Safety Assessment of Hexamidine and Hexamidine Diisethionate as Used in Cosmetics

Status:	Re-Review for Panel Consideration
Release Date:	September 1, 2022
Panel Meeting Date:	September 26 – 27, 2022

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

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Priya Cherian, Senior Scientific Analyst/Writer, CIR
Date: September 1, 2022
Subject: Re-Review of the Safety Assessment of Hexamidine and Hexamidine Diisethionate

The Expert Panel for Cosmetic Ingredient Safety (Panel) first published a review of the safety of Hexamidine and Hexamidine Diisethionate in 2007 (identified as *originalreport_Hexamidine_092022* in the pdf), with the conclusion that these ingredients are safe at concentrations up to 0.1%. Because it has been 15 years since this report was published, in accord with Cosmetic Ingredient Review (CIR) Procedures, the Panel should consider whether the safety assessment of Hexamidine and Hexamidine Diisethionate should be re-opened. An exhaustive search of the world's literature was performed for studies dated 2000 forward. An historical overview, comparison of original and new use data, the search strategy used, and a synopsis of notable new data are enclosed herein (*newdata_Hexamidine_092022)*.

New studies were found for several toxicological endpoints (e.g., acute oral toxicity, genotoxicity, dermal irritation and sensitization, ocular irritation). Of note, are hypersensitivity case reports following Hexamidine exposure, and positive patch test results to Hexamidine in atopic patients.

Also included for your review is a table of current and historical use data (*usetable_Hexamidine_092022).* The frequency of use of Hexamidine Diisethionate has increased from 38 to 52, according to 2002 and 2022 VCRP data, respectively. The concentration of use of this ingredient has remained the same (≤ 0.1%). There were previously and currently no reported uses for Hexamidine.

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

Re-Review - Hexamidine and Hexamidine Diisethionate - History and New Data

(Priya Cherian – September 2022 Meeting)

Ingredients (2)	Citation	Conclusion	Use - New Data	Results	Use - Existing Data	Results	Notes
Hexamidine **Hexamidine Diisethionate**	JACT 1IJT 26(Suppl. 3):79-88, 2007	safe as used at concentrations up to 0.1%	Hexamidine frequency of use (2022) conc of use (2022) Hexamidine Diisethionate frequency of use (2022) conc of use (2022)	0 not reported 52 ≤ 0.1%	Hexamidine frequency of use (2002) conc of use (2004) Hexamidine Diisethionate frequency of use (2002) conc of use (2004)	0 not reported 38 ≤ 0.1%	no changes frequency of use increased, but concentration of use has stayed the same

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
https://ec.europa.eu/growth/tools-databases/cosing/index.cfm?fuseaction=search.simple	European Union legislation – CosIng	Hexamidine and Hexamidine Diisethionate are listed in annex V/47 of EU Cosmetics Regulation 2007/17/EC – list of preservatives allowed in cosmetic products – maximum concentration in ready for use preparation is 0.1%	EU restrictions not reported in previous report
https://echa.europa.eu/registration-dossier/-/registered-dossier/26729/	acute toxicity – oral	OECD TG 423; female Sprague-Dawley rats (3-6 animals/group); administered 300 or 2000 mg/kg bw Hexamidine Diisethionate in DMSO; LD_{50} was considered to be higher than 300 mg/kg bw and lower than 2000 mg/kg bw; study authors estimated the LD_{50} cut-off to be 500 mg/kg bw	yes; the lowest oral LD_{50}s for mice and rats in previous report were 710 and 750 mg/kg, respectively
Opinion of the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers Concenring Hexamidine and its salts – SCCNFP 2002	subchronic toxicity – oral	90-d assay in male rats; 200, 400, and 800 mg/kg Hexamidine via gavage; no-effect level of 200 mg/kg	no subchronic toxicity data on Hexamidine was provided in original report
https://echa.europa.eu/registration-dossier/-/registered-dossier/26729/	genotoxicity – in vitro	OECD TG 471; Ames assay; *E. coli* WP2 uvr A; Hexamidine Diisethionate at up to 5000 µg/plate; with and without metabolic activation; non-genotoxic	no
https://echa.europa.eu/registration-dossier/-/registered-dossier/26729/	genotoxicity – in vitro	OECD TG 471; Ames assay; *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100; Hexamidine Diisethionate at up to 5000 µg/plate; with and without metabolic activation; non-genotoxic	no
https://echa.europa.eu/registration-dossier/-/registered-dossier/26729/	dermal irritation – in vitro	OECD TG 431; reconstructed human epidermis; Hexamidine Diisethionate with physiological saline to improve contact; non-corrosive to skin	in vitro dermal irritation data not provided in original report; in vivo data suggested slight irritation in animals
https://echa.europa.eu/registration-dossier/-/registered-dossier/26729/	dermal irritation – in vitro	OECD TG 431; reconstructed human epidermis; Hexamidine Diisethionate with water to improve contact; non-corrosive/non-irritating to skin	in vitro dermal irritation data not provided in original report; in vivo data suggested slight irritation in animals
Opinion of the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers Concerning Hexamidine and its salts – SCCNFP 2002	dermal irritation – in vivo	rabbits exposed to 0.2% or 0.5% Hexamidine for 4 h; slight erythema at both concentrations; considered to be non-irritating	no dermal irritation data for Hexamidine was provided in original report

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
https://echa.europa.eu/registration-dossier/-/registered-dossier/26729/	dermal sensitization – in vitro	OECD TG 442D; KeratinoSens assay; Hexamidine Diisethionate up to 2000 µM in 1% DMSO; non-sensitizing	in vitro dermal sensitization data not provided in original report; in vivo data was negative
https://echa.europa.eu/registration-dossier/-/registered-dossier/26729/	dermal sensitization – in vitro	OECD TG 442 E; human cell line activation assay; Hexamidine Diisethionate up to 31.80 µM in DMSO; non-sensitizing	in vitro dermal sensitization data not provided in original report; in vivo data was negative
https://echa.europa.eu/registration-dossier/-/registered-dossier/26729/	dermal sensitization – in vitro	OECD TG 442C; direct peptide reactivity assay; 100 mM Hexamidine Diisethionate in acetonitrile and water; non-sensitizing	in vitro dermal sensitization data not provided in original report; in vivo data was negative
https://echa.europa.eu/registration-dossier/-/registered-dossier/26729/	ocular irritation – in vitro	OECD TG 438; isolated chicken eye test; 30 mg Hexamidine Diisethionate; non-irritant	in vitro ocular irritation data not provided in original report; in vivo data suggested slight, reversible irritation in animals
Le Seac'h A, Castagna J, Chantran Y, Kurihara F, Amsler E, Soria A, Barbaud A. Occurrence of immediate and delayed hypersensitivity to hexamidine. Contact Dermatitis. 2021 Nov;85(5):580-582.	case report - hypersensitivity	66-yr-old man developed a grade II anaphylactic reaction 5 min after intranasal application of Hexamidine; a few days later, lotion containing Hexamidine applied to neck, contact dermatitis observed; positive patch test reactions to 0.1% Hexamidine	no; some case reports of hypersensitivity following Hexamidine exposure was provided in original report
Mullins RJ. Systemic allergy to topical hexamidine. Med J Aust. 2006 Aug 7;185(3):177. doi: 10.5694/j.1326-5377.2006.tb00513.x.	case report – hypersensitivity	7-yr-old boy with generalized urticarial and facial swelling after eating peanut-containing product and application of Hexamidine Isethionate-containing lotion to elbow; 6 mo later, same cream applied to chest resulted in urticarial welt; positive skin prick tests to cream, but not the other active constituents in cream	no; some case reports of hypersensitivity following Hexamidine exposure was provided in original report
Mailhol C, Lauwers-Cances V, Rancé F, Paul C, Giordano-Labadie F. Prevalence and risk factors for allergic contact dermatitis to topical treatment in atopic dermatitis: a study in 641 children. Allergy. 2009 May;64(5):801-6. doi: 10.1111/j.1398-9995.2008.01890.x. Epub 2009 Jan 31.	clinical – single-center study	patch testing in 41 patients with atopic dermatitis; 3 patients displayed positive reaction to Hexamidine	no clinical studies were provided in original report
Barbaud A, Vigan M, Delrous JL, Assier H, Avenel-Audran M, Collet E, Dehlemmes A, Dutartre H, Géraut C, Girardin P, Le Coz C, Milpied-Homsi B, Nassif A, Pons-Guiraud A, Raison-Peyron N; Membres du Groupe du REVIDAL. Allergie de contact aux antiseptiques: 75 cas analysés par le réseau Revidal de dermato-allergovigilance [Contact allergy to antiseptics: 75 cases analyzed by the dermato-allergovigilance network (Revidal)]. Ann Dermatol Venereol. 2005 Dec;132(12 Pt 1):962-5. French.	clinical – retrospective multi-center study	patch testing in 75 patients with contact dermatitis; 20 patients displayed a positive reaction to Hexamidine	no clinical studies were provided in original report

