range considered to be respirable.[12] However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays.

According to the European Union Cosmetics Directive, Chlorphenesin is listed among the preservatives that cosmetic products marketed in the European Union may contain. The maximum authorized use concentration for this ingredient is 0.3%.[13]

Noncosmetic

Chlorphenesin is one of the ingredients in an antimicrobial product identified as Miol cream.[14]

TOXICOKINETICS

Absorption, Distribution, Metabolism, and Excretion

The absorption, distribution, and metabolic fate of Chlorphenesin was evaluated using male Sprague-Dawley rats and beagle dogs.[15] In the first experiment (4 rats), a16.7 mg oral dose of Chlorphenesin-1,3-^{14}C (in physiological saline) was administered via stomach tube, after which concentrations in the blood were determined. In a second experiment, Chlorphenesin-1,3-^{14}C (15.2 mg) was administered i.p. to one rat, and the distribution of administered radioactivity was determined. A third experiment was performed to isolate Chlorphenesin metabolites from the urine. Non-radioactive Chlorphenesin (500 mg/kg) was administered orally to 2 beagle dogs and urine was collected for 24 h. Urine from 2 beagle dogs was also collected after the animals received 2 i.p. injections of non-radioactive Chlorphenesin (250 mg/kg, 6 h apart). In an experiment to identify conjugated metabolites, 4 male rats were injected i.p. with Chlorphenesin UL-ring-^{14}C (30 mg) and urine was collected for 24 h. The relative radioactivity corresponding to each of the major metabolites was determined quantitatively in additional experiments in which Chlorphenesin-1,3-^{14}C was administered to rats (capsule form) and beagle dogs (i.p. dose).

Following oral ingestion, Chlorphenesin-^{14}C was absorbed rapidly in the rat. Radioactivity reached a peak blood concentration in 30 min, and the half-life of serum radioactivity was approximately 140 minutes. Results of the distribution experiment indicated that over half of the administered i.p. dose of Chlorphenesin-1,3-^{14}C in the rat was excreted in the urine after 4 h. The remainder was found primarily in the gastrointestinal tract and carcass. A small portion of the radioactivity was recovered as respiratory CO_2. The urinary end products identified after administration of the drug to rats or dogs were: 3-p-chlorophenoxylactic acid, p-chlorophenoxyacetic acid, and unchanged Chlorphenesin. Additional urinary end products identified as a conjugate of chlorophenol and a conjugate of Chlorphenesin were observed after rats were injected i.p. with Chlorphenesin UL-ring-^{14}C.[15]

Percutaneous Absorption

The percutaneous absorption of ^{14}C-Chlorphenesin was evaluated using 16 male rats of the Sprague-Dawley CD strain (~ 6 weeks old).[16] ^{14}C-Chlorphenesin (in 0.05% w/w cold cream; mean dose = 1.14 mg/kg [~ 14 µCi]) was applied topically to shaved skin on the back (9 cm^2). Application sites were occluded with aluminum foil until the animals were killed. After test substance application, the animals were placed in individual metabolism cages for the collection of urine

and feces. Pairs of animals were killed at various intervals, beginning at 1 h and ending at 96 h. The mean total recovery of radioactivity (application site, excreta, selected tissues, and residual carcass) was 92.35% dose + 3.11 standard deviation (SD) after dosing. The proportion of administered ^{14}C-Chlorphenesin dose that remained at the application site (in and on the skin) decreased from ~ 89% at 1 h to ~ 43% at 96 h. During the 0 to 96 h time period, ~ 48% (mean value) of the applied dose was excreted in the urine. Approximately 0.5% was excreted in the feces and ~ 0.7% was recovered in cage washings. Thus, practically all of the absorbed dose was excreted over a period of 96 h.

Not more than 1% of the applied dose was present in any of the tissues during the 1 h to 96 h time frame, though up to 57% of the dose was absorbed. At 96 h, ~ 7 to 8% of the administered dose remained and was subsequently eliminated from the body. Apparently, the radioactivity was absorbed biphasically, with initial and terminal half-lives for absorption of ~ 4 h and 126 h, respectively. The urinary excretion rate was proportional to plasma radioactivity concentrations during 0 to 96 h, suggesting that the renal clearance of radioactivity was constant. The terminal excretion half-life (~ 22 h) was considerably shorter than the terminal absorption half-life (~ 126 h). Thus, the excretion of radioactivity was absorption-rate limited.[16]

TOXICOLOGY

Acute Oral Toxicity

The acute oral toxicity of Chlorphenesin (in 0.5% carboxymethylcellulose aqueous gel) was evaluated using 5 groups of 10 (5 males, 5 females/group; ~ 6 weeks old) Sprague Dawley rats.[17] The 5 groups received single oral doses of 1200, 1620, 2187, 2952, and 3985 mg/kg, respectively. Dosing was followed by a 14-day observation period, after which all surviving animals were killed. The following signs were observed after test substance administration of each dose: dyspnea, decrease in spontaneous activity, hypotonia, piloerection, and loss of reflex. Necropsy findings for animals that died were mainly an intestinal meteroism and lung congestion. A mean LD50 of 3,000 mg/kg 95% confidence interval: 2830 to 3180 mg/kg) was reported.

