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Final Report on the Safety Assessment of Chlorhexidine/Chlorhexidine Diacetate/Chlorhexidine Dihydrochloride/Chlorhexidine Digluconate

ABSTRACT

Chlorhexidine and its Diacetate and Digluconate salts are used in cosmetics as preservatives. Chlorhexidine Digluconate was slightly toxic in oral and inhalation studies. At cosmetic use concentrations, Chlorhexidine Digluconate was not irritating to the eyes or skin. Positive sensitization reactions were cited in provocative patch testing at 1.0% concentration in patients with eczema, but not in predictive patch testing of 0.05% in normal subjects. In bacterial assays, Chlorhexidine tested both positive and negative for mutagenesis. In two mammalian systems, Chlorhexidine Digluconate was not genotoxic. *p*-Chloroaniline is a degradation product of Chlorhexidine salts. A study of the degradation of Chlorhexidine revealed minor amounts of *p*-chloroaniline after 36 weeks storage. Chlorhexidine Digluconate was not carcinogenic in a 2-year drinking water study. On the basis of the data presented in this report, it is concluded that Chlorhexidine and its salts are safe for use in cosmetic products at concentrations up to 0.14% calculated as Chlorhexidine free base; 0.19% as Chlorhexidine Diacetate; 0.20% as Chlorhexidine Digluconate; and 0.16% as Chlorhexidine Dihydrochloride.

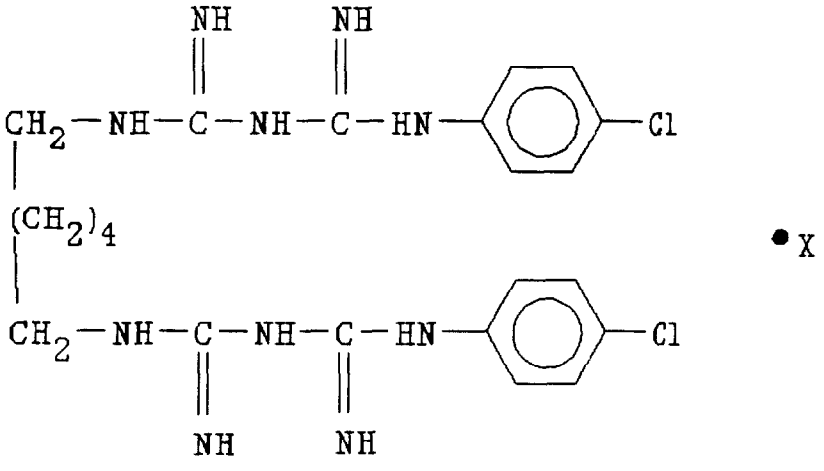
INTRODUCTION

CHLORHEXIDINE AND ITS DIACETATE and Digluconate salts are used in a variety of cosmetic products as a preservative. These ingredients, particularly the Digluconate salt, have extensive use in dentistry to inhibit plaque formation and in surgical soaps as a disinfectant. Clinical experiences in the use of Chlorhexidine are sufficiently similar that many investigators treat these three ingredients as a group rather than distinct entities (Bruze and Fregert, 1983). This literature review includes the medical and dental test data and clinical experiences relating to cosmetic application and use of Chlorhexidine.

CHEMISTRY

Definition and Structure

Chlorhexidine and its salts are organic compounds that conform to the following formula:



where X for the salts may be: $(CH_3COOH)_2$ for the Diacetate or $(C_6H_{12}O_7)_2$ for the Digluconate. Chlorhexidine (CAS No. 55-66-1) is also known as N,N'-Bis(Chlorophenyl)-3,12-Diimino-2,4,11,13-Tetraazatetra-decanediimide. The CAS numbers for the salts are Diacetate, 56-95-1; Digluconate, 18472-51-0 (Estrin et al., 1982).

Properties

Chlorhexidine is an odorless white crystalline powder with a molecular weight of 505.48. It is unstable at temperatures above 70°C or at pH below 5. The solubilities of the salts in water are Digluconate, >70%; Diacetate, 1.8%. Chlorhexidine is soluble in alcohol, glycerol, propylene glycol, and polyethylene glycols. Chlorhexidine is a strongly alkaline compound (Dolby et al., 1972; Kabara, 1984; Windholz, 1983).

Bruze and Fregert (1983) and Bruze et al. (1985) report that Chlorhexidine has an ultraviolet (UV) absorption maximum of 259 nm, and absorbs in the UVB (290–320) but not the UVA wavelength (320–400). However, subsequent UV spectra run on Chlorhexidine show maxima at 205 nm and 258 nm, with no absorption in the UVB (CIR, 1991).

Method of Manufacture

Chlorhexidine can be synthesized by combining *p*-chloroaniline, hexamethylene-bis[dicyandiamide], and $[NCNHC(:NH)\text{-}NH(CH_2)_3]_2$ in 2-ethoxyethanol and refluxing at 130–140°C for 2 h. Neutralizing this base with the appropriate acids will result in the Digluconate and Diacetate salts (Osol, 1980).

Analytical Methods

A large number of assay techniques are available to detect and quantitate the amounts of Chlorhexidine and its salts in various mixtures. Polarographic methods have

been used to determine these ingredients in the presence of surface active agents (Thomas et al., 1983). Dual wavelength UV spectrophotometric assay procedures have been used to analyze Chlorhexidine Dihydrochloride (Zhang, 1985), and Chlorhexidine Digluconate in drugs (Van de Vaart et al., 1980). De Kruijf et al. (1987) reported on the advances in thin layer chromatographic methods for the routine analysis of Chlorhexidine and other preservatives in cosmetic formulations.

Impurities and Stability

p-Chloroaniline, a component for the synthesis of Chlorhexidine and a degradation product of Chlorhexidine Diacetate and Digluconate, is routinely detected in Chlorhexidine after prolonged storage. At low pH and high temperatures, conditions that can exist during sterilization for clinical use, degradation is accelerated. Chlorhexidine Digluconate tended to decompose more than Chlorhexidine Diacetate (Dolby et al., 1972).

The stability of a formulation containing 20% Chlorhexidine Digluconate was investigated in a 156 week study (ICI, 1992a). Five liter samples were stored in high-density polyethylene bottles (eight samples, four different batches) in the dark at room temperature, 25°C, and 30°C, or in amber bottles (two samples, two different batches) at room temperature. The samples were diluted, coupled with N-(1-naphthyl) ethylenediamine and the resulting azo dyes, and measured at 560 nm. This was done initially and at 36 weeks for the two samples in the amber bottles. Initial *p*-chloroaniline measurements ranged from 12 ppm to 31 ppm. The largest concentration of *p*-chloroaniline found throughout the study was 492 ppm (see Table 1).