EU = European Union; DMSO = dimethyl sulfoxide; LD_{50} = median lethal dose; OECD TG = Organisation for Economic Co-operation and Development Test Guidelines

Search (from 1997 on)

PubMed

(((“hexamidine”) OR (3811-75-4[CAS Number])) AND (("2000"[Date - Publication] : "2022"[Date - Publication])) – 63 hits; 7 useful hits

(((“hexamidine diisethionate”) OR (“hexamidine isethionate”) OR(659-40-5[CAS Number])) AND (("2000"[Date - Publication] : "2022"[Date - Publication])) – 63 hits;

Table 1. Current and historical frequency and concentration of use of Hexamidine Diisethionate according to duration and exposure

	# of Uses		*Max Conc of Use (%)*	
	Hexamidine Diisethionate			
	2022[1]	**2002[2]**	**2022[3]**	**2004[2]**
Totals*	**52**	**38**	**0.036 – 0.1**	**0.03 – 0.1**
Duration of Use				
Leave-On	*34*	*23*	*0.036 – 0.1*	*0.03 – 0.1*
Rinse-Off	*18*	*15*	*NR*	*0.05 – 0.06*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*
Exposure Type				
Eye Area	2	1	NR	NR
Incidental Ingestion	NR	NR	NR	NR
Incidental Inhalation-Spray	9[a]; 8[b]	9[a]; 5[b]	NR	0.1[a]; 0.05[b]
Incidental Inhalation-Powder	1; 8[b]; 3[c]	5[b]; 3[c]	0.036[c]	0.05[b]
Dermal Contact	37	32	0.036 – 0.1	0.04 – 0.1
Deodorant (underarm)	3[a]	NR	0.05	0.1[a]
Hair - Non-Coloring	14	5	NR	NR
Hair-Coloring	1	NR	NR	NR
Nail	NR	NR	NR	0.03
Mucous Membrane	1	NR	NR	NR
Baby Products	5	5	0.036	NR

[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.
[b] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories
[c] It is possible these products are powders, but it is not specified whether the reported uses are powders.
NR – no reported use

References

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

2. Andersen F.A. (ed). Final report on the safety assessment of Hexamidine and Hexamidine Diisethionate. *Int J Toxicol.* 2007;26 Suppl 3:79-88.

3. Personal Care Products Council. 2022. Concentration of Use by FDA Product Category: Hexamidine and Hexamidine Diisethionate. (Unpublished data submitted to Personal Care Products Council on July 7, 2022.)

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Final Report on the Safety Assessment of Hexamidine and Hexamidine Diisethionate[1]

Hexamidine Diisethionate functions as a biocide in cosmetics at concentrations of 0.03% to 0.1% in 38 cosmetic products. Hexamidine functions as a biocide and preservative in cosmetics, but is not in current use in cosmetics, but it is used in over-the-counter (OTC) drug products. Hexamidine was poorly absorbed by human cadaver skin when in water-oil formulations or in a gel that simulated a cosmetic product formulation. Hexamidine Diisethionate was poorly absorbed by the skin of live rats and was not stored in any tissue type. Hexamidine Diisethionate given to rats intravenously was rapidly metabolized to Hexamidine. Excretion was primarily via the feces, with a small amount excreted in the urine. Acute oral LD_{50} values of Hexamidine Diisethionate were 0.71 to 2.5 g/kg in mice and 0.75 g/kg in rats. Dermal exposure to 4 g/kg Hexamidine Diisethionate in rats or up to 9.4 ml/kg of a 0.1% Hexamidine Diisethionate solution under occlusion in rabbits produced no mortality or other signs of toxicity. The no-observed-effect level (NOEL) for oral subchronic toxicity of Hexamidine Diisethionate in rats was 50 mg/kg/day. No signs of toxicity were observed with 2% Hexamidine Diisethionate in subchronic studies using rabbits. Application of 0.1 ml of 0.11% Hexamidine Diisethionate in aqueous solution to the eyes of rabbits produced transient reactions; 0.05% produced no reactions. Slight erythema was observed with 0.10% Hexamidine Diisethionate applied to the abraded skin of 1/11 albino rabbits. A 40% solution of Hexamidine Diisethionate applied to 10% of the body surface of rats produced slight erythema, slight edema, and scabbing in some animals at varying times after treatment. Hexamidine Diisethionate was not a sensitizer in the guinea pig maximization test or in an intracutaneous guinea pig sensitization test. Hexamidine Diisethionate was not a photosensitizer in albino rabbits. Hexamidine Diisethionate was not mutagenic in a bacterial reverse mutagenicity assay or clastogenic in mammalian cells. Hexamidine Diisethionate at 0.10% did not provoke primary irritation, inflammation, or sensitization in a clinical test of 200 human subjects. One case report of photosensitivity to Hexamidine and one of contact sensitivity to Hexamidine were reported. There were nine case reports of contact sensitivity to Hexamidine Diisethionate. A European safety assessment recommended a limit of 0.1% Hexamidine Diisethionate in leave-on and rinse-off cosmetic products. In considering the available data, the Cosmetic Ingredient Review (CIR) Expert Panel acknowledged the lack of carcinogenicity and reproductive/developmental toxicity data. Because genotoxicity studies were negative, and there were no structural alerts, the Panel concluded that it was unlikely that these ingredients would be carcinogenic. Because the rate of absorption of Hexamidine and Hexamidine Diisethionate is slow, there is no tissue accumulation, and excretion is rapid and complete, and there was no toxicity in a subchronic study, the Panel concluded that dermal exposures would not likely present a risk of reproductive/developmental toxicity. The Panel noted that a guinea pig maximization study using Hexamidine Diisethionate produced no dermal reactions and that a clinical test at 0.1% produced no irritation or sensitization. The Panel also expressed concern regarding the possible presence of 1,4-dioxane as an impurity, and stressed that the cosmetic industry should continue to use the necessary purification procedures to remove these impurities from the ingredient before blending into cosmetic formulations. The Panel noted that there are no data for concentration of use for eye makeup and baby products, and was concerned that there should not be unrestricted concentration levels in these product categories. Although there are gaps in knowledge about product use, the overall information available on the types of products in which these ingredients are used and at what concentration indicate a pattern of use. Within this overall pattern of use, the Expert Panel considers all ingredients in this group to be safe at concentrations up to and including 0.1%.