Repeated Dose Toxicity

A repeated dose oral toxicity study on Chlorphenesin was performed using 4 groups of 16 rats of the Charles River Crl : CD(SD) BR strain (8 males, 8 females/group; 47 days old).[18] Chlorphenesin (suspension in 1% aqueous methylcellulose) was administered by gavage to 3 groups at doses of 10, 100, and 1000 mg/kg/day (dose volume = 10 ml/kg/day), respectively, for 28 consecutive days. Control rats were dosed similarly with 1% aqueous methylcellulose. Except for one animal killed, on humane grounds, during week 4, the animals were killed on day 29. Microscopic examination of the rat (high-dose male) killed during week 4 revealed renal tubular dilatation and necrosis of the papillary tip, both treatment-related. No microscopic changes were observed in high-dose female rats or the remaining high-dose male rats. Clinical findings in the highest dose group included: hunched posture, abnormal gait, pallor, lethargy, ptosis, a badly groomed appearance, noisy respiration, and piloerection. A badly groomed appearance was also observed in rats of the low dose (not toxically significant) and intermediate dose groups, and increased salivation was also observed in the intermediate dose group. Compared to controls, a statistically significant reduction ($P < 0.01$) in body weight gain was noted for male and female rats of the highest dose group. Significantly lower hemoglobin levels were reported for high-dose males and females and intermediate-dose males.

Statistically significant increases ($P < 0.01$) in glutamic pyruvic transaminase (GPT) were reported for high-dose males and females. Alkaline phosphatase levels in high dose males were slightly higher when compared to controls, but the difference was not statistically significant. Potassium and calcium ion concentrations were significantly lower ($P < 0.05$) in high-dose females. IgG and IgM serum levels in high-dose females, when adjusted for pre-dose levels, were significantly higher than control values at the end of dosing. These changes were considered a reflection of hematological and biochemical changes due to treatment with Chlorphenesin, and not a specific effect on the immune system. Absolute spleen weights (high-dose males and females) and thymus weights (high-dose males) were significantly lower ($P < 0.05$ or $P < 0.01$) when compared to controls. At macroscopic examination, general brown staining of the fur was observed in all 5 high-dose female rats examined, compared to the absence of this finding in controls. The only microscopic finding (in kidney) is mentioned in the preceding paragraph. The reported changes in the high and intermediate dose groups were considered treatment-related. A dose of 10/mg/kg/day was considered the no adverse effect level in this study.[18]

Ocular Irritation

The ocular irritation potential of Chlorphenesin (1% [w/v] in distilled water) was evaluated using 3 New Zealand albino rabbits (ages not stated).[19] The test substance (0.1 ml) was instilled into the right eye of each animal, and the lids were held together for approximately 10 seconds. Untreated left eyes served as controls. The eyes were examined for ocular reactions at 1 h and then at days 1, 2, 3, 4, and 7 post-instillation. Slight conjunctival irritation (enanthema, chemosis, and lacrimation) was reported for each rabbit and these reactions had cleared by 24 h post-instillation. Chlorphenesin was classified as a weak ocular irritant (maximum ocular irritation index = 6 [at 1 h post-instillation]).

Skin Irritation

The skin irritation potential of Chlorphenesin was evaluated using 6 male New Zealand albino rabbits (ages not stated). A 2.5 x 2.5 cm occlusive patch containing Chlorphenesin (1% [w/v] in distilled water, 0.5 ml) was applied to the shaved flanks of each animal.[19] The right flank was abraded and the left remained intact. Patches were secured with fastening tape and the trunk was wrapped with an elastic bandage secured with adhesive tape. At 24 h, the patches were removed. Slight, reversible erythema was observed in 2 rabbits, and there was no evidence of structural modification. Chlorphenesin was classified as a non-irritant (primary irritation index [PII] = 0.1).

A study was performed to investigate the side effects of cosmetic preservatives by evaluating objective and subjective skin irritants.[20] In a 24 h occlusive patch test involving 30 subjects (20 females, 10 males; mean age = 33.7 years), 2% Chlorphenesin (20 µl) was applied to filter paper discs on IQ test chambers, and patches remained in contact with the forearm for 24 h. Reactions were evaluated at 30 minutes and 1 day after patch removal. A mean irritation score of 0.17 ± 0.38 was reported. A cumulative skin irritation test was performed using 15 healthy subjects (8 females, 7 males; mean age = 29.7 years). The formulations tested were emulsion bases with a preservative mixture consisting of 0.2% methylparaben, 0.1% propylparaben, and 0.25% Chlorphenesin (Type 1) and emulsion bases containing 0.2% methylparaben, 0.1% propylparaben, 0.3% phenoxyethanol, and 0.25% Chlorphenesin (Type 2). Each formulation (20 µl) was applied according to the preceding method 3 times per week over a 21-day period. Each subject received 9 applications (same site) of the test substance. For Type 1 formulations tested, the highest reported total cumulative irritation mean score was 0.40 ± 0.91. For Type 2 formulations, a mean score of 0.87 ± 1.19 was the highest reported.