The National Cancer Institute (1979) performed a 78-week feeding study on *p*-chloroaniline. Dosage groups were 250 and 500 ppm in Fischer 344 rats and 2500 and 5000 ppm in $B_6C_3F_1$ mice. There was insufficient evidence to conclude that *p*-chloroaniline was carcinogenic for Fischer rats or $B_6C_3F_1$ mice. Subsequently, Chhabra et al. (1991) reviewed a National Toxicology Program (NTP) (1989) 2-year gavage study of *p*-chloroaniline hydrochloride. Dosage groups were 0, 2, 6, and 18 mg/kg in Fischer 344 rats; 0, 3, 10, and 30 mg/kg in $B_6C_3F_1$ mice; dosages were

Table 1. Concentration of p-Chloroaniline (in ppm) in 20% Chlorhexidine Digluconate

Weeks stored	Amber bottles		High-density polyethylene bottles							
			Batch 1		Batch 2		Batch 3		Batch 4	
	Batch 1	Batch 2	Room temp.	30°C	25°C	30°C	25°C	30°C	25°C	30°C
0	29	27	31	31	21	21	14	14	12	12
12			193	386						
13					71	122	65	108	58	98
18			236	492						
24			255							
26					110	204	100	182	91	169
36	226	235	347							
52			192	385	266	299	255	277	229	260
78			235	491						
104			255							
156			346							

Source: ICI, 1992a.

calculated as the free base. There was a significant increase in proliferative mesenchymal lesions in the spleen in male rats, especially splenic sarcomas in high-dose male rats. There was also a significant increase of hepatocellular neoplasms and hemangiosarcomas (liver and spleen) in male mice. *p*-Chloroaniline was considered carcinogenic in male rats and male mice. Japanese Standards for Cosmetic Ingredients (1985) require that Chlorhexidine Digluconate must contain less than 0.002% aromatic amines.

USE

Cosmetic

Chlorhexidine and its Diacetate and Digluconate salts are used as preservatives in a variety of cosmetic products (Nikitakis, 1988). Although the free base form of Chlorhexidine is listed, products containing this very alkaline compound would have to be buffered, resulting in a Chlorhexidine salt. Cosmetic products containing Chorhexidine may be used on many parts of the body, including the ocular region. They may be applied repeatedly throughout the day or over an extended period of time, and may remain in contact with the skin or be rinsed off. Their uses are summarized in Table 2.

Voluntary filing of product formulation data with the Food and Drug Administration (FDA) by cosmetic manufacturers and formulators conforms to the prescribed format of preset concentration ranges and product categories as described in Title 21, Part 720.4 of the Code of Federal Regulations (21 CFR 720.4). Because data are only submitted within the framework of preset concentration ranges, opportunity exists for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one at the highest end of

TABLE 2. PRODUCT FORMULATION DATA

Product category	*Total no. of formulations in category*	*Total no. containing ingredient*	*No. of product formulations within each concentration range (%)*		
			>1–5	*>0.1–1*	*<0.1*
Chlorhexidine digluconate					
Eye shadow	2582	1	—	1	—
Eye makeup remover	81	1	—	—	1
Makeup foundations	740	6	—	—	6
Cuticle softeners	32	1	—	—	1
Skin cleansing preparations (cold creams, lotions, and pads)	680	3	—	—	3
Face, body, and hand care preparations (excluding shaving preparations)	832	6	—	—	6
Night skin care preparations	219	4	—	—	4
Paste masks (mud packs)	171	6	—	—	6
1986 Totals		**28**	—	**1**	**27**
Chlorhexidine					
Mouthwashes and breath fresheners (liquids and sprays)	53	1	1	—	—
Skin fresheners	260	1	—	1	—
1986 Totals		**2**	**1**	**1**	—

Source: FDA, 1986.

that range, thus introducing the possibility of a 2–10-fold error in the assumed ingredient concentration. Some cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, and, therefore, the value reported by the cosmetic manufacturer or formulator may not necessarily reflect the actual concentration of the finished product; the actual concentration in such a case would be a fraction of that reported to the FDA.

Noncosmetic

Chlorhexidine has been widely used in medical practice since the 1950s. It has been extensively used in topical antiseptics for burn prophylaxis or presurgical scrubs. Chlorhexidine Digluconate has been used on intact skin in hand washes at a concentration of 4.0%, as an antiseptic on damaged skin at 1.0%, in obstetrical procedures at 1.0%, and in peritoneal cavity and bladder procedures, including irrigation, at 0.02% (Rushton, 1977). Chlorhexidine Digluconate was used in contact lens soaking solutions at a concentration of 0.05% (Coward et al., 1984). A commercial product containing 4.0% Chlorhexidine Digluconate is approved by the FDA for prescription drug use (Berman et al., 1984).

Dental uses include mouth rinses (0.2%), gels (1.0%), and a toothpaste (concentration not reported) (Fardal and Turnbull, 1986). The FDA has approved the drug use of a mouthrinse containing 0.12% Chlorhexidine Digluconate, with the sponsor's labeling advising that "periodic dental examinations are needed as part of a [Chlorhexidine mouthrinse] treatment program." This mouthrinse was subsequently accepted by the Council on Dental Therapeutics (Procter & Gamble, 1991).

There have been a number of studies on the long-term effectiveness of 0.12% and 0.2% Chlorhexidine Digluconate in mouthwashes (Loe et al., 1976; JADA, 1988; Banting et al., 1989; Sanz et al., 1989).

As of April 20, 1989, Chlorhexidine and its salts are no longer listed as active ingredients in the OTC Drug Review (CIR, 1990; Federal Register, 1988).

International

Chlorhexidine and its Dihydrochloride and Digluconate salts are approved for use in Japan in cosmetic ingredients. The upper concentration limits are listed in Table 3.

Chlorhexidine and its Digluconate and Diacetate salts are approved for use in cosmetic products by the European Economic Commission at concentrations not to exceed 0.3%, defined as Chlorhexidine free base (EEC, 1988).

GENERAL BIOLOGY

Bactericidal

Chlorhexidine, a cationic compound, interacts with the negatively charged surface of the bacterial cell. At pH 7, the bacterial adsorption is rapid and extensive. The bactericidal effect is due to the inhibition of membrane functions such as electron transfer and the activity of membrane bound ATPase. Chlorhexidine is effective against a wide variety of gram-positive and gram-negative bacteria at concentrations of

TABLE 3. MAXIMUM ALLOWABLE CONCENTRATION (%) BY JAPANESE STANDARDS FOR COSMETIC INGREDIENTS

	Chlorhexidine	*Chlorhexidine Dihydrochloride*	*Chlorhexidine Digluconate*
Soap	[a]	0.1	0.1
Dentifrice	[a]	0.01	0.01
		0.05[b]	0.05[b]
Lip cream	[a]	0.05	0.05
Shampoo	[a]	[a]	0.1
Hair rinse	[a]	0.1	0.1
Eye shadow	0.05	0.05	0.05
Mascara	0.05	0.05	0.05
Eyebrow	[a]	[a]	0.05
Eyeliner	[a]	[a]	0.05
Hair care	[a]	0.05	0.05

[a] No approved use listed.

[b] Allowed for products designed to be diluted to 0.01%.

Source: Watanabe, 1989.

0.1–4.0% (Harold et al., 1969). It has an optimum biocidal effect between pH 6.5 and 7.5 (Kabara, 1984).

Cytotoxicity

Goldschmidt et al. (1977) studied the cytopathologic effects of Chlorhexidine Digluconate on HeLa cells. Three different methods to determine cell injury and death were used: trypan blue uptake, ^{51}Cr release, and inhibition of [^{3}H]leucine incorporation. Cell cultures were exposed to Chlorhexidine for a minimum of 3 h before analysis. By three methods, the cytotoxicity of Chlorhexidine Digluconate at concentrations of 0.006% or greater was demonstrated.