INTRODUCTION

The safety of Hexamidine (CAS no. 3811-75-4) and Hexamidine Diisethionate (CAS no. 659-40-5), which function as cosmetic biocides and/or preservatives in cosmetic products, is reviewed in this report.

CHEMISTRY

Definition and Structure

As given in the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and McEwen 2004), Hexamidine (CAS no. 3811-75-4) is the organic compound that conforms to the formula displayed in Figure 1. Synonyms for Hexamidine include:

- Benzamidine, 4,4′-(hexamethylenedioxy)di-;
- Benzenecarboximidamide, 4, 4′-[1,6-hexanediylbis (oxy) Bis-];
- 2-Deoxyphenobarbitol;
- 4,4′-Diamidino-1,6-diphenoxyhexane; and
- 4,4′-[1,6-Hexanediylbis(oxy)bisbenzene carboximidamide.

As given in the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and McEwen 2004), Hexamidine

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[1]Reviewed by the Cosmetic Ingredient Review Expert Panel.

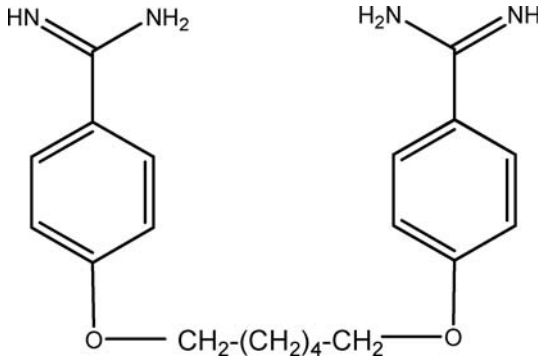

FIGURE 1
Structure of Hexamidine (Gottschalck and McEwen 2004).

Diisethionate (CAS no. 659-40-5) is the organic salt that conforms to the formula in Figure 2. Synonyms for Hexamidine Diisethionate include:

- Benzamidine, 4,4′-(hexamethylenedioxy)di-, bis(2-hydroxyethanesulfonate) and
- Ethanesulfonic acid, 2-hydroxy-, compd. with 4,4′(1,6-hexanediylbis(oxy)) bis(benzene-carboximidamide) (2:1).

Physical and Chemical Properties

According to the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumer (SCCNFP), a scientific advisory body of the European Commission, Hexamidine has a molecular weight of 354.54 Da and is soluble in water and insoluble in organic solvents (SCCNFP 2002). The antimicrobial activity of Hexamidine is optimum at pH 5 to 9 and may be inactivated by chloride or sulfate ions and some proteins (Hill 1995).

Hexamidine Diisethionate is a white solid that is soluble in 1% aqueous methylcellulose and in saline (Zavorskas and Tozer 2004) and has a peak ultraviolet (UV) absorption at 265 nm (Laboratories Serobiologiques no date).

Method of Manufacture

Hexamidine Diisethionate is produced by adding ammonium isethionate to a chloroformic solution of 1,6(4-butoxycarbonimidoylphenoxy)hexane. The mixture is warmed and then gradually cooled. Hexamidine Diisethionate precipitates and is washed in ethanol (with 2.5% toluene as alcohol denaturant). The product is crystallized in a mix of ethanol and water, which is pressed and dried (Laboratories Serobiologiques 2004).

Analytical Methods

Taylor et al. (1983) found high-performance liquid chromatography to be a rapid and precise method for detection and quantitative analysis of Hexamidine Diisethionate in four pharmaceutical preparations.

Zavorskas and Tozer (2004) measured Hexamidine Diisethionate in 1% methylcellulose, in saline, and in heparinized rat plasma by high-performance liquid chromatography with UV detection.

Impurities

British Pharmacopeia Convention (2001) listed 4-[{6-(4-carbamimidoylphenoxy) hexyl}-oxy] benzamide as an impurity to Hexamidine Diisethionate (Isethionate) but does not specify an amount.

The European Pharmacopoeia (Pharmeuropa 2003) listed 4-[{6-(4-carbamimidoylphenoxy)hexyl}oxy]-benzamide, 4-[{6-(4-carbamimidoylphenoxy)hexyl}oxy]benzoate ethyl, 4-[{6-(4-carbamimidoylphenoxy)hexyl}-oxy]benzimidoate ethyl and 4-imino-9,16-dioxa-3-azatricyclo[15.2.2.2$^{5.8}$] tricosal(10),2.5.7.17.20.22-heptaen-2-amine as impurities.

The Council of Europe (2002) listed 4-[6-{4-ethoxy(imino) methylphenoxy}hexyloxy]phenyl(imino)-methylamine as an impurity at not more than 0.3% and other detectable impurities at not more than 0.1%.

Laboratoires Serobiologiques (2004) found less than 10 ppm heavy metal impurities.

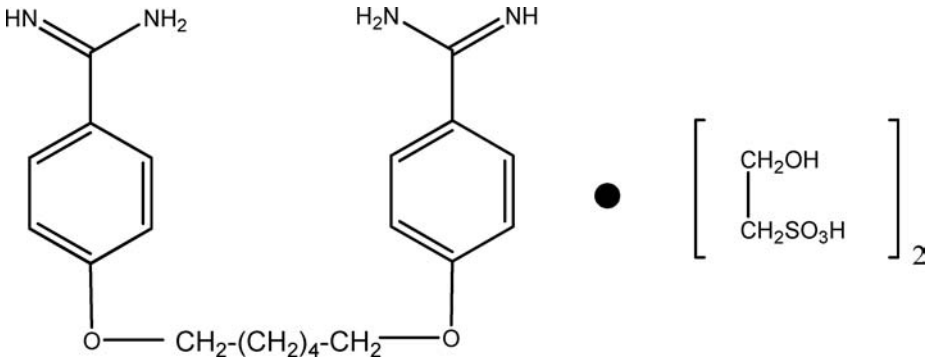

FIGURE 2
Structure of Hexamidine Diisethionate (Gottschalck and McEwen 2004).

TABLE 1
Metal impurities in Hexamidine Diisethionate (Füling 2003)

Assayed metal	Hexamidine Diisothionate sample no. SO03008004 (mg/kg)	Hexamidine Diisothionate sample no. SO030270007 (mg/kg)
Arsenic	<0.1	<0.1
Antimony	<0.1	<0.1
Lead	<0.1	<0.1
Bismuth	<0.1	<0.1
Chromium	<0.1	<0.1
Cadmium	<0.1	<0.1
Iron	2.2	<1
Cobalt	<0.1	<0.1
Copper	0.2	<0.1
Nickel	0.1	<0.1
Silver	<0.1	<0.1
Mercury	<0.1	<0.1
Zinc	1.1	0.5
Tin	<0.1	<0.1
Manganese	<0.1	<0.1

In an analysis of two batches of Hexamidine Diisethionate for metal impurities, by Inductively Coupled Plasma–Optical Emission Spectrometry and Inductively Coupled Plasma–Mass Spectrometry, Füling (2003) showed that none of the metals tested were above 1.9 mg/kg, most were below 0.1 mg/kg as given in Table 1. This author also reported trace levels of 1,4-dioxane, chloroform, dichloromethane, and trichloroethene in four samples of Hexamidine Diisethionate as given in Table 2.