A sensory irritation test was performed using 16 healthy subjects (6 females, 10 males; mean age = 28.3 years). A cotton swab soaked with 0.4% Chlorphenesin (in 0.5% carbopol solution, 0.5 ml volume) was rubbed briskly and applied (under occlusion) to each side of the nasolabial fold and cheek. Any evidence of a stinging/burning reaction was recorded over a period of 9 minutes. Carbopol (0.5%) solution served as the vehicle control. The sensory irritation potential of 0.4% Chlorphenesin (mean score = 0.54) was greater than the control (mean score = 0.22). Emulsion bases (with or without Chlorphenesin in preservatives mixture) were tested according to the same procedure. Sensory irritation induced by the formula containing methylparaben, propylparaben, and Chlorphenesin was greater when compared to the same formula without Chlorphenesin.[20]

The skin irritation potential of 0.3% Chlorphenesin (in water) was evaluated using 25 subjects (20 females, 5 males; 19 to 62 years old). An occlusive patch containing the test substance (0.1 ml) was applied to the back of each subject for 48 h. Reactions were scored 20 minutes after patch removal. Faint, minimal erythema (score = ±) was observed in 2 subjects and erythema (score = 1) was observed in a third subject Chlorphenesin was classified as having negligible dermal irritation potential.[21]

Skin Irritation and Sensitization

Prior to initiation of the sensitization study below, a test was performed to determine the maximal non-irritant concentration of Chlorphenesin.[22] The test involved 3 male albino Dunkin Hartley guinea pigs (ages not stated). A dorsal surface area of ~ 60 cm^2 was clipped free of hair, and, on both sides of the spinal column, 3 symmetrical intradermal injections (0.1 ml) of the following preparations were made: (1) 50% Freund's Complete Adjuvant (FCA) in distilled water, (2) distilled water, and (3) a 50/50 mixture of 1 and 2. Sites were clipped free of hair 7 days later, and the following concentrations of Chlorphenesin (0.5 ml volume) were applied under an occlusive patch for 24 h: 0.1 %, 0.25 %, 0.5 %, and 1.0% in distilled water. Irritation reactions were scored at 24 h and 48 h after patch removal. Irritation was not induced by any of the concentrations tested. Test concentrations of 0.5% and 1.0% were designated for use during the challenge phase of the sensitization study.

The skin sensitization potential of Chlorphenesin was evaluated in a modified guinea pig maximization test using 30 female albino Dunkin Hartley guinea pigs (ages not stated). Test and control groups consisted of 20 and 10 guinea pigs,

respectively. Dorsal skin was clipped free of hair, and 3 symmetrical intradermal injections (0.1 ml) of 1% Chlorphenesin (in distilled water), 1% Chlorphenesin (in a mixture of Freund's complete adjuvant [FCA] and distilled water), and a mixture of FCA and distilled water, respectively, were made on both sides of the spinal column (scapular level) during induction of test animals. During induction, control animals were injected with FCA/distilled water mixtures and distilled water. Induction injections were followed by a single 48 h application of an occlusive patch (2 x 4 cm) moistened with 1% Chlorphenesin in distilled water (0.5 ml, test animals) or distilled water (0.5 ml, controls). During the challenge phase, Chlorphenesin (1% or 0.5% in distilled water, 0.5 ml) was applied, under occlusive patch (2 x 2 cm), to a new test site for 24 h. Reactions were evaluated at 24 h and 48 h after patch removal. Chlorphenesin did not induce sensitization at a concentration of 0.5% or 1.0%.[22]

A human repeated insult patch test was used to evaluate the skin irritation and sensitization potential of a test material containing 5 to 9% Chlorphenesin.[23] Fifty-five male and female subjects (between 27 and 67 years old) completed the study. Three of the original 58 subjects withdrew for reasons unrelated to test material application. During induction, a 1 inch x 1 inch semi-occlusive patch containing the test material (0.2 mg/cm^2) was applied to the back, between the scapulae, of each subject. Patches were removed at 24 h, and any irritation reactions scored 24 h after patch removal. The scoring of reactions was followed by application of a new patch that remained for 24 h. This cycle was repeated for a total of 9 consecutive patch applications (i.e., 3-week induction phase). The 4-day challenge phase was initiated after a 10- to 14-day non-treatment period. A new patch containing 0.2 ml or 0.2 g of the test material was applied (24 h) to a new test site on the back. Reactions were scored at 48 h and 72 h post-application. Neither irritation nor sensitization reactions were observed during the study, and it was concluded that the test material did not have dermal irritation or allergic contact sensitization potential.