The cytotoxicity of Chlorhexidine (free base) for human erythrocytes and neutrophils was studied by Gabler et al. (1987). Trypan blue staining and lactic dehydrogenase release were used to determine neutrophil damage; cell lysis was used for erythrocytes. Chlorhexidine at concentrations above 0.02% was toxic for these cells.

Absorption and Excretion

The metabolism of orally administered radioactive Chlorhexidine (free base) was studied in five animal species and assayed in one human volunteer (Winrow, 1973). Two ^{14}C compounds, one with the radioactivity in the aromatic ring and the second with the ^{14}C in the central hexamethylene chain, were used. The volunteer was given the ring radioactive compound in a mouthwash, and the laboratory test animals (rat, mouse, dog, marmoset, and rhesus monkey) were given a gelatin capsule containing a compound with radioactivity in either the ring or chain portion of the molecule. In these laboratory animals, 90–106% of the initial radioactivity was excreted in the feces. Small amounts were detected in the urine. Approximately 82% of the radioactivity was detected in the feces and 0.3% in the urine of the human volunteer. There was no

significant difference in the recovery rates of radioactivity from either the ring or chain of test compounds. The authors concluded that Chlorhexidine is poorly absorbed.

Bonesvoll (1977) used [^{14}C]Chlorhexidine Digluconate at 0.2% concentration in a mouthwash and 30% of the radioactivity was retained in the mouth of 12 volunteers. Of this amount, more than 70% of the test compound was retained after three 1-min after-rinses. The concentration of Chlorhexidine Digluconate in the saliva fell rapidly in the first 4–8 h; this was followed by a very slow release, with Chlorhexidine Digluconate still present in the saliva after 24 h.

Chlorhexidine Digluconate absorption was studied in infants bathed with a detergent containing 4.0% Chlorhexidine Digluconate (Cowen et al., 1979). Infants, 41 test and 10 control, were bathed with 1:10 dilution of a detergent containing 0.4% Chlorhexidine Digluconate (O'Neill et al., 1982). Group 1 was bathed one time with a wash cloth containing the test compound, then rinsed with a wash cloth saturated with water. Group 2 was treated in a similar manner to Group 1, but on 3 consecutive days. Group 3 was treated only once, but not rinsed, only dried. Trace amounts (0.27 and 0.28 μg/ml) of Chlorhexidine Digluconate was detected in the blood of two infants by a gas-liquid chromatographic method with electron-capture detection. Very low amounts of Chlorhexidine Digluconate were detected in the stool samples in 23 of the 41 exposed infants. This was attributed to the contact of the fecal samples with treated skin. These authors considered that the blood concentrations reported by Cowen et al. (1979) were the result of contamination that occurred during the removal of blood from the heel; the results reported by O'Neill et al. (1982) were obtained from blood samples taken from an untreated area on the infant's head. Results similar to those reported by O'Neill et al. (1982) were reported by Johnsson et al. (1987). These latter investigators monitored neonates whose mothers underwent skin disinfection before delivery; the neonates were also treated with a 4.0% Chlorhexidine Digluconate solution in routine cord care. Immediately following birth, blood samples from the cord were taken. Of the 32 infants delivered vaginally, 7 had detectable amounts of Chlorhexidine, ranging in concentration from 26 to 249 ng/ml of blood. Venous blood samples of 21 of these infants were taken on day 5 after delivery. One positive sample was reported, with a concentration of 496 ng/ml. Of the 32 infants delivered by Cesarean section, one had detectable amounts of Chlorhexidine. Venous blood samples of 23 of these infants were taken on day 5 after delivery. No positive samples were reported. No skin irritation was observed.

ANIMAL TOXICOLOGY

Acute

Butler and Iswaran (1980) reported that the LD_{50} of Chlorhexidine Digluconate in female and male mice at the end of the 14-day observation period was 2.5 g/kg (oral), 0.02 g/kg (intravenous), and 0.63 g/kg (subcutaneous). For female and male rats, the 14-day LD_{50} was >3.0 g/kg (oral), 0.02 g/kg (intravenous), and >1.0 g/kg (subcutaneous). Groups of 10 mice and 5 rats in each treatment category were used.

In another study, 6 groups of 5 rats were given by gavage a 20% Chlorhexidine Digluconate solution; weights were measured on the day of intubation and again on day 7. Dosages varied between 1.0 and 15.0 g/kg. It was concluded by the authors that the test material was slightly toxic according to the toxicity rating scale of Gleason et al.

(1976) (CTFA, 1990b). In a third study, 5 male and 5 female rats were given a 0.05% Chlorhexidine Digluconate solution equivalent to 2% of their body weights. No toxic effects were noted in the 14 day test period (CTFA, 1990a).

Chronic

Four test groups of 112 male and 112 female rats (strain not stated) were given water *ad libitum* containing 5 mg/kg, 25 mg/kg, or 50 mg/kg Chlorhexidine Digluconate (calculated as the free base), or 50 mg/kg Chlorhexidine Digluconate (calculated as the free base) plus 0.125 mg/kg *p*-chloroaniline for 24 months. A group of 128 male and 125 female rats served as controls. Water consumption was significantly reduced at the highest concentration of Chlorhexidine. Feed consumption was not affected. The results of hematologic and chemical evaluations of the four test groups were that no changes resulted from the administration of the test compound. The combined organ weight/body weight ratio of the high-dosage groups was reduced. No abnormalities attributable to the test compound were detected. The only significant abnormality found during the microscopic examination was giant cells in the mesenteric lymph nodes. No evidence of neoplastic or other toxic effects was observed (Case, 1977).

Inhalation

In 4 separate studies, the subchronic inhalation toxicity of 13 different products containing 0.20–0.25% Chlorhexidine Digluconate was studied in rats. In a controlled environment, selected dosages (conforming to anticipated human use concentrations) were discharged by aerosol cans for 2–8 sec every 5 min, 4 h/day, 5 days/week, for 65 days. Body weights were measured weekly. Blood and urine samples were collected during weeks 7 and 13 for a number of different tests, including measurement of serum enzymes, erythrocytic and leukocytic cell counts, glucose, and pH. At the end of the test period, animals were killed and specific organs were weighed before fixation. There were some differences in the test and control groups in some areas of testing, but in all cases the authors concluded that these differences were neither statistically significant nor attributable to any toxic effects of the aerosol (CTFA, 1990b).

Eight Beagle dog littermates were organized into two groups and placed in separate, environmentally controlled rooms. The treated groups was fogged with a Chlorhexidine Diacetate solution twice a day for 30 days. Data such as weight, temperature, hemoglobin, serum transaminase, and blood urea nitrogen levels were recorded twice before, once during, and twice following the fogging period. No adverse clinical effects due to Chlorhexidine Diacetate were noted (Andrews and Paul, 1977).