USE

Cosmetic Use

Hexamidine functions in cosmetic products as a cosmetic biocide and as a preservative (Gottschalck and McEwen 2004); however, industry reports to the Food and Drug Administration (FDA) did not indicate any current uses (FDA 2002a), nor were any current use concentrations reported by the Cosmetic, Toiletry, and Fragrance Association (CTFA) in an industry survey (CTFA 2004).

Hexamidine Diisethionate functions as a cosmetic biocide. Industry reported to FDA that this ingredient is used in 38 cosmetic products—this is the frequency of use (FDA 2002a). CTFA (2004) reported that Hexamidine Diisethionate is used at concentrations of 0.03% to 0.1%. The frequency of use and concentration of use of Hexamidine Diisethionate in specific product categories are listed in Table 3.

The European Union Cosmetics Directive 76/768/EEC, Annex VI, allows Hexamidine Diisethionate as a preservative for cosmetics and toiletries up to a maximum concentration of 0.1% (European Economic Community 2001). The SCCNFP reviewed the question of increasing the concentration limit of Hexamidine and Hexamidine Diisethionate for use in leave-on and rinse-off cosmetic products from the current limit of 0.1% to a proposed 0.2% (SCCNFP 2002) . The committee concluded that there was not sufficient data available to evaluate the safety of 0.2% Hexamidine or its salts in cosmetic products.

Hexamidine and Hexamidine Diisethionate are permitted for use in cosmetics in Chile at concentrations of up to 0.1% (Instituto de Salud Publica de Chile 2003).

Noncosmetic Use

Hexamidine had been used since 1950 as a biocide in powders, ointments, alcohol solution, or percutaneous penetrating vehicles (Robin 1978). However, in 2002, the FDA rejected an application for U.S. marketing of a French-manufactured drug Desomedine because the ingredient Hexamidine Diisethionate "appears to be a new drug without an approved new drug application" (FDA 2002b). The Cosmetic Toiletry and Fragrance Association (CTFA 2004) reports that Hexamidine is used at 0.05% in over the counter baby products.

Antimicrobial Activity

Granel et al. (1996) successfully used Hexamidine and fusidic acid to treat a cutaneous infection of *Tsukamurella paurometabolum*, a gram-positive aerobic bacillus.

TABLE 2
Determination of the trace organic impurities in Hexamidine Diisothionate (aka Diisethionate) using USP Method 467 IV (Füling 2003)

Name of assayed compound	Hexamidine Diisothionate sample no. SO02008001 (μg/g)	Hexamidine Diisothionate sample no. SO023080015 (μg/g)	Hexamidine Diisothionate sample no. SO030080004 (μg/g)	Hexamidine Diisothionate sample no. SO030270007 (μg/g)
1,4-Dioxane	<50	<50	<50	<50
Chloroform	100	280	210	250
Dichlormethane	<50	<50	<50	<50
Trichlorethene	<50	<50	<50	<50

TABLE 3
Current cosmetic product uses and concentrations for Hexamidine Diisethionate

Product category (total number of products in each category) (FDA 2002a)	Ingredient uses in each product category (FDA 2002a)	Use concentrations (CTFA(%) 2004)
Baby products		
Shampoos (29)	1	—
Lotions, oils, powders, and creams (60)	3	—
Other (34)	1	—
Eye makeup		
Mascara (195)	1	—
Noncoloring hair care products		
Conditioners (651)	1	—
Shampoos (884)	3	—
Nail Care Products		
Nail creams and lotions	—	0.03
Personal hygiene products		
Underarm deodorants (247)	—	0.1
Shaving products		
Aftershave lotions (231)	1	—
Skin care products		
Skin cleansing creams, lotions, liquids, and pads (775)	7	0.05
Face and neck creams, lotions, powders, and sprays (310)	3	0.05
Body and hand creams, lotions, powders, and sprays (840)	2	—
Moisturizers (905)	9	0.1
Paste masks/mud packs (271)	3	0.06
Other (725)	3	0.04–0.1
Total uses/ranges for Hexamidine Diisethionate	38	0.03–0.1

Hexamidine was found to be effective in treatment of corneal co-infections of *Acanthamoeba*, *Valkampfia*, and *Hartmanella* amoeba species. The mean minimal cysticidal concentrations ranged from 200 to 280 μg/ml, and the mean amoebicidal concentrations were 5 to 15 μg/ml (Aimard et al. 1998).

Enyedy et al. (2001) reported that Hexamidine inhibited the proteolytic activity of thrombin ($K_i = 224$ nM) and matripase ($K_i = 924$ mM), a trypsin-like protease.

Hexamidine Diisethionate (1% solution in 30% alcohol) reduced by 70% to 90% the bacterial flora from the antecubital fossa of 20 healthy volunteers. The flora consisted of halotolerant and non-halotolerant micrococci, corneobacteria, *Staphyloccus epidermis*, *Propionibacterium acnes*, and other microorganisms (Michel et al. 1986).

Fourteen out of 208 strains of *Listeria* were resistant to the antimicrobial effects of Hexamidine Diisethionate. The resistance was plasmid-mediated and transferrable between *Listeria* species and *Staphylococcus aureus* (Lemaître et al. 1998).

Absorption, Distribution, Metabolism, and Excretion

Human

Oblong et al. (2004) studied the in vitro penetration of Hexamidine through human cadaver skin. Dorsal skin from a male Caucasian donor, less than 2 months postmortem, was prepared in Franz-type cells with an orifice size of 0.79 cm^2. The integrity of the skin was ensured with tritiated water. Radiolabeled Hexamidine was tested in two formulations. One formulation tested was 3 or 5 mg/cm^2 [^{14}C]Hexamidine (0.1%) in a gel. The other formulation consisted of 0.0023 μg/cm^2 0.1% or 0.3% [^{14}C]Hexamidine in an oil-water skin care emulsion formulation. The doses were applied to the donor side (stratum corneum) of the skin samples. Aliquots of the receptor fluid were collected and analyzed by liquid scintillation counting to detect the presence of [^{14}C]Hexamidine.

Results of the analysis of the receptor fluid indicated that 0.027% of the Hexamidine in the gel formulation penetrated the skin samples in 72 h. In the 0.1% and 0.3% water-oil formulations, 0.0321% and 0.0208% of the Hexamidine dose, respectively, penetrated the skin samples in 72 h. The authors suggested that because of the low skin penetration observed in this study, bioavailability of Hexamidine from topical cosmetic formulations would be minimal (Oblong et al. 2004).

Animal

Chasseaud et al. (1990) studied the percutaneous absorption of Hexamidine Diisethionate in rat skin. A 0.1% formulation

of [^{14}C]Hexamidine Diisethionate was applied to a 5 × 5-cm area on the shaved backs of Sprague-Dawley rats ($n = 21$). The amount of Hexamidine Diisethionate applied was 56 μg/cm^2. Dose sites were occluded with aluminum foil, and animals were placed in metabolism cages for collection of urine, feces, and expired air. At 1, 2, 4, 8, 24, 48, and 96 h after dose application, two animals for each time point were anesthetized for atrial blood collection and killed for separation of tissues. All tissues and fluids, were analyzed for radioactivity by liquid scintillation.

Less than 2% of the applied radioactivity was absorbed over 96 h of occluded dermal exposure. Less than 1% was excreted in the urine over the 96-h period, with no radioactivity detected in the urine before 24 h of exposure. Total fecal excretion of radioactivity (0.29% of dose) occurred between the 72- and 96-h time points. Radioactivity was detected only in the organs of excretion (liver and kidney) and in the gastrointestinal tract. Hexamidine Diisethionate was considered to be poorly absorbed in rat skin and was not stored in any tissue type (Chasseaud et al. 1990).