The skin irritation and sensitization potential of a different test material containing 12 to 17% Chlorphenesin was evaluated using 53 male and female subjects (between 18 and 66 years old).[24] Three of the original 56 subjects withdrew from the study, and it was stated that one of the subjects withdrew for reasons unrelated to test material application. The test material (0.2 ml or 0.2 g) was applied using a semi-occlusive patch according to the test procedure immediately above. In one subject, barely perceptible erythema (score = 0.5) was observed on day 19 of induction and mild erythema (score = 1) was observed on day 22. The mild erythema observed was classified as a transitory, weak response that could be considered clinically insignificant.

Case Reports

A 38-year-old female developed widespread acute dermatitis after using a proprietary antifungal powder and cream, both containing Chlorphenesin.[25] Signs included severe maceration of the toe webs, with severe eczema of the foot. A generalized rash on the legs, forearms, and hands was also observed. Patch testing of individual constituents of the products used revealed a positive response only to 1% Chlorphenesin in petrolatum. No reaction to this test concentration was observed in 3 control subjects.

A 60-year old atopic female developed facial eczema within several hours after applying a foundation (cosmetic) containing Chlorphenesin.[26] Patch testing revealed an allergic response (++ reaction) to 1% Chlorphenesin in petrolatum. The patient was not patch tested with the foundation. In a second report, a 33-year old female who used a proprietary moisturizing cream containing Chlorphenesin had a 1 –month history of facial eczema. The eczema eventually involved the entire face and spread to the neck, upper chest, and upper arms. The patient had no personal or family history of atopy. Patch test results indicated a + reaction to 1% Chlorphenesin in petrolatum and a +++ reaction to the moisturizer (as is). Both reactions were observed by day 2 and persisted to day 4.

In another case report, a 24-year-old male applied an ointment containing Chlorphenesin to his feet twice daily to relieve itching.[27] Following 3 days of treatment, a symmetrical vesciculo-bullous eruption was observed on the dorsa of the feet. This reaction extended to the ankles and was accompanied by extensive eczema on the trunk and arms within 24 h. Patch testing resulted in a ++++ reaction to 0.5% Chlorphenesin in white soft paraffin and to the ointment.

Chronic dermatitis of the axillae was reported for a 29-year-old female who used a deodorant that contained Chlorphenesin.[28] She also had a past history of allergy to metallic jewelry. Patch results for the deodorant were positive at 48 h (+ reaction) and 96 h (+ reaction), and patch test results for 1% Chlorphenesin in petrolatum were positive at 48 h (+ reaction) and 96 h (++ reaction). Positive reactions were not observed in 5 control subjects patch tested with 1% Chlorphenesin in petrolatum.

A 43-year old female experienced burning discomfort and developed a florid eczema after applying a facial moisturizer containing Chlorphenesin.[29] The patient had a history of hay fever, but no history of medicament or cosmetic

intolerance. Patch test reactions were positive (++) for Chlorphenesin on days 2 and 4. Positive patch test reactions were also reported for the product on day 2 (++) and day 4 (+).

Photoallergenicity

Eleven patients photoallergic to ketoprofen were photo patch tested with Chlorphenesin.[30] Testing was initiated on day 0 and the subjects were irradiated with UVA light (5 J/cm^2) at day 2. Readings were performed on days 3 and 4 according to International Contact Dermatitis Research Group recommendations. There were no positive reactions in patients photo patch tested with Chlorphenesin. Additional study details will be included after this study has been translated.

Immunosuppression

The immunosuppressive activity of Chlorphenesin was evaluated using groups of 3 to 4 albino rabbits.[31] The groups were immunized with 1 ml of antigen (gram-positive bacteria (CA+) alone or antigen + Chlorphenesin). A total of 3 i.v. injections of each was made on days 0, 3, and 7 according to the following procedure: Group 1 (control) received the mixture of one part of CA(+) antigen (final dilution of 1:100) and 9 parts of buffer. Group 2 received a mixture of one part of antigen and 9 parts of Chlorphenesin at concentrations of 0.01, 0.1, 1, or 10 mg/ml. Prior to injection, these mixtures were incubated (37°C) for 30 minutes. Group 3 received the same antigen-Chlorphenesin mixtures without prior incubation. The fourth group received antigen and Chlorphenesin, albeit separate injections. When tested at a concentration of 1 or 10 mg/ml, but not 0.01 or 0.1 mg/ml, Chlorphenesin markedly inhibited the CA (+) hemagglutinin response. It was also noted that injection of the non-incubated mixture and separate administration of the 2 materials into separate ear veins caused an undiminished immune response. The results of additional experiments indicated that Chlorphenesin suppressed antibody formation less effectively when larger amounts of antigen were used. With smaller amounts of antigen, Chlorphenesin partially inhibited the antibody response, even at a concentration of 0.1 mg/ml.