Nephrotoxicity and Hepatotoxicity

Groups of adult and 4-week-old male rats were given a single dose of 10 ml/kg of solutions containing either varying concentrations of Chlorhexidine Digluconate or of the vehicle only. At a preselected time, rats were anesthetized with ether, and the kidneys were removed. Cortical slices were prepared and incubated in either *p*-amino-hippurate (pAH) or [^{14}C]N-methylnicotinamide (NMN) in a balanced salt solution. At 24 h after treatment, NMN accumulation by renal slices of both adult and 4-week-old animals was decreased by Chlorhexidine at dosages of 0.5 g/kg and greater. pAH uptake was less affected in the 4-week-old animals than in the adults, although uptake by both sets of slices occurred at the greater doses. A 4–16-fold increase in plasma

transaminase activities indicated hepatic damage in the rats treated with either 1.0 g/kg or greater Chlorhexidine Digluconate solution. The renal slice uptake data along with an elevation of blood urea nitrogen indicated impaired renal function (Chow et al., 1977).

Ototoxicity

Aursnes (1980) reported that 0.5% Chlorhexidine (free base; in both water and 70% ethanol solutions) was ototoxic to guinea pigs when introduced through the bulla into the cavity of the middle ear. Solutions of 0.1% Chlorhexidine were not ototoxic. Aursnes (1982) reported extensive fibrous tissue and neuroepithelial damage when Chlorhexidine was introduced into the vestibular part of the inner ear; the extent of the damage depended on the concentration and the duration of the exposure. Galle and Venker-Van Haagen (1986) reported that 0.5 ml of a commercial product containing 0.015% Chlorhexidine Digluconate and 0.15% cetrimide produced acute vestibular dysfunction in the middle ear of 2 guinea pigs.

When 2.0% Chlorhexidine Digluconate was introduced into the tympanic cavity of cats by means of epidural tubes, it damaged the sensory neurons of the cochlear nerve ganglion. Some cellular damage, but little surface effect, was induced at a concentration of 0.05%. Chlorhexidine Digluconate at concentrations of 0.05 and 2.0% was ototoxic to the labyrinthine vestibule. The sensory cell-nerve complex was most affected (Igarashi and Suzuki, 1985; Igarashi and Oka, 1988).

Ocular Irritation

Chlorhexidine Digluconate was applied to the cornea of rabbits and cats at concentrations of less than 0.01%. The dosed animal were killed 30–40 min after exposure. Corneal damage occurred at all concentrations tested, although the amount of damage was minimal at clinical use concentrations (from 0.0025 to 0.005%). There was little difference between the apparent sensitivity of the cat and the rabbit. The authors concluded the rapid binding of Chlorhexidine Digluconate to the corneal epithelium offset any reduction of effects in the cat due to the increased blinking and lacrimation, as compared to the rabbit (Burstein, 1980). Similar results were reported in rabbits for 0.1–0.5% Chlorhexidine Digluconate concentrations (Dormans and Van Logten, 1982). Chlorhexidine Digluconate was rapidly bound to proteins (Hjeljord et al., 1973). The simultaneous application of 0.05% Chlorhexidine and a 1.0% albumin solution to the cornea of rabbits produced neither swelling nor significant degeneration of corneal epithelial cells. These results were similar to those obtained during the *in vivo* use of 0.05% Chlorhexidine Digluconate as a disinfectant. In this use, the compound was bound by the proteinaceous material in the tear film. The rapid binding of Chlorhexidine Digluconate by tear protein thus provided protection to the cornea from possible membrane-induced effects of the disinfectant (Green et al., 1980).

A Draize eye test was performed using 9 female rabbits. A 0.1 ml dose of a product with 0.05% Chlorhexidine Digluconate was instilled once in the conjunctival sac of the eye. There were no adverse reactions noted (CTFA, 1990a). In two similar tests, a product containing 0.04% Chlorhexidine Digluconate produced only minimal irritation (CTFA, 1990b).

In another study, a Draize eye test was performed on 6 rabbits with 20% Chlorhexidine Digluconate in water. The product was applied once, and the rabbits

were observed for 7 days. The irritation scores were over 85 (of 110 maximal value) on each day, with no recovery by the end of the 7 day period. At this concentration, the authors concluded that the ocular irritation potential was extreme (CTFA, 1990b).

Dermal Irritation and Sensitization

Chlorhexidine Digluconate produced discrete white lesions and hyperplasia of the cheek pouches of Chinese hamsters when it was applied topically at a concentration of 2.0%, but not at a concentration of 0.2% (Harvey et al., 1984).

A modified Draize dermal test was performed using 6 female rabbits. A 0.5 ml dose of a product containing 0.05% Chlorhexidine Digluconate was applied on the back and sides of the clipped rabbits 3 times at 24 h intervals. The rabbits were observed an additional 48 h after dosing. No positive responses were noted (CTFA, 1990a).

Injections of a 0.2% solution of Chlorhexidine Digluconate were given to 6 guinea pigs on alternate days for 1 week, and challenged 3 weeks later. Each immunized animal had a positive reaction for antibody formation at 4 days postchallenge (Haugen and Johansen, 1974). Similar data have been reported by Tolo and Rolla (1972).

Goodwin et al. (1981) compared 3 guinea pig sensitization injection procedures for the detection of 19 human contact sensitizing agents (maximization, single injection adjuvant test, and a modified Draize procedure). Weak sensitization responses in the guinea pigs were obtained in the maximization and single injection adjuvant test procedures with a 2.5% injection and a 12.5% challenge of Chlorhexidine Digluconate. No sensitization was detected in the 10 animals that were tested with the modified Draize procedure using an induction dose of 3.125% Chlorhexidine Digluconate and a challenge dose of 25%.

Layton et al. (1986) tested the primary antibody response to the hapten Chlorhexidine Digluconate and its N-chloro derivative in female BALB/c mice. N-chloro Chlorhexidine was derived from the addition of 2 mM chlorine water to Chlorhexidine Digluconate in distilled water. Mice were injected intraperitoneally with 25 or 100 μg/mouse of Chlorhexidine Digluconate in distilled water or in *Bordella pertussis* (BP) or alum adjuvant vaccine. Normal mouse sera (NMS), obtained from mice immunized with BP, was used as control. On day 14 after injection, blood was taken by cardiac puncture from ether-anesthestized mice. IgG and IgE concentrations were measured by enzyme-linked immunosorbent assay (ELISA). N-chloro Chlorhexidine, which binds covalently to its protein carrier, induced a dose-dependent increase in both IgG and IgE concentrations. Chlorhexidine Digluconate, which binds electrostatically to its protein carrier, induced low concentrations of IgG antibody only. Both N-chloro Chlorhexidine and Chlorhexidine Digluconate failed to produce an immune response when not complexed with a protein carrier.

In another study, Layton et al. (1987a) tested the immune response to Chlorhexidine Digluconate both electrostatically and covalently linked to keyhole limpet hemocyanin (KLH). The previous procedures were used in preparing and measuring antibodies. N-chloro Chlorhexidine was prepared by diluting Chlorhexidine Digluconate with five dilutions of chlorine water (0–2.5 mM). Dosages were 100 μg/mouse Chlorhexidine Digluconate or N-chloro Chlorhexidine and 50 μg/mouse KLH or 500 μg/mouse Chlorhexidine Digluconate or N-chloro Chlorhexidine and 50 μg/mouse KLH. At 0 mM chlorine (Chlorhexidine electrostatically linked to KLH), IgG, but not IgE, antibodies were induced. IgE antibodies to covalently linked Chlorhexidine and KLH

were related in a dose-response manner to chlorine concentrations. Free Chlorhexidine did not induce an immune response.