Zavorskas and Tozer (2004) performed a pharmacokinetic analysis of Hexamidine Diisethionate following intravenous or oral administration to female Sprague-Dawley rats. In the intravenous study, five rats received 10 mg/kg Hexamidine Diisethionate in a volume of 2 ml/kg saline by an infusion pump into the femoral vein over a dosing period of 15 min. In the oral study, rats received a singe dose of 50 or 200 mg/kg Hexamidine Diisethionate in 10 ml of 1% aqueous methylcellulose by oral gavage ($n = 5$ rats per dose level). Blood was collected from each rat prior to dosing and at intervals up to 24 h after dosing. The blood samples were analyzed by liquid chromatography/ mass spectrometry to determine the plasma concentration of Hexamidine for each time point. The lower detection limit for this analytical method was 1.00 ng/ml.

After intravenous infusion of 10 mg/kg Hexamidine Diisethionate, the maximum plasma concentration (C_{max}) of Hexamidine was 2190 ng/ml; the mean residence time (MRT) was 5.0 h; the mean clearance was 10,700 ml/h/kg; the steady state volume of distribution was 389,000 ml/kg; and the half-life was 27.3 h.

After an oral dose of 50 mg/kg Hexamidine Diisethionate, the mean concentration-time profile was erratic, with plasma concentrations of Hexamidine decreasing after the first post-dose sample collection and then increasing at the 2-h time point before declining again below the detection limit. In the rats receiving 200 mg/kg Hexamidine Diisethionate by oral gavage, the plasma concentration peaked after dosing, followed by a multiphasic decline measurable through 8 h post-dose. In both oral dose levels, the time to C_{max} was 15 min, indicating rapid absorption. The C_{max} at 50 mg/kg was 3.10 ng/ml, and the C_{max} at 200 mg/kg was 14.8 ng/ml. Plasma concentrations of Hexamidine were measurable up to 2 h after the 50 mg/kg dose and up to 8 h at 200 mg/kg. Oral bioavailability of Hexamidine was 0.10% at 50 mg/kg and 0.17% at 200 mg/kg (Zavorskas and Tozer 2004).

ANIMAL TOXICOLOGY

Acute Toxicity

Oral

Kynoch and Lloyd (1976a) used CFLP strain mice to evaluate the acute oral toxicity of Hexamidine Diisethionate. The median lethal dose (LD_{50}) was 2.5 g/kg. Deaths occurred within 42 h of dosing. Observations at the 4.0 g/kg dose level included lethargy and piloerection shortly after dosing and ataxia and body tremors the day after treatment. Hemorrhage of the liver was observed in the animals that died.

Lamarche (1972) reported rat and mouse oral LD_{50} values of 750 mg/kg and 710 mg/kg Hexamidine Diisethionate, respectively.

Dermal

Kynoch and Lloyd (1976b) tested the acute percutaneous toxicity of Hexamidine Diisethionate in CYF rats. A 40% solution of Hexamidine Diisethionate in aqueous methylcellulose (1%) was applied to a clipped (not shaved) area equivalent to 10% of each animal's body surface area. The applied dose of Hexamidine Diisethionate was 4 g/kg. The area was occluded with aluminum foil under bandage for 24 h, after which the dressing was removed and the dose site was washed. The animals were observed daily for signs of toxicity and dermal irritation. There were five animals per sex in the treated group, and the same number in a vehicle control group. Body weight gains of treated animals were similar to those of controls. There were no remarkable findings at necropsy. The percutaneous LD_{50} for Hexamidine Diisethionate in rats was greater than 4 g/kg. Results of the irritation scoring are presented in the Dermal Irritation and Sensitization section later in this review.

Lamarche (1972) reported an acute dermal toxicity study of Hexamidine Diisethionate in rabbits. The trunks of the rabbits were clipped free of hair. Half of the animals from each dose group received epidermal abrasions prior to dosing. Rabbits were placed in rubber sleeves and immobilized. Each rabbit then received a single dose of 3.9, 6, or 9.4 ml/kg of 0.10% Hexamidine Diisethionate introduced under the rubber sleeve by means of a microsyringe. The rubber sleeves were removed after 24 h. The volume of nonabsorbed solution was measured, and the exposure sites were observed. Animals were cleaned and returned to their cages for 2 weeks prior to necropsy. Light erythema was observed in abraded skin at all doses. There were no signs of irritation in intact skin. No mortalities were observed. There were no treatment-related effects on clinical chemistry, urine, or hematological parameters. There were no gross or microscopic findings in the liver, kidney, spleen, pericardium, lung, or skin.

Subchronic Toxicity

Oral

Colipa (1982) reported a study in which male rats received 0, 200, 400, or 800 mg/kg/day Hexamidine Diisethionate by oral

gavage, 5 days per week for 12 weeks ($n = 20$ rats/dose level). There was no mortality in the 0 or 200 mg/kg/day groups; mortality rates were 30% at 400 mg/kg/day and 100% at 800 mg/kg at 4 weeks after treatment. There was a delay in body weight increases that appeared to be dose dependent. A decrease in weight gain first appeared in the 200 mg/kg/day group at week 11, in the 400 mg/kg/day group at week 6, and in the 800 mg/kg/day group at week 4. Slight anemia was seen in animals at the two highest doses. Except for increased transaminase activity and reduced renal clearance of creatinine, liver and kidney functions were not affected/impaired. Hexamidine showed no effect in the tissues examined histologically in any dose group.

Powell et al. (1987) conducted a four-week oral toxicity study of Hexamidine Diisethionate in Sprague-Dawley rats. The animals were given 0, 50, 100, or 200 mg/kg/day Hexamidine Diisethionate (suspended in 1% aqueous methylcellulose) by oral gavage for 28 days ($n = 5$ rats/sex/dose level). Blood was collected for analysis before the animals were killed for necropsy.

No treatment-related deaths occurred. Body weights and food consumption were not affected by treatments. Salivation was the primary observation noted in all dose groups, with a slightly reduced incidence in the 50 mg/kg/day group. Associated wetness around the mouth with isolated incidences of brown oral staining began during the latter part of week 2 of dosing and continued to study termination. Males of the 200 mg/kg/day group had elevated mean total white blood cell counts attributable to lymphocytes. Increased alanine aminotransferase and serum calcium occurred in male rats of the 100 and 200 mg/kg/day groups. Increased aspartate aminotransferase was reported in the highest dose only. At necropsy, organ weights were similar between treated and control groups. Cecal distentions were noted in all treated rats, an effect attributed to the antimicrobial properties of Hexamidine Diisethionate. The no-observed-adverse-effect level (NOAEL) in this study was 50 mg/kg/day (Powell et al. 1987).

Dermal

Lamarche (1972) reported a 90-day dermal toxicity study of Hexamidine Diisethionate in rabbits. The trunks of the animals were clipped free of hair. A glass rod was used to apply 4 ml/kg of 0.05%, 0.1%, 0.2%, or 0.4% Hexamidine Diisethionate over a 240-cm^2 area ($n = 3$ rabbits/dose level). Applications were repeated daily for 90 days. No mortalities occurred. No reactions or signs of dermal irritation were observed. No remarkable observations were made at gross necropsy or microscopic examination of tissues. Hematological parameters were normal for all dose groups. The no-observed-effect level (NOEL) was 0.4% Hexamidine Diisethionate in this study.

Chronic Toxicity

No published data were available on the chronic toxicity of Hexamidine or Hexamidine Diisethionate.