The immunosuppressive activity of Chlorphenesin was studied using a wide variety of *in vitro* assays for cellular immunity in both humans (25 to 40 years old) and mice (6 to 11 months old) of the following strains: BALB/c, C57Bl/6, and BDF_1 (C57Bl x DBA) F_1 mice.[32] At doses of 20 to 50 µg/ml, Chlorphenesin inhibited mitogenic responses of B and T cells from mice and humans. Exposure to these doses for 72 h did not result in death of B or T cells. Mixed lymphocyte reactions in cells from inbred strains of mice and unrelated humans were also inhibited at concentrations of approximately 50 µg/ml. In light of these results, the generation of cytotoxic T cells in cell-mediated lympholysis assays was not inhibited to the same extent as proliferation in mixed lymphocyte reactions. Also, the cytotoxic potential of pre-sensitized mouse T cells for allogeneic targets was totally unaffected. The results of these studies suggest that Chlorphenesin may have a broad spectrum of suppressive effects on both B and T lymphocytes and that the predominant inhibition of proliferative responses in these lymphocytes may reduce the expansion of clones of immunocompetent cells *in vivo*.

The effect of Chlorphenesin on the immune response in mice, rabbits, and guinea pigs was studied.[33] Male Swiss Webster mice were injected with sheep red blood cells (S-RBC) or chicken red blood cells, after which potassium penicillin G (KPG) conjugated to keyhole limpet hemocyanin was injected intravenously (i.v.). An assay for localized hemolysis was then performed, in which the degree of hemolysis was determined after 2 h. Groups of 4 to 8 New Zealand White rabbits were used to determine the presence of circulating antibodies. The antigens were injected into the hind footpads and subcutaneously (s.c.) over each shoulder. The rabbits were bled and tested for antibody titers for up to 21 days post-immunization. Male albino guinea pigs were sensitized with bacillus Calmette-Guérin (BCS) vaccine intradermally and challenged intradermally with old tuberculin at 5 weeks post-sensitization. In the localized hemolysis assay, the joint administration of an antigen with Chlorphenesin greatly reduced the number of antibody-forming cells in the spleen. The simultaneous administration of antigen with Chlorphenesin also resulted in suppression of formation of humoral antibodies in mice and rabbits. Chlorphenesin was effective as an immunosuppressive agent only when administered jointly with an antigen, did not affect existing antibody levels or the secondary response, and did not increase the susceptibility of the animals to infections. If administered at the time of challenge, Chlorphenesin affected the BCS reaction, i.e., significantly reduced the tuberculin reaction in guinea pigs.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

The effect of Chlorphenesin (suspension in 1% methylcellulose) on pregnancy and *in utero* development of the rat was evaluated using 4 groups of 25 sexually mature, Specific Pathogen Free female rats of the Crl: CD®BR VAF/Plus strain

(8 to 10 weeks old).[34] Three groups received oral doses (gavage; dose volume = 10 ml/kg body weight) of 10, 50, and 100 mg/kg, respectively, once daily on days 6 to 15 post-coitum. The control group was dosed with the vehicle (1% methylcellulose) according to the same procedure. The animals were killed on day 20 and necropsy was performed to identify any congenital abnormalities or macroscopic pathological changes in maternal organs. Tissues were preserved for microscopic examination. There was no evidence of maternal toxicity at either of the 3 administered doses, and neither maternal body weight gain nor food intake was affected by treatment. Increased fur loss and transient post-dosing salivation were observed in the highest dose (100 mg/kg/day) group. Based on necropsy results, it was considered unlikely that fur loss was test substance-related. At all doses administered, Chlorphenesin had no adverse effect on embryo-fetal survival, growth, or development *in utero*. The no observed effect level for selective toxicity to the developing fetus was considered to be 100 mg/kg/day.

GENOTOXICITY

The genotoxicity of Chlorphenesin was evaluated in the Ames test (bacterial reverse gene mutation assay) using the following *Salmonella typhimurium* strains: TA 98, TA 100, TA 1535, TA 1537, and TA 1538.[35] Test concentrations up to 5,000 µg/plate were evaluated with and without metabolic activation . 2-Aminoanthracene served as the positive control for metabolic activation cultures and 2-nitrofluorene, 9-aminoacridine, and N-ethyl-N'-nitro-N-nitrosoguanidine served as positive controls for non-activation cultures. Chlorphenesin was not genotoxic with or without metabolic activation over the range of concentrations tested. The positive controls were genotoxic. The same conclusion was reached in another Ames test evaluating the genotoxicity of Chlorphenesin in *Salmonella typhimurium* strain TA 102 and *Escherichia coli* strain WP2 uvrA over the same test concentration range (with and without metabolic activation).[36] Both positive controls (2-aminoanthracene and methyl methane sulfonate [non-activation]) were genotoxic to both strains.