As in previous experiments, antibodies to covalently linked Chlorhexidine and KLH were produced in mice (Layton et al., 1987b). Antibodies were also produced to a "semi-Chlorhexidine," 1-(*p*-chlorophenyl)-5-hexamethylene succinamic acid biguanide, linked to human serum albumin (HSA). IgG and IgE antibodies to N-chloro Chlorhexidine bound to the semi-Chlorhexidine-HSA antigen. Free Chlorhexidine Digluconate inhibited IgG and IgE antibody binding. IgG and IgE antibodies to N-chloro Chlorhexidine-KLH bound to covalently linked semi-chlorhexidine-HSA. *p*-Chlorophenyl biguanide sequences in Chlorhexidine were the recognized epitopes and N-chlorination does not affect its specificity as an immunogen or antigen.

Reproductive Effects

Chlorhexidine (free base) dosages up to 68.5 mg/kg/day were administered by gastric intubation to pregnant rats on day 6–15 of gestation. The rats were killed on day 20 and the fetuses examined. No adverse effects were observed (Gilman and De Salva, 1979).

Cutting et al. (1964) tested a number of related compounds for their effects on fertility of mice. Chlorhexidine (free base) was administered in the drinking water at a concentration of 0.2% for 1 week; subsequently the sexes were mixed and the litters counted. Mice that absorbed or aborted litters were not differentiated from mice that did not become pregnant. Chlorhexidine reduced the number of litters by half, but did not influence the number of mice in each litter. The authors suggest that this effect may have its origins in the early stimulation of ovarian activity along with its later depression. Although the authors exclude overt toxicity, they say that minor toxicity was difficult to rule out.

MUTAGENICITY

Suessmuth et al. (1979) reported that Chlorhexidine Digluconate induced mutation in *Salmonella typhimurium* TA 1535 and TA 1538 at concentrations of 0.4 μM (280 μg/L), a very low concentration to induce mutation; metabolic activation did not significantly alter the effect. These results were confirmed by the repair assay of the DNA-polymerase-deficient strain of *Escherichia coli*. *p*-Chloroaniline was also tested, but was not mutagenic. Additional studies of the mechanism for the mutagenic effect and the DNA damaging capabilities of Chlorhexidine Digluconate indicate a possible decomposition scheme that involves a reactive biguanide cation (Ackermann-Schmidt et al., 1982a,b).

Using a liquid rec-assay, Sakagami et al. (1988a) reported that Chlorhexidine Digluconate produced slight damage to DNA, both with and without metabolic activation. Subsequent studies were done with a μmμ test. This test, based on a procedure reported by Oda et al. (1985), employs *S. typhimurium* TA 135/pSK1002, in which the plasmid (pSK1002) carries the fused gene μmμC'-'lacZ. Expression of this gene, measured by β-galactosidase activity, indicated mutagenesis induced by either chemicals or radiation. This test did not indicate that Chlorhexidine Digluconate was a mutagen (Sakagami et al., 1988b).

The preceding summaries of data from bacterial mutagenic assays of Chlorhexidine Digluconate contain both positive and negative results. The Environmental Protection

Agency (EPA) concluded that bacterial systems are not appropriate for determining the mutagenicity of biocides in mammals. The EPA stated that studies using mammalian systems capable of using natural activation and detoxification pathways were appropriate for mutagenicity testing for biocides (Federal Register, 1988).

An *in vivo* micronucleus assay was conducted using 3 groups of 10 male Swiss mice and concentrations of 10, 20, and 30 mg/kg of Chlorhexidine Digluconate in a dimethyl sulfoxide (DMSO)/glycerol vehicle. Vehicle and positive control (2 mg mitomycin) groups of 6 animals each were included in the test protocol. Two applications of the material were made at 24 h intervals. Under the test condition, no mutagenic activity was detected (COLIPA, 1984).

A mammalian cytogenic test using Chinese hamster ovary cells was used to evaluate the clastogenic potential of Chlorhexidine Digluconate. The test included positive and negative controls, and cells were treated with 1, 10, and 100 μg/ml of Chlorhexidine Digluconate, both with and without metabolic activation. No treatment related effects were observed with Chlorhexidine Digluconate in the absence of metabolic activation. With metabolic activation, the number of breaks remained unchanged; at the middle dosage, however, there was a slight increase in the number of exchanges. The high dosage produced a significantly enhanced frequency of gaps. These positive results were considered the result of comparison with abnormally low control values. The authors concluded that Chlorhexidine Digluconate did not produce clastogenic effects (COLIPA, 1987).

CARCINOGENICITY

The carcinogenicity of Chlorhexidine Digluconate was studied in a 2-year drinking water study (ICI, 1992b). Groups of 112 male and 112 female Wistar-derived specific pathogen-free rats were given Chlorhexidine Digluconate-dosed drinking water in concentrations of 5, 25, and 50 mg of Chlorhexidine (calculated as the free base) per kg of body weight per day. In addition, another group of rats received 50 mg/kg/day Chlorhexidine and 0.125 mg/kg/day *p*-chloroaniline. Due to problems with palatability, the two high-dose groups received approximately 40 mg/kg/day; the amount of *p*-chloroaniline received by the dually dosed group was calculated to be about 0.178 mg/kg/day. Dosed drinking water was prepared from 5 different batches of 20% aqueous Chlorhexidine Digluconate solution. These batches were analyzed for *p*-chloroaniline before and after use within the study (see section on Impurities and Stability, above). The authors concluded that Chlorhexidine Digluconate, along with Chlorhexidine Digluconate fortified with *p*-chloroaniline, did not induce an increase in neoplasms in this study.

CLINICAL ASSESSMENT

Predictive Tests

A single-insult occlusive patch test was performed on 19 human volunteers with a 0.04% Chlorhexidine Digluconate solution. No skin irritation was demonstrated (CTFA, 1990b).

A repeated insult patch test (RIPT) was performed using 155 men and women with a product containing 0.05% Chlorhexidine Digluconate. An occlusive patch was

applied to the test site 3 times a week for 3 weeks, followed by a 2 week nontreatment period, followed by 2 consecutive 48 h challenge patches applied adjacent to the test site. No allergic responses were noted (CTFA 1990a).

The Council on Dental Therapeutics of the American Dental Association (1988) accepted the result of studies that demonstrated the safety of a mouthrinse containing Chlorhexidine Digluconate. A 6-month study used 158 school children between the ages of 10 and 12 years. Four groups were established. One rinsed with a 0.2% Chlorhexidine Digluconate solution 6 times per week, a second rinsed with the same concentration 2 times per week, and a third rinsed with a 1.0% solution 6 times per week. A fourth group rinsed 6 times a week with a placebo. A second 6-month study was composed of 430 adults. The test group (215) rinsed with a 0.12% Chlorhexidine Digluconate solution and the control (215) rinsed with a placebo. Some minor side effects were noted in the evaluation of these clinical studies, including minor irritation, superficial desquamation of the epithelium of the oral mucosa, and changes in taste perception. All effects were deemed reversible when the use of the product was discontinued.