Ocular Toxicity

Lamarche (1972) instilled 0.1 ml of 0.05% or 0.10% Hexamidine Diisethionate in aqueous solution to the right eye of albino rabbits ($n = 9$ rabbits/dose level). For each dose level, three animals' eyes were rinsed with lukewarm water exactly 2 s after application of the test material, three animals' eyes were rinsed after 4 s, and three animals' eyes were not rinsed. The left eye of each animal served as control. The eyes were scored for signs of irritation 24, 48, and 72 h and 4 and 7 days after the treatment. No reactions were observed in the 0.05% dose group. Slight reactions were observed in eyes not rinsed of 0.10% Hexamidine Diisethionate at 24, 48, and 72 h, but this observation disappeared after 72 h. No reactions were observed in eyes rinsed of 0.10% Hexamidine Diisethionate 2 or 4 s after instillation.

Dermal Irritation

Lamarche (1972) clipped areas on the flanks of 12 albino rabbits. The right flank of each rabbit was abraded with repeated tape strippings of the skin, whereas the left flank was left intact. Then, 0.50 ml of 0.10% or 0.05% Hexamidine Diisethionate in aqueous solution was applied to each flank (area of exposure not reported; $n = 6$ rabbits/dose level). Dose sites were occluded for 24 h and then scored for signs of irritation. Scoring was done again at 72 h after initial application. "Light erythema" was reported in one abraded site exposed to 0.10% Hexamidine Diisethionate at the 24-h observation, but not at 72 h. The respective primary irritation indices were 1/12 and "null" for the 0.10% and 0.05% treatments.

Kynoch and Lloyd (1976b) tested the dermal irritation potential of Hexamidine Diisethionate in CYF rats. A 40% solution of Hexamidine Diisethionate in aqueous methylcellulose (1%) was applied to a clipped (not shaved) area equivalent to 10% of each animal's body surface area. The applied dose of Hexamidine Diisethionate was 4 g/kg. The area was occluded with aluminum foil under bandage for 24 h, after which the dressing was removed and the dose site was washed. The animals were observed daily for signs of toxicity and dermal irritation. There were five animals per sex in the treated group, and the same number in a vehicle control group. On the day following treatment three females showed slight erythema and one male had slight edema. Slight erythema and slight edema were observed in two male rats on day 2. By day 3, there were no signs of irritation. However, slight erythema recurred in two females on days 6 to 9. Slight scabbing was seen in four rats on day 3 and in one rat on days 8 to 14.

Dermal Sensitization

Lamarche (1972) treated white male guinea pigs intracutaneously with 0.50 ml of 0.05% or 0.10% Hexamidine Diisethionate into skin clipped free of hair ($n = 10$ guinea pigs/dose level).

Each injection site was scored for signs of irritation 24 h after the injection. Then, 0.10 ml of the same dose per animal was injected every other day for a total of 10 injections per animal, and the sites were scored 24 h after each reaction. It was not clear whether all injections were at the same site or at different locations. The animals were then given a 2-week rest period before a challenge injection of 0.05 ml of the same dose level for each animal. Neither 0.05% nor 0.10% Hexamidine Diisethionate produced any sign of irritation or sensitization in this guinea pig test.

Kynoch and Clifford (1976) used a Magnusson-Kligman protocol to study delayed contact hypersensitivity of Hexamidine Diisethionate in albino guinea pigs. The dorsal area of ten female Hartley/Dunkin guinea pigs was clipped to remove hair, and three pairs of intradermal injections were made simultaneously. The three induction injections were Freund's complete adjuvant (50% in water), 1% Hexamidine Diisethionate in liquid paraffin, and a mixture of 1% Hexamidine Diisethionate in liquid paraffin 50:50 with Freund's complete adjuvant. One week after the injections, the same dorsal area was clipped free of hair, and 0.4 ml of 50% Hexamidine Diisethionate was applied to a 3 $\times$ 6-cm^2 area. The site was then occluded with a nonabsorbent paper and secured with adhesive tape and an elastic bandage. The dressing was left in place for 48 h. Two weeks later, a challenge dose of 25% Hexamidine Diisethionate in liquid paraffin was applied to a 2 $\times$ 2-cm^2 area of clipped skin on the left flank, occluded for 24 h. The challenge site was evaluated for erythema and edema 24, 48, and 72 h after removal of the challenge patch.

Hexamidine Diisethionate alone produced no sign of dermal irritation following the intradermal injections. However, Freund's complete adjuvant with and without Hexamidine Diisethionate elicited dermal irritation. Scoring of irritation response to the topical application of 50% Hexamidine Diisethionate was obscured by the reaction to the intradermal injections of Freund's complete adjuvant. However, the 25% challenge dose produced no signs of erythema or edema. The authors concluded that, in this study, Hexamidine Diisethionate did not produce any evidence of delayed contact hypersensitivity (Kynoch and Clifford 1976).

Photosensitization

Lamarche (1972) treated three male and three female adult albino rabbits with a 5-cm circular cotton patch saturated with a 0.10% solution of Hexamidine Diisethionate, secured with tape for 24 h (presumably, the dose site was clipped, but this was not stated). The animals were then exposed 5 min/day for 10 days to a UV lamp. The radiation parameters were not described, but the UV exposure was such that it produced a light transient suberythema on three control animals. No difference was noticed in the reactions of the treated and control animals. Hexamidine Diisethionate was not a photosensitizer in this study.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

No published data were available on the reproductive or developmental toxicity of Hexamidine or Hexamidine Diisethionate.

GENOTOXICITY

Hexamidine

Bailly et al. (1997) found that Hexamidine binds selectively to nucleotide sequences composed of at least four A-T base pairs.

Hexamidine Diisethionate

Jones and Fenner (1987) tested Hexamidine Diisethionate in the Ames bacterial reverse mutagenicity assay using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 with and without S9 microsomal activation. The concentrations evaluated were 50, 150, 500, 1500, and 5000 μg/plate. Toxicity occurred at $\geq$500 μg/plate, so a second assay was prepared using 5, 15, 50, 150, and 500 μg/plate. Hexamidine Diisethionate did not induce reverse mutations in these assays.

Allen et al. (1987) evaluated whether Hexamidine Diisethionate would cause chromosomal aberrations in Chinese hamster ovary (CHO) cells. CHO cells were incubated with the test material at concentrations of 42, 210, 350, and 420 μg/ml with S9 microsomal activation and 3.4, 17, 27.5, and 34 μg/ml without S9 activation. There were no chromosomal aberrations after treatment without activation. In the presence of S9, a slight increase in the incidence of chromosomal aberrations was seen at the lowest dose but not in the higher doses. This finding was not considered to be indicative of a clastogenic effect. The authors concluded that Hexamidine Diisethionate had no evidence of clastogenic activity in this assay.

CARCINOGENICITY

No published data were available on the carcinogenicity of Hexamidine or Hexamidine Diisethionate.

CLINICAL ASSESSMENT OF SAFETY

Skin Sensitization

Lamarche (1972) reported a sensitization study in 100 male and 100 female subjects (ages 15 to 60 years). A 1-inch-square patch containing 0.5 ml of 0.10% Hexamidine Diisethionate in aqueous solution was applied to the dorsal area of the left hand of each subject. A 1-inch-square patch containing 0.5 g of 0.10% Hexamidine Diisethionate in a cold cream formulation was applied to the dorsal area of the right hand of each subject. Patches remained in place for 24-h periods, and the exposure sites were scored for edema, erythema, and eschar formation upon patch removal. The 24-h exposures were repeated 10 times for each subject, with a day of rest between each exposure. After the 10th exposure, the subjects were not exposed to the test materials for a rest period of 2 weeks. Then, challenge patches of 0.10% Hexamidine Diisethionate in 0.50 ml aqueous solution

or 0.50 g in cold cream were applied to the same sites for 24 h, and the sites were scored for signs of irritation. Of the 200 people tested, no reaction was observed after the primary applications or the challenge tests. Thus, 0.10% Hexamidine Diisethionate did not provoke primary irritation, inflammation, or sensitization in human subjects.