CARCINOGENICITY

Antitumorigenicity

In a study involving groups of Strain A (inbred strain) female mice, immune competence during initiation-promotion carcinogenesis was determined by the length of time required to reject allografts of tail skin and by the incorporation of [^{3}H]thymidine by lymphocytes stimulated with the mitogens phytohemoagglutinin (PHA) and pokeweed mitogen (PWM).[37] During initiation-promotion carcinogenesis, mice were also treated with Chlorphenesin, predicated on its reported effects to increase immunological reactivity, particularly cellular immunity. The skin grafting experiment involved 5 groups of mice. Initially, 2.5% croton oil (20 µl) was applied to the intrascapular area twice per week for 30 weeks, and mice were treated with a single application of 7,12-dimethylbenzanthracene (DMBA, 100 µg) 10 days later. The mice were then separated into 2 groups, with and without tumors, respectively. In order to study the effect of the initiating and promoting agents, DMBA (100 µg) was applied to the interscapular area of each animal in the third group at 10 days before grafting. The fourth group was treated with 2.5% croton oil (20 µl) according to the same procedure, and the fifth group served as the untreated control group.

The experiment using lymphocyte cultures also involved 5 groups of mice. Groups 1 and 2 were treated with DMBA and croton oil, respectively (same doses), and Group 3 received two 2.5 mg doses of Chlorphenesin i.p. (same day). Group 4 received a dermal application of croton oil and two i.p. doses of Chlorphenesin, and Group 5 served as the untreated control group. The mitogenic response of lymphocytes to PHA and PWM was determined using whole blood lymphocyte cultures. The tumor initiation-promotion experiment involved 2 groups of 30 Swiss mice. In the first group, DMBA (100 µg) was applied to the interscapular area of each animal, and, after 3 weeks, 2.5% croton oil was applied to the skin twice weekly for 20 weeks. Group 2 animals received applications of DMBA and croton oil plus two 2.5 mg injections of Chlorphenesin i.p. (same day) at the same time that croton oil was applied. The animals were necropsied at 20 weeks. The carcinogen 7,12-dimethylbenzanthracene inhibited the cellular immune competence of mice, and lymphocytes from mice treated with croton oil had enhanced PWM response. Chlorphenesin inhibited tumorigenesis in initiation-promotion skin carcinogenesis when injected during promotion.[37]

Anticarcinogenicity

Female Swiss mice were injected i.p. (day 0) with 0.2 ml of Rauscher murine leukemia virus (RMLV) or Friend murine leukemia virus (FMLV) suspension and distributed randomly into paired groups of 18 to 20 mice each.[38] Chlorphenesin in warm Hank's balanced salt solution (HBSS) was then injected i.p. (dose = 100 mg/kg in 0.5 ml) in the

morning and late afternoon on each day of treatment. Chlorphenesin was injected into the RMLV mice on days 1, 2, 3, 4, 7, and 8, and FMLV mice received injections on days 1, 2, 6, 7, 9, 12, and 13. Control mice were injected with HBSS only after virus injection according to the same schedules. Injected virus routinely resulted in 80% mortality in leukemic control groups within 50 to 60 days. Chlorphenesin caused a pronounced sparing effect on mortality due to leukemia after infection with RMLV. Delayed onset of early death in Chlorpehensin-treated mice was observed, but the most characteristic finding was the marked sparing effect in later stages of the disease. Mortality in mice dosed with Chlorphenesin leveled off at 40%; however, controls continued to die at a nearly linear rate.

Additional experiments evaluating antiviral activity suggested that Chlorphenesin was probably acting on malignant cells rather than against the transforming virus. In an effort to confirm this, Leukemia L-1210 in ascites form was implanted s.c. into B6DF1 mice, and results indicated that Chlorphenesin had little effect against conventional massive i.p. doses of this highly malignant cell line. However, when the system was modified by using reduced numbers of cells implanted s.c., the sparing effect was readily demonstrable. More than 40% of the treated mice survived until the experiment was terminated at 50 days, at which time there was no visible evidence of residual tumor.

Clinical trials involving cancer patients were conducted by the Clinical Screening Group of the European organization for Research on Treatment of Cancer. Patients (31) with a wide range of neoplasms had been treated with Chlorphenesin for periods ranging from 1 to 6 weeks. Oral doses ranged from 1 to 6 g daily, with a usual dose of 4 g/day. Treatment with Chlorphenesin was ineffective in 16 cases of carcinoma (cervix, uterus, tonsil, esophagus, and lung) and in 4 cases of sarcoma. However, in 9 cases of squamous cell carcinoma of the skin, complete remission was achieved in one patient and substantial, though incomplete, remission was achieved in 4 other patients. For 2 patients with basal cell carcinoma, no benefit was observed.[38]