Ten patients had periodontal surgery on the left and right sides of the jaw. One side receiving a dressing containing 0.2% Chlorhexidine Diacetate in 12% methylcellulosum 1500 (ADA, Sweden). The other side received a placebo with only 12% methylcellulosum 1500. Healing was studied on days 5, 8, 11, 14, 21, 28, and 35 postsurgery. Gingival exudate, bleeding tendency, and the Gingival Index was scored. Beginning on day 11, patients rinsed twice daily with a 0.2% Chlorhexidine Digluconate solution. During the observation period, the side that had received the Chlorhexidine Diacetate dressing had less gingival exudate, less bleeding, and a higher Gingival Index than the placebo side (Asboe-Joergensen et al., 1974). During a 6-week study using a 0.12% Chlorhexidine Digluconate or placebo mouthwash in 40 patients after periodontal surgery, the only adverse reactions to Chlorhexidine were staining of teeth (8 of 17 patients) and a "burning sensation" or "a too strong taste" (5 of 17) (Sanz et al., 1989).

In long-term studies (1 and 2 years), 0.12% and 0.2% Chlorhexidine Digluconate mouthwash produced significant reactions neither in the blood parameters (Rindom-Schiott et al., 1976) nor in the oral musoca (Mackenzie et al., 1976). The only side effect noted was staining of teeth (Loe et al., 1976; Banting et al., 1989).

The American Heart Association recommends use of Chlorhexidine Digluconate mouth rinse among other means to reduce the chances of bacterial endocarditis during dental procedures likely to cause gingival bleeding (Dajani et al., 1990; Council on Dental Therapeutics , 1991).

Male and female volunteers used a mouthrinse containing 0.12% Chlorhexidine Digluconate or a placebo for up to 9 months. Of 363 original volunteers, 224 completed the study. Of those not completing the study, 17 cited reasons related to the Chlorhexidine Digluconate (staining of teeth, taste). Subjects were skin prick tested with 2.01 mg/ml Chlorhexidine Digluconate, ragweed antigen, house dust mite antigen, and the vehicle (50% glycerin in water). Tests were performed before use of the mouthwash and, after 6 months and 9 months of use. There were no allergic reactions to the Chlorhexidine Digluconate throughout the study. Results of testing with ragweed and house dust mite antigens identified 32% of the volunteers as atopic. There was no significant change in atopic status throughout the study (Procter & Gamble, 1991).

The skin prick procedure was used on a population of 683 male and female volunteers. These subjects were asked to use 15 ml of a mouthwash containing

Chlorhexidine Digluconate twice daily. Initial dosages of Chlorhexidine Digluconate in mouthwash were 0.035, 0.082, and 0.12%. Skin prick tests were performed before mouthwash use and after 3 months, 6 months, and 24 months of use. At 3 months, the group receiving 0.035% Chlorhexidine Digluconate was instead given a concentration of 0.12%. At 6 months, the 0.082% dose group was discontinued. Of the 683 volunteers, 258 completed the study. Of those leaving for reasons due to the effect of Chlorhexidine Digluconate, 83 cited staining of teeth or changes in taste, 4 cited either irritated or sore gums, and 1 each cited parotid irritation, gastric intolerance, irritated tongue and sore throat, lip irritation, film on teeth, irritation of the oral mucosa, stinging of the tongue, and irritation of the mouth and dermatitis of the hands. In all cases, skin prick tests with Chlorhexidine Digluconate were negative (Procter & Gamble, 1991).

A population of 89 dental school faculty and students were skin prick tested, as above, for immediate hypersensitivity to Chlorhexidine Digluconate and other nitrogen-containing compounds. Subjects completed a questionnaire indicating their exposure to 14 products containing Chlorhexidine Digluconate. Only three subjects reported no exposure to any of the products. Environmental antigens were used to determine atopy (38 subjects were considered atopic). There were no immediate hypersensitivity reactions to the Chlorhexidine Digluconate (Procter & Gamble, 1991).

Provocative Tests

Osmundsen (1982) reported the results of a 3 year clinical study in which 14 of 551 patients had a strong contact dermatitis reaction when patch tested with 1.0% Chlorhexidine Digluconate in water. With use of the same concentration for the patch test, Bechgaard et al. (1985) reported 48 positive reactions among a population of 2,061 patients who were patch tested. A greater percentage of positive reactions was reported for males, and an overall greater percentage of positive results for patients with eczema was noted. In another study, 52 of 1063 patients with eczema had a positive reaction to 1.0% Chlorhexidine Digluconate in water and/or petrolatum (Anderson and Brandrup, 1985). Upon retest of 29 of the patients who had a positive reaction, the number of the positive reactions was decreased by 28%. There was no apparent difference in results between the two vehicles. The retest using Chlorhexidine Diacetate produced similar results. Bajaj and Gupta (1986) reported the results of the patch testing of 314 patients suspected to have contact hypersensitivity to Chlorhexidine Dihydrochloride. Ten patients had positive results. Patients with leg ulcers and stasis eczema were patch tested using the International Contact Dermatitis Research Group (ICDRG) standard technique with 1.0% aqueous Chlorhexidine Diacetate and 1.0% Chlorhexidine Digluconate (Knudsen and Avnstorp, 1991). Of the 297 patients tested, 3 reacted only to the Digluconate, 21 reacted only to the Diacetate, and 15 reacted to both Chlorhexidine Diacetate and Digluconate solutions.

Waclawski et al. (1989) documented two cases in which occupational asthma was related to a Chlorhexidine Digluconate and alcohol aerosol disinfectant. In one case, a 54-year old woman who smoked, with no history of asthma, had attacks of coughing and wheezing within minutes of exposure to the Chlorhexidine Digluconate disinfectant. Spirometric tests were normal, but a 9.2 g/L concentration of histamine reduced forced expiratory volume by 20%. This indicated borderline hyperresponsiveness of the airways. A second exposure to the disinfectant yielded a 13% reduction in forced expiratory volume. In the second case, a 43-year old nonsmoker with no history of asthma who had been prescribed a solbutamol inhaler experienced chest tightness after

exposure to the same disinfectant. Spirometric tests were normal, and a >16 g/L concentration of histamine reduced forced expiratory volume by 20%. A challenge test with the disinfectant induced a 22% reduction in forced expiratory volume.

Ohtoshi et al. (1986) tested a patient who went into anaphylactic shock after application of a 0.5% Chlorhexidine Digluconate solution to an abraded wound on his elbow for specific antibodies to Chlorhexidine. A skin prick test with 0.02% Chlorhexidine Digluconate in saline was negative. Three sites on the patient were injected intradermally with 0.1 ml of the patient's serum; three other sites were tested with 0.1 ml of normal serum. Each set of three sites was initially challenged with 0.002% or 0.0002% Chlorhexidine Digluconate in saline. Sensitized sites were those that had received an injection of the patient's serum and had been challenged with Chlorhexidine Digluconate. Seventy-two hours after the initial challenge, 0.0002% Chlorhexidine Digluconate in saline was injected into each of the 6 sites. Sites previously injected with normal serum had no reaction. Sites injected with the patient's serum and previously challenged with 0% or 0.002% Chlorhexidine Digluconate in saline were sensitized in the second challenge. The site that had been sensitized with 0.02% Chlorhexidine Digluconate had no reaction to the second challenge. IgE radioallergosorbent technique (RAST) and RAST inhibition assays were performed on the sera of this and seven other patients who had a shock reaction after the application of Chlorhexidine. The mean RAST count of the patients' sera was 15.6%, compared to 2.3% of normal sera.