Case Reports

Hexamidine

Boulitrop-Morvan et al. (1993) described a case of a 19-year-old male eczema patient who showed a photosensitivity reaction to a Hexamidine solution. Topographical lesions developed on the patient only in sun-exposed skin. Patch tests for Hexamidine without UV exposure were negative. However, patch tests with Hexamidine exposed to UVA irradiation were positive.

Brand and Ballmer-Weber (1995) reported a case of a 13-year-old boy whose atopic eczema was made worse when it was treated with Imacort cream. Each ingredient of Imacort cream was patch-tested on the child's skin. The boy showed sensitivity to Hexamidine (1% in petrolatum), prednisolone acetate, PEG-1500 stearate, glycerol-poly-(oxyethylene)-6-alkanoate, and clotrimazole.

Hexamidine Diisethionate

Robin (1978) reported eight cases of contact sensitivity to Hexamidine Diisethionate in male and female patients in an age range of 25 to 81 years. Three cases were on the face, one on the leg, and four cases of allergies to Hexamidine were disseminated. The authors noted that allergy to Hexamidine was not very common.

Dooms-Goossens et al. (1989) reported a case of an 8-year-old girl who developed a sensitivity to Hexamidine Diisethionate when an ointment containing the compound was applied to the groin as treatment for an "irritation." The child was also sensitive to ethylenediamine.

SUMMARY

Hexamidine Diisethionate functions as a biocide in cosmetics at concentrations of 0.03% to 0.1% in 38 cosmetic products. Hexamidine functions as a biocide and preservative in cosmetics, but is not in current use in cosmetics, but it is used in over-the-counter (OTC) drug products.

Hexamidine was poorly absorbed by human cadaver skin when in water-oil formulations or in a gel that simulated a cosmetic product formulation. Hexamidine Diisethionate was poorly absorbed by the skin of live rats, was not stored in any tissue type, and what was absorbed was excreted via the urine and feces. Hexamidine Diisethionate given to rats intravenously was rapidly metabolized to Hexamidine, had an elimination half-life of 27.3 h, and had a clearance rate of 10,700 ml/h/kg. The oral bioavailability of Hexamidine Diisethionate was 0.10% or 0.17% with an acute oral dose of 50 or 200 mg/kg, respectively, in rats.

Reported acute oral LD_{50} values of Hexamidine Diisethionate were 710 mg/kg to 2.5 g/kg in mice and 750 mg/kg in rats. Dermal exposure to 4 g/kg Hexamidine Diisethionate in rats or up to 9.4 ml/kg of a 0.1% Hexamidine Diisethionate solution under occlusion in rabbits produced no mortalities or other signs of toxicity.

Observations in subchronic oral toxicity studies of Hexamidine Diisethionate in rats included excessive salivation, elevated lymphocyte counts, increased alanine aminotransferase and serum calcium in rats given 100 or 200 mg/kg/day and increased aspartate aminotransferase at 200 mg/kg/day. Mortality rates were 30% and 80% in rats given 400 and 800 mg/kg/day Hexamidine Diisethionate, respectively, for 12 weeks. The NOEL for oral subchronic toxicity (4 weeks) of Hexamidine Diisethionate in rats was 50 mg/kg/day.

No signs of toxicity were observed when 4 ml/kg of 0.4% Hexamidine Diisethionate was applied to a 240-cm^2 area of rabbit skin for 90 days.

Application of 0.1 ml of 0.11% Hexamidine Diisethionate in aqueous solution to the eyes of rabbits produced slight reactions that disappeared after 72 h. The same volume of 0.05% Hexamidine Diisethionate produced no observable reaction.

Slight erythema was observed when 0.50 ml of 0.10% Hexamidine Diisethionate was applied to the abraded skin of an albino rabbit. No other observations were noted in 11 other rabbits given this treatment. When a 40% solution of Hexamidine Diisethionate was applied to 10% of the body surface of rats, slight erythema, slight edema, and scabbing were reported in some animals at varying times after treatment.

Hexamidine Diisethionate was not a sensitizer in the guinea pig maximization test or in an intracutaneous guinea pig sensitization test. Hexamidine Diisethionate was not a photosensitizer in albino rabbits.

Hexamidine Diisethionate was not mutagenic in a bacterial reverse mutagenicity assay up to the toxic dose (500 μg/plate). Up to 420 μg/ml Hexamidine Diisethionate was not clastogenic in CHO cells.

Hexamidine Diisethionate at 0.10% did not provoke primary irritation, inflammation, or sensitization in a clinical study of 200 human subjects. One individual case report of photosensitivity to Hexamidine and one case report of contact sensitivity to Hexamidine were reported. There were nine reported case reports of contact sensitivity to Hexamidine Diisethionate.

Hexamidine Diisethionate is approved at 0.1% in leave-on and rinse-off cosmetic products sold in the European Union.

DISCUSSION

After reviewing the available data on Hexamidine and Hexamidine Diisethionate, the Cosmetic Ingredient Review (CIR) Expert Panel agreed that there are sufficient data for evaluation of their safety.

The Panel acknowledged the lack of carcinogenicity and reproductive/developmental toxicity data. Because genotoxicity studies were negative, and there were no structural alerts,

the Panel concluded that it was unlikely that these ingredients would be carcinogenic. Because the rate of absorption of Hexamidine and Hexamidine Diisethionate is slow, there is no tissue accumulation, and excretion is rapid and complete, and there was no toxicity in a subchronic study, the Panel concluded that dermal exposures would not likely present a risk of reproductive/developmental toxicity. The Panel noted that a guinea pig maximization study using Hexamidine Diisethionate produced no dermal reactions and that clinical testing at 0.1% produced no irritation or sensitization.

The Expert Panel also expressed concern regarding the possible presence of 1,4-dioxane as an impurity. They stressed that the cosmetic industry should continue to use the necessary purification procedures to remove these impurities from the ingredient before blending into cosmetic formulations.

The Panel noted that there are no data for concentration of use for eye makeup and baby products, and was concerned that there should not be unrestricted concentration levels in these product categories. This led the Panel to establish a concentration limitation.

The Expert Panel recognized that certain ingredients in this group are reportedly used in a given product category, but the concentration of use is not available. For other ingredients in this group, information regarding use concentration for specific product categories is provided, but the number of such products is not known. In still other cases, an ingredient is not in current use, but may be used in the future. Although there are gaps in knowledge about product use, the overall information available on the types of products in which these ingredients are used and at what concentration indicate a pattern of use. Within this overall pattern of use, the Expert Panel considers all ingredients in this group to be safe if used at concentrations less than or equal to 0.10%.

CONCLUSION

Hexamidine and Hexamidine Diisethionate are safe for the use in cosmetic products in the practices and concentrations of use as described in this safety assessment if used at concentrations less than or equal to 0.10%.

REFERENCES

Aimard, L., P. Brasseur, L. Favennec, D. Perrine, L. Watt, and G. Brasseur. 1998. Amebic keratitis due to mixed infection with *Acanthamoeba* and *Hartmanella* species. *Clin. Infect. Diseases* 26:187–188.