BIOLOGICAL EFFECTS

Effect on Second Messenger

Chlorphenesin (3 to 6 mM) inhibited the hydrolysis of cyclic AMP by guinea-pig lung phosphodiesterase, and this inhibition was reversed by the addition of exogenous magnesium ions.[39] The incubation of monkey lung tissue in the presence of 400 µg/ml Chlorophenesin (2 mM) caused a 40% increase in the level of intracellular cyclic AMP.[40]

Effect on Histamine Release

The effect of Chlorphenesin on histamine release was evaluated using isolated human leukocytes.[41] Cells from donors sensitive to ragweed or grass pollen were added to tubes containing a variable concentration of Chlorphenesin and a constant concentration of ragweed antigen or rye grass Group I antigen. The percent inhibition was calculated by comparing the percentage of histamine release in tubes containing Chlorophenesin with that noted in control tubes without the drug. Chlorphenesin at a concentration between 0.3 and 0.6 mM caused 50% inhibition of histamine release. In another study, after passive sensitization, monkey lung tissue was incubated for 10 minutes with varying concentrations of Chlorphenesin (50 to 400 µg) in Tyrode's solution.[40] Incubation was followed by the addition of either the dialyzed timothy pollen extract (40 µg protein) or the green pea albumin (40 µg protein) and a 15 minute incubation period. The supernatant was removed and assayed for histamine and the slow reacting substance of anaphylaxis (SRS-A). Chlorphenesin (400 µg in 0.5 ml Tyrode's solution) completely inhibited the release of histamine and SRS-A from monkey lung tissue passively sensitized with reagin-containing sera from timothy-sensitive patients. Chlorphenesin also inhibited the release of histamine and SRS-A from monkey lung tissue passively sensitized with sera of patients sensitive to green pea and challenged with the appropriate allergen.

DATA NEEDS

After reviewing the published literature, it became apparent that CNS depression, respiratory depression, and skeletal muscle relaxation effects forming the basis for the FDA's request for an accelerated review of Chlorphenesin (CAS No. 104-29-0) by the Cosmetic Ingredient Review (CIR) are associated with the drug, chlorphenesin carbamate (CAS No. 886-754-8, also known as Maolate). However, the extent, if any, to which these drug effects would be associated with Chlorphenesin (CAS No. 104-29-90) remains unknown. Therefore, CIR is requesting any data addressing the muscle relaxant activity of Chlorphenesin or its effects on the nervous system, including information pertaining to dose- or concentration-effect/response relationships to enable a comparative evaluation of Chlorphenesin relative to chlorphenesin carbamate or other esters of chlorphenesin. Additionally, study data demonstrating or characterizing the purported biocidal

activity of Chlorphenesin in cosmetics or other consumer products, including effective biocidal concentrations, would be valuable.

SUMMARY

Chlorphenesin, a biocide, is produced by condensing equimolar amounts of p-chlorophenol and glycidol in the presence of a tertiary amine or a quaternary ammonium salt as a catalyst. According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP) in 2011, Chlorphenesin was being used in cosmetic products. Furthermore, results from a survey of ingredient use concentrations provided by the Personal Care Products Council in 2011 indicate that Chlorphenesin was being used at concentrations up to 0.32% (rinse-off products) and up to 0.3 % (leave-on products). Similarly, the maximum authorized use of this ingredient in cosmetic products marketed in the European Union is 0.3%.

The results of a toxicokinetic study involving rats and dogs indicated that Chlorphenesin was rapidly absorbed and excreted mainly in the urine. Urinary end products identified included 3-p-chlorophenoxylactic acid, p-chlorophenoxyacetic acid, and unchanged Chlorphenesin. In an *in vivo* percutaneous absorption study involving rats, practically all of the absorbed dose was excreted over a period of 96 h.

In an acute oral toxicity study (rats), a mean oral LD50 of 3,000 mg/kg was reported for Chlorphenesin. Repeated oral dosing of rats with Chlorphenesin for 28 days caused a significant reduction in body weight gain and significantly lower hemoglobin levels in the highest dose group (1,000 mg/kg/day) when compared to controls. Significantly reduced spleen and thymus weights were also reported for this group. The only treatment-related microscopic finding in the study, renal tubular dilatation/necrosis, occurred in one male rat from the highest dose group. A dose of 10 mg/kg/day was considered the no adverse effect level in this study.

Chlorphenesin was classified as a weak ocular irritant when instilled into the eyes of rabbits at a concentration of 1%. The same test concentration did not induce skin irritation when applied, under an occlusive patch, to rabbits for 24 h. Negligible dermal irritation was observed in 3 of 25 subjects tested with 0.3% Chlorphenesin in a 48 h occlusive patch test. In a sensory irritation test involving 16 healthy subjects, irritation induced by a formula containing methylparaben, propylparaben, and Chlorphenesin was greater when compared to the same formula without Chlorphenesin. In the guinea pig maximization test, Chlorphenesin did not induce sensitization at a concentration of 0.5% or 1%. These 2 concentrations were classified as non-irritating in a preliminary test to determine the maximal irritant concentration.