Sera from Chlorhexidine-sensitive patients and hospital staff of Japanese origin and hospital staff of British origin were tested for IgG and IgE antibodies to Chlorhexidine (Layton et al., 1989). Antibodies were measured using the RAST employing a semi-Chlorhexidine benzoate derivative complexed with HSA as the solid phase antigen or ELISA. IgE antibodies were found only in those Japanese patients who had a previous sensitivity to Chlorhexidine. IgG antibodies were found in nonsensitive Japanese and British donors as well as Chlorhexidine-sensitive patients.

Eight subjects were recruited to participate in a skin prick test, using the method previously described. Three subjects were previously positive for delayed contact hypersensitivity to Chlorhexidine Digluconate in a repeated insult patch test using abraded skin as the application site. Two subjects had no reaction to the same test. Three of the subjects had no known exposure to Chlorhexidine. Chlorhexidine Digluconate did not produce a reaction in any of the subjects. Two participants were considered atopic by environmental antigen testing (Procter & Gamble, 1991).

Thune et al. (1988) reported the results of a Scandinavian photopatch test study using 1,993 patients with suspected photodermatosis. Patients were screened for UVA and UVB sensitivity by exposure to a xenon light source and filter (UVA: Schott WG + Schott KG 1; UVB: Schott WG 295 + Schott KG 1) and any erythema at 24 h recorded. These scores were used as a reference to determine a patient's threshold for UV-induced erythema. Each test substance was applied to two areas of the back using Finn chamber aluminum discs and Scanpor tape. One series of patches was removed in dim light after 24 h and the subjects inspected for reactions. The sites of the other set of patches was exposed to either 5 J/cm^2 of light from a Waldmann psoralen/UVA (PUVA)-500 fluorescent tube (major output in the range of 320–400 nm) or a light source with the same light qualities. After 48 h from the end of irradiation, the subjects with both light-exposed patches were examined for reactions (Jansen et al., 1982). Six patients had positive contact dermatitis reactions and 2 patients had photocontact dermatitis reactions to a 0.5% Chlorhexidine Digluconate solution in petrolatum (Table 4).

Table 4. Contact and Photocontact Dermatitis to Chlorhexidine

Ingredient	Concentration	Vehicle	No. of patients	Positive reactions		References
				Contact	Photocontact	
Chlorhexidine Digluconate	1.0%	Water	551	14 (2.5%)	—	Osmundsen, 1982
Chlorhexidine Digluconate	1.0%	Unknown	2061	45 (2.3%)	—	Bechgaard et al., 1985
Chlorhexidine Digluconate	1.0%	Water and/or petrolatum	1063	52 (5.4%)	—	Anderson and Brandrup, 1985
Chlorhexidine Digluconate	0.5%	Water	1993	6 (0.3%)	2 (0.1%)	Thune et al., 1988
Chlorhexidine Dihydrochloride	Powder	Powder	314	10 (3.2%)	—	Bajaj and Gupta, 1986

Case studies of delayed and immediate hypersensitivity to Chlorhexidine were reviewed (Bergquist-Karlsson, 1988). The author noted that whereas the number of cases of immediate sensitivity to Chlorhexidine are few considering its extensive use, care should be taken in applying the ingredient to wounds or abraded areas. Okano et al. (1989) reported that Chlorhexidine Digluconate was confirmed as the causative agent by scratch, epicutaneous, or intradermal test in six patients who developed urticaria, dyspnea, and anaphylactic shock following topical use of the compound as a disinfectant.

A product containing 0.05% Chlorhexidine Digluconate was applied to the periorbital area of 53 women at least once a day for 4 weeks. There were five positive reactions to the treatment. Of these, four were considered subjective and insignificant. One subject had dryness and swelling of the eyelids, but these changes were considered responses to the surfactant content of the product (CTFA, 1990a).

Delayed hypersensitivity to the fluid used for soft contact lenses has been obseved in only a few patients with conjunctivitis. Chlorhexidine Digluconate tested at 1.0% gave a positive patch test in 1 of 15 patients (van Ketel and Melzer-van Riemsdijk, 1980) and 3 of 41 patients with conjunctivitis (Rietschel and Wilson, 1982).

Population Study

Interviews were conducted with 866 patients with cancer of the oral cavity and 1,249 control volunteers to determine the risk of regular mouthwash use with respect to primary oral and pharyngeal cancer (Winn et al., 1991). Patients with cancer were from 4 metropolitan areas and ranged in age from 18 to 79 years. Patients were diagnosed with cancer between January 1, 1984, and March 31, 1985. Excluded from the study were patients with cancers of the lip, salivary glands, and nasopharynx. Controls were chosen to match geographic, age, race, gender, education, smoking habits, alcoholic beverage drinking habits, and dietary fruit intake by random digit dialing (18–64 years old) and files from the Health Care Financing Administration (65–79 years old). After adjustment was made for the above parameters, statistically significant increases of cancer of the oral cavity were seen in the population that regularly used mouthwash with an alcohol content of $\geq$25% (odds ratio of 1.6 compared to 1.0 of control). This increased risk, however, was not seen in the population that regularly used mouthwash with an alcohol content $\leq$25% (odds ratio of 0.7).

SUMMARY

Chlorhexidine and its Diacetate and Digluconate salts are used in cosmetics as a preservative. Chlorhexidine is an odorless white crystalline powder with UV absorption maxima of 205 and 258 nm and no absorption in the UVB range, and is unstable at temperatures above 70°C or pH below 5. *p*-Chloroaniline is routinely detected as an impurity after prolonged storage. In a 3 year study, the largest concentration of *p*-chloroaniline found in a 20% Chlorhexidine Digluconate formulation was 492 ppm. An NTP study reported clear evidence of the carcinogenicity of *p*-chloroaniline in male mice and male rats.

Chlorhexidine and its salts are used in eye makeup preparations, makeup foundations, skin care products, mouthwashes, hair care products, hair bleaches, and hair dyes requiring caution statements. Noncosmetic uses include topical antiseptics, surgical scrub preparations, toothpaste, mouthwash, and soaking solutions for contact lenses.

Chlorhexidine has a strong bactericidal effect. Chlorhexidine Digluconate was cytotoxic at concentrations of 0.006% and greater.

Radioactive Chlorhexidine administered orally to five animal species and one volunteer was primarily recovered in the feces. In a mouthwash study, some radioactive Chlorhexidine remained in the mouth after 24 h.

A number of studies evaluated the blood and feces of infants bathed with a Chlorhexidine Digluconate solution for the presence of Chlorhexidine. Chlorhexidine Digluconate was detected in some studies. This positive result was considered due to the contamination of the blood and fecal samples by the antiseptic on the skin.

The LD_{50} of Chlorhexidine in mice was 2.5 g/kg (oral), 0.02 g/kg (intravenous), and 0.63 g/kg (subcutaneous); in rats: >3.0 g/kg (oral), 0.02 g/kg (intravenous), and >1.0 g/kg (subcutaneous). It was concluded that upon oral application, Chlorhexidine is slightly toxic by the toxicity rating scale of Gleason et al. (1976).

Male and female rats were used to assess the chronic toxicity of Chlorhexidine in a 24 month drinking water study. No evidence of neoplastic or other toxic effects was observed.