Allen, J. A., P. C. Brooker, D. M. Birt, and A. Howell. 1987. Analysis of metaphase chromosomes obtained from CHO cells cultured in vitro and treated with MX 202. HP100-501-27. Unpublished data submitted by CTFA on January 15, 2004. 16 pages.[2]

Anon. 2003. *Monograph, Pharmeuropa*, 688–689.

Bailly, C., D. Perrine, J. C. Lancelot, C. Saturnino, M. Robba, and M. J. Waring. 1997. Sequence-selective binding to DNA of bis(amidophenoxy)alkanes related to propamidine and pentamidine. *Biochem. J.* 323:23–31.

Boulitrop-Morvan, C., E. Collet, S. Dalac, N. Bailly, M. Jeanmougin, and O. Lambert. 1993. Photoallergy to hexamidine. *Photodermatol. Photoimmunol. Photomed.* 9:154–155.

Brand, C. U. and B. K. Ballmer-Weber. 1995. Contact sensitivity to 5 different ingredients of a topical medicament (Imacort® cream). *Contact Dermatitis* 33:137.

British Pharmacopoeia Commission. 2001. *British Pharmacopoeia*. Stationery Office Publication 843–844.

Chasseaud, L. F., A. M. Hackett, and C. Webber. 1990. The percutaneous absorption of ^{14}C-MX202 in the rat. HP100-502-26. Unpublished data submitted by CTFA on January 15, 2004. 60 pages.[2]

Colipa. 1982. Hexamidine and its salts. Unpublished data submitted on March 8, 2004. 35 pages.[2]

Cosmetic, Toiletry, and Fragrance Association (CTFA). 2004. Ingredient use data - hexamidine and hexamidine diisethionate. Unpublished data submitted by CTFA on February 6, 2004. 1 page.[2]

Council of Europe. 2002. Certification of suitability of monographs of the European Pharmacopoeia: Certificate No. RO-CEP 1999-106-REV 02. 2 pages.[2]

Dooms-Goossens, A., M. Vandaele, R. Bedert, and K. Marien. 1989. Hexamidine isethionate: A sensitizer in topical pharmaceutical products and cosmetics. *Contact Dermatitis*. 21:270.

Enyedy, I. J., S. L. Lee, A. H. Kuo, R. B. Dickson, C. Y. Lin, and S. Wang. 2001. Structure-based approach for the discovery of bis-benzamidines as novel inhibitors of matripase. *J. Med. Chem.* 44:1349–1355.

European Economic Community (EEC). 2001. EEC Cosmetics Directive 76/768/EEC, as amended, Annexes I through VII. Brussels: EEC.

European Pharmacopoeia. 2003. Hexamidine (diisétionate d'); Heamidini diisetionas. *Pharmaeuropa* 15:688–689.

Food and Drug Administration (FDA). 2002a. Frequency of use data. *FDA database*. Washington, DC: FDA.

FDA. 2002b. Refusal actions by FDA as recorded in OASIS for France. Available at http://www.fda.gov/ora/oasis/9/ora_oasis_c_fr.html [accessed September 11, 2003]. 6 pages.

Füling, R. 2003. Analysis of two batches of Hexamidine Diisethionate for metals. Unpublished data submitted by CTFA on November 29, 2004. 4 pages.[2]

Gottschalck, T. E. and G. N. McEwen, Jr., eds. 2004. *International cosmetic ingredient dictionary and handbook*, 10th ed., Washington, DC: CTFA.

Granel, F., A. Lozniewski, A. Barbaud, C. Lion, M. Dialloux, M. Weber, and J. L. Schmutz. 1996. Cutaneous infection caused by *Tsukamurella paurometabolum*. *Clin. Infect. Diseases*. 23:839–840.

Hill, G. 1995. Preservation of cosmetics and toiletries. In: *Handbook of biocide and preservative use*, ed. H.W. Rossmore, 349–415. New York: Chapman and Hall.

Instituto de Salud Publica de Chile. 2003. List of permitted preservatives in cosmetic products. Available at http://www.ispch.cl/ctrl/reg/cosmeticos/doc/lis_preservantes.pdf [accessed September 11, 2003].

Jones, E. And L. A. Fenner. 1987. Ames metabolic activation test to assess the potential mutagenic effect of MX 202. HP100-501-28. Unpublished data submitted by CTFA on January 15, 2004. 16 pages.[2]

Kynoch, S. R. and R. Clifford. 1976. Screening test for delayed contact hypersensitivity with MX 202 in the albino guinea pig. HP100-502-22. Unpublished data submitted by CTFA on January 15, 2004. 7 pages.[2]

Kynoch, S. R., and G. K. Lloyd. 1976a. Acute oral toxicity to mice of MX 202. HP100-502-21. Unpublished data submitted by CTFA on January 15, 2004. 5 pages.[2]

Kynoch, S. R., and G. K. Lloyd. 1976b. Acute percutaneous toxicity to rats of MX 202. HP100-502-23. Unpublished data submitted by CTFA on January 15, 2004. 5 pages.[2]

Laboratories Serolbiologiques. No date. Hexamidine Diisethionate. Manufacturing process of la mesta chemie fine. Unpublished data submitted by CTFA on June 17, 2004. 6 pages.[2]

[2]Available from the Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 412, Washington, DC 20036, USA.

Laboratoires Serobiologiques. 2004. Data Profile Elestab® HP 100. Unpublished data submitted by CTFA on November 29, 2004. 4 pages.[2]

Lamarche, M. 1972. Hexamidine Di-isethionate. Unpublished data submitted by CTFA on January 15, 2004. 32 pages.[2]

Lemaître, J. P., H. Echchannaoui, G. Michaut, C. Divies, and A. Rousset. 1998. Plasmid-mediated resistance to antimicrobial agents among Listeriae. *J. Food Protection*. 61:1459–1464.

Michel, G., M. Truquet, J. Mondain, and P. Poitou. 1986. Comparison of in vivo antibacterial activity of hydro-alcoholic solutions of hexamidine and chlorhexidine. *J. Int. Med. Res*. 14:205–209.

Oblong, J. E., S. Tozer, S. J. McPhail, and M. Neinbar. 2004. In vitro cadaver skin penetration profile of radiolabeled hexamidine. Unpublished data submitted by CTFA on January 23, 2004. 8 pages.[2]

Powell, L. A. J., M. Coleman, R. Heywood, C. Gopinath, W. A. Gibson, and D. Crook. 1987. MX 202 Toxicity to rats by repeated oral administration for 4 weeks (final report). HP100-502–24. Unpublished data submitted by CTFA on January 15, 2004. 134 pages.[2]

Robin, J. 1978. Contact dermatitis to hexamidine. *Contact Dermatitis* 4:375–376.

Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP). 2002. Opinion of the SCCNFP concerning hexamidine and its salts, including di-isethionate and di(p-hydroxybenzoate). Available at http://europa.eu.int/comm/food/fs/sc/sccp/out161_en.pdf [accessed September 5, 2003].

Taylor, P., P. D. Braddock, and S. Ross. 1983. Quantitative determination of hexamidine isethionate in pharmaceutical preparations by high-performance liquid chromatography. *J. Pharmaceut. Sci*. 72:1477–1478.

Zavorskas, P. A., and S. Tozer. 2004. Pharmacokinetics of hexamidine diisethionate following intravenous and oral administration to female Sprague-Dawley rats. Unpublished data submitted by CTFA on February 5, 2004. 162 pages.[2]