In a human repeated insult patch test (HRIPT) involving 55 subjects, a test material containing 5 to 9% Chlorphenesin did not have skin irritation or allergic contact sensitization potential. A test material containing 12 to 17% Chlorphenesin induced clinically insignificant erythema in one of 53 subjects in another HRIPT. When 11 patients photoallergic to ketoprofen were photo patch tested with Chlorphenesin, results were negative. In case reports, positive patch test reactions to 0.5% and 1% Chlorphenesin were reported.

In a study evaluating the immunosuppressive activity of Chlorphenesin in albino rabbits, marked inhibition of the CA (+) hemagglutinin response was observed at test concentrations of 1 or 10 mg/ml, but not 0.01 or 0.1 mg/ml. In other animal studies, the simultaneous administration of antigen with Chlorphenesin resulted in suppression of formation of humoral antibodies in mice and rabbits. When the immunosuppressive activity of Chlorphenesin was studied using a wide variety of *in vitro* assays for cellular immunity in both humans and mice, the results suggested that it may have a broad spectrum of suppressive effects on both B and T lymphocytes.

Chlorphenesin had no adverse effect on embryo-fetal survival, growth, or development *in utero* when administered orally to rats at doses up to 100 mg/kg/day on days 6 to 15 post-coitum. In the Ames test, Chlorphenesin was not genotoxic to the following bacterial strains when tested at concentrations up to 5,000 µg/plate with or without metabolic activation: *Salmonella typhimurium* strains TA 98, TA 100, TA 102, TA 1535, TA 1537, and TA 1538, and *Escherichia coli* strain WP2 uvrA.

In an initiation promotion experiment, DMBA (100 µg) was applied to the interscapular area of each of 30 mice, and, after 3 weeks, 2.5% croton oil was applied to the skin twice weekly for 20 weeks. A second group of 30 mice received applications of DMBA and croton oil plus two 2.5 mg injections of Chlorphenesin i.p. (same day) at the same time that croton oil was applied. Chlorphenesin inhibited tumorigenesis in initiation-promotion skin carcinogenesis when injected during promotion. In another study, mice previously injected with murine leukemia virus (RMLV or FMLV) were injected

i.p. with 100 mg/kg Chlorphenesin for up to 7 days. Chlorphenesin caused a pronounced sparing effect on mortality due to leukemia after infection with RMLV. Thirty-one cancer patients received Chlorphenesin orally at a usual daily dose of 4 g/day for 1 to 6 weeks. Treatment was ineffective in 16 cases of carcinoma (cervix, uterus, tonsil, esophagus, and lung) and in 4 cases of sarcoma. However, in 9 cases of squamous cell carcinoma of the skin, complete remission was achieved in one patient and substantial, though incomplete, remission was achieved in 4 other patients.

The inhibition of cyclic AMP hydrolysis in the presence of Chlorphenesin has been demonstrated *in vitro*. In addition, Chlorphenesin completely inhibited the release of histamine from monkey lung tissue.

Table1. Properties of Chlorphenesin

Form	White powder with bitter taste. Almost odorless.[42]
Molecular Weight	202.63[5]
Density	0.70 to 0.75[42]
Solubility	Soluble in 200 parts water and in 5 parts alcohol (95%); soluble in ether; slightly soluble in fixed oils;[42] Solubility in water < 1%[5]
Melting Range	78 to 81°C[42]
Flash Point	100°C[42]
Assay (Dried Basis)	Contains not less than 99.0% $C_9H_{11}ClO_3$[42]
Loss on Drying	Not more than 1.0%[42]
Sulfated ash	Not more than 0.10%[42]
Chlorophenol	Complies with British Pharmacopoeia specifications[42]
pH Value	7 to 7.50[42]

Table 2. Current Frequency and Concentration of Use According to Duration and Type of Exposure Provided in 2011[7,8]

	Chlorphenesin	
	# of Uses	Conc. (%)
Exposure Type		
Eye Area	168	0.02 to 0.3
Incidental Ingestion	3	0.3 to 0.3
Incidental Inhalation-sprays	25	0.2 to 0.3
Incidental Inhalation-powders	47	0.2 to 0.3
Dermal Contact	1054	0.00004 to 0.32
Deodorant (underarm)	NR	NR
Hair - Non-Coloring	47	0.0003 to 0.3
Hair-Coloring	NR	0.000008 to 0.003
Nail	0.0003	0.2
Mucous Membrane	0.00004	0.3
Baby Products	2	NR
Duration of Use		
Leave-On	998	0.0003 to 0.3
Rinse off	145	0.000008 to 0.32
Diluted for (bath) Use	3	0.0006 to 0.3
Totals*/Conc. Range**	1146	0.000008 to 0.32

NR = Not Reported; Totals = Rinse-off + Leave-on Product Uses
NOTE: Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure type uses may not be equal to sum total uses.

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