The short-term inhalation toxicity of Chlorhexidine and Chlorhexidine Diacetate was tested in rats and Beagle dogs, respectively. In both studies, no adverse effect due to Chlorhexidine was noted.

Groups of adult and 4-week-old mice receiving 1.0 g/kg or greater Chlorhexidine Digluconate had hepatic damage as well as impaired renal function.

Chlorhexidine and Chlorhexidine Digluconate were ototoxic to cats and guinea pigs when introduced into the tympanic cavity at concentrations greater than 0.5%.

Corneal damage was produced in rabbits and cats by concentrations of Chlorhexidine Digluconate as low as 0.0025%, but damage was minimal at concentrations of use. Minimal irritation was observed in similar tests using 0.04% Chlorhexidine Digluconate solution.

Upon topical application, Chlorhexidine produced discrete white lesions and epidermal hyperplasia of the cheek pouch of Chinese hamsters.

No signs of dermal irritation were observed in a modified Draize dermal study and a single occlusive patch test with 0.004% Chlorhexidine Digluconate. Guinea pigs were weakly sensitized to Chlorhexidine when 0.2% and 2.5% solutions were injected. No sensitization was produced when 3.125% Chlorhexidine was used in a modified Draize procedure.

Chlorhexidine is a hapten and, when covalently bound to a protein carrier, it raised IgE as well as IgG antibody responses in BALB/c mice. The epitope for Chlorhexidine immune response is the *p*-chlorophenyl structure.

In mice, Chlorhexidine in drinking water reduced the number of litters, but not the number of mice within each litter. No other reproductive effect due to Chlorhexidine was observed.

Chlorhexidine Digluconate was mutagenic in *Salmonella typhimurium* TA1535 and TA1538, with and without metabolic activation. Micronucleus and μmμ tests were negative. Chlorhexidine Digluconate was not clastogenic in Chinese hamster ovary cells.

Chlorhexidine Digluconate produced no carcinogenic effects in a 2-year drinking water study using rats.

There was no skin irritation potential reported in two RIPTs using Chlorhexidine.

In a 6-month study, volunteers used either a 0.12% or 0.2% Chlorhexidine Digluconate mouthrinse 6 times a week. Some minor side effects were noticed in the epithelium of the oral mucosa, but these were deemed reversible with discontinued use of the product.

In two postperiodontal surgery studies, a Chlorhexidine mouthwash was tested against a placebo. The only adverse reaction to Chlorhexidine reported was a burning sensation.

In long-term studies with a Chlorhexidine mouthwash, no significant reaction to the Chlorhexidine was found in the blood parameters and oral mucosa.

Skin prick tests were performed on participants in 9-month and 24-month studies of mouthwash containing Chlorhexidine. No allergic reaction to Chlorhexidine was produced throughout the studies.

A population with prior exposure to Chlorhexidine was tested for sensitivity. No immediate hypersensitivity reaction to Chlorhexidine was observed.

In clinical studies, neither skin irritation nor sensitization was seen in patch tests using 0.05% Chlorhexidine Digluconate. When 1.0% Chlorhexidine Digluconate was used, 14 of 551 and 48 of 2,061 volunteers had positive reactions to patch tests. In a similar study with 1.0% Chlorhexidine Digluconate, 52 of 1,063 patients with eczema had positive reactions. In a UVA photopatch study of 1993 volunteers, 0.5% Chlorhexidine Digluconate produced a contact dermatitis reaction in 6 volunteers and a photocontact dermatitis reaction in 2 volunteers.

Two cases were reported of allergic asthma reactions due to Chlorhexidine Digluconate in an aerosol.

Sera from Chlorhexidine-sensitive and non-Chlorhexidine-sensitive patients were tested for IgG and IgE antibodies. IgE antibodies were found only in the sensitized population. IgG antibodies were found in both populations.

Eight subjects, three with a known sensitivity to Chlorhexidine, had no reaction to an RIPT on abraded skin.

Six case studies have been reviewed in which a Chlorhexidine Digluconate topical disinfectant was the causative agent of urticaria, dyspnea, and anaphylactic shock. Delayed hypersensitivity to contact lens solution containing Chlorhexidine Digluconate has been reported in some patients with conjunctivitis.

A population study using interviews of patients with oral and pharyngeal cancer and volunteers discovered an increase in oral cavity cancers among the population that regularly used a high alcohol-content mouthwash.

DISCUSSION

In the Expert Panel's review of the effects of Chlorhexidine on the mucous membrane, there was concern about its implication in cases of anaphylactic shock reaction after some patients were treated with Chlorhexidine Digluconate as a disinfectant. After a careful review of the clinical data, the Expert Panel members agreed that the data presented did not support their initial apprehension; only one cited case could possibly be classified as an anaphylactic shock reaction. Therefore, the initial recommendation that Chlorhexidine not be used on damaged skin or mucous membrane was deleted.

Also of concern was the dermal irritation and sensitization data on Chlorhexidine Digluconate. Positive reactions were cited in provocative patch testing at 1.0% concentration in patients with eczema, but not in predictive patch testing of 0.05% in normal subjects. The Expert Panel recognized that testing in patients suspected of sensitization often yields more irritation reactions in provocative patch testing than normal controls in predictive patch testing. Because Chlorhexidine does not absorb light in the UVB range, phototoxic reactions would not be expected in the UVB range. Photosensitization reactions to Chlorhexidine in the UVA range were not significant in the studies available to the Panel. Oral test data from use tests of 0.2% Chlorhexidine Digluconate in mouthwash formulations proved uneventful. The human safety test data on Chlorhexidine Digluconate, along with the diminished concern of anaphylactic reaction, indicate that Chlorhexidine Digluconate could be safely used at 0.2% without qualifications. These results extrapolate to the safety of Chlorhexidine as a free base at 0.14%, Chlorhexidine Diacetate, 0.19%, and Chlorhexidine Dihydrochloride, 0.16%.

Chlorhexidine Digluconate, in some bacterial assays, tested positive for mutagenesis and, in others, tested negative. However, the Expert Panel is aware of the evaluation of the Environmental Protection Agency's Scientific Advisory Panel on the use of mutagenic assays in determining the safety of biocides. This Advisory Panel recommended that only studies using mammalian systems capable of using natural activation and detoxification pathways were appropriate for mutagenicity testing for biocides. The two mutagenicity studies on Chlorhexidine Digluconate using mammalian systems were negative.

p-Chloroaniline is a degradation product of Chlorhexidine salts that is routinely detected in formulations containing Chlorhexidine after prolonged storage. *p*-Chloroaniline has been shown to be a carcinogen in animal studies. The Expert Panel requested and received additional information on the stability of Chlorhexidine Digluconate in formulation with respect to the degradation to *p*-chloroaniline and a carcinogenicity study on Chlorhexidine Digluconate. This would extrapolate to less than 5 ppm *p*-chloroaniline in a formulation containing 0.20% Chlorhexidine Digluconate.

CONCLUSION

On the basis of the data presented in this report, the CIR Expert Panel concludes that Chlorhexidine and its salts are safe for use in cosmetic products at concentrations up to 0.14% calculated as Chlorhexidine free base; 0.19% as Chlorhexidine Diacetate; 0.20% as Chlorhexidine Digluconate; and 0.16% as Chlorhexidine Dihydrochloride.

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