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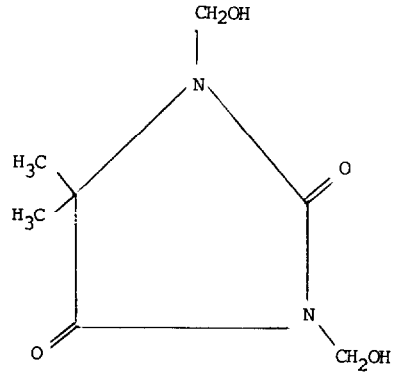
Final Report on the Safety Assessment of DMDM Hydantoin

DMDM Hydantoin is a preservative, which is used in cosmetic products at concentrations up to 1%. This ingredient is a formaldehyde donor containing up to 2% of the free aldehyde in equilibrium with the hydantoin. When ^{14}C-DMDM Hydantoin was applied to the middorsal area of Sprague-Dawley rats, more than 98% of the recovered radioactivity was confined to the dose site. The LD_{50} dermal and oral toxicity of DMDM Hydantoin was greater than 2 g/kg. No significant toxic effects were noted in a subchronic oral toxicity study. In skin irritation studies using product formulations, results ranged from non-irritating to moderate skin irritation. At most, transient minimal irritation was noted in albino rabbits treated with DMDM Hydantoin formulations. The mutagenicity of DMDM Hydantoin formulations varies in accordance with the test system. The ingredient was not mutagenic in one *Salmonella*/microsome assays but was in another. Both positive and negative mutagenic activities were reported when DMDM Hydantoin was tested in the L5178y TK± mouse lymphoma assay. The ingredient was mutagenic in the chromosome aberrations and unscheduled DNA synthesis assays but was not mutagenic in unscheduled DNA synthesis and DNA strand breaks/crosslinks assays. A comparison of Ames test results from studies of DMDM Hydantoin product and formaldehyde indicates a similar number of revertants per formaldehyde equivalent. In clinical studies, skin irritation ranged from none to observations of intense erythema and edema when various formulations containing DMDM Hydantoin were applied. DMDM Hydantoin formulations did not induce sensitization in some clinical studies. DMDM Hydantoin formulations were neither phototoxic nor photoallergenic. Use of DMDM Hydantoin at its current concentration of use in cosmetic products would not expose the consumer to levels of formaldehyde above the limit previously considered as acceptable in cosmetic products. Based on the available data included in this report, it is concluded that DMDM Hydantoin is safe as a cosmetic ingredient in the present practices of use.

CHEMISTRY

DMDM Hydantoin (CAS No. 6440-58-0) is an organic compound having the following structure[1]:



Synonyms for this compound include 1,3-dimethylol-5,5-dimethyl hydantoin, 1,3-Bis (Hydroxymethyl)-5,5-Dimethyl-2,4-Imidazolidenedione, and 2,4-Imidazolidenedione, 1,3-Bis (Hydroxymethyl)-5,5 Dimethyl-.[1] It is supplied as a 55.0% solution.[2]

DMDM Hydantoin is a formaldehyde donor containing up to 2% of the free aldehyde in equilibrium with the hydantoin.[3] It is stable over a wide range of pH and temperature conditions. For example, when maintained at −18°C, 24°C, and 50°C for 1 year, the amounts of free and total formaldehyde did not change.[2,3] Also, no changes in free and total formaldehyde were detected in DMDM Hydantoin after 32 days of storage at pHs of 5, 7, and 9.[2] Additional properties of DMDM Hydantoin appear in Table 1. Properties of a 55% DMDM Hydantoin solution* are listed in Table 2.

TABLE 1. Properties of DMDM Hydantoin[2]

Molecular weight	188.19
Combined formaldehyde (%)	31.19
Appearance	White crystal
Odor	Very slight
Melting point (°C)	102-104
Boiling point (°C)	Decomposes
Vapor pressure (60°C, mm)	0.5
Solubility (g/100 g of solvent)	
Water (20°C)	177.3
Water (30°C)	>200.0
Methanol	107.5
Acetone	20.2
Ethanol	56.4
Isopropanol	15.3
Chloroform	1.52
Methylene chloride	0.93
Toluene	0.09
Hexane	0.02

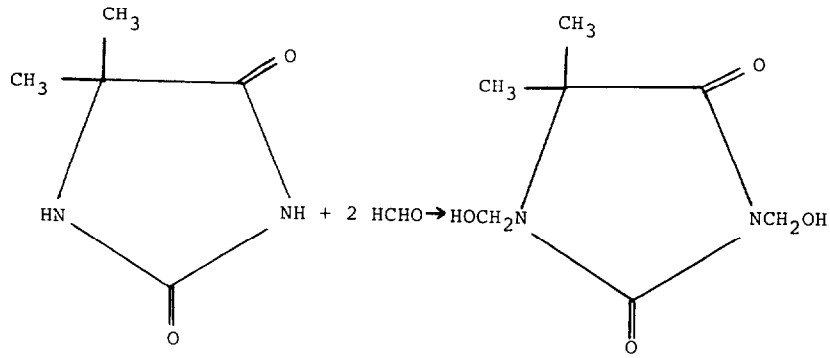
*All 55% DMDM Hydantoin solutions discussed in the text are from the same batch.

TABLE 2. Properties of a 55% DMDM Hydantoin Solution[2]

Appearance	Colorless liquid
Odor	Mild
Freezing point (°C)	−11
Density (25°C)	1.158
pH (25°C)	
As is	6.9
1:10 dilution	6.0
1:20 dilution	5.9
Viscosity (cP)	
15°C	8.4
25°C	5.5
Formaldehyde content	
Combined (%)	17.0–17.6
Free (%)	0.5–2.0

Methods of Production

DMDM Hydantoin is produced by reacting 3–5 moles of formaldehyde, as the 37% by weight aqueous solution, with 1 mole of dimethylhydantoin at 84°C.[4] A highly concentrated aqueous solution of the compound is prepared by reacting 2 moles of formaldehyde, as 37% formalin, with dimethylhydantoin at 38–50°C, pH 8.1–8.3.[5]



Analytical Methods

DMDM Hydantoin has been identified via the following techniques: gas chromatography, infrared spectroscopy, ultraviolet spectroscopy, nuclear magnetic spectroscopy, and differential scanning calorimetry.[2] The ultraviolet spectrum of a 55% DMDM Hydantoin formulation has been recorded at concentrations of 4.0×10^3 mg/L and 1.0×10^2 mg/L (water solvent). There was no significant absorbance above 260 nm for either solution.[6]

Impurities

The composition of DMDM Hydantoin as determined by gas chromatography is as follows: 94–98% DMDM Hydantoin, 2.5–3.0% monomethyloldimethylhydantoin, and other dimethylhydantoin formaldehyde products comprise the balance.[2]

USE

Purpose in Cosmetics

DMDM Hydantoin is a cosmetic preservative.[7] It is described as being a broad-spectrum antimicrobial agent, effective against fungi, yeast, and gram-positive and gram-negative bacteria.[3]

The cosmetic formulation listing that is made available by the Food and Drug Administration (FDA) is compiled through voluntary filing of such data in accordance with Title 21 part 720.4 of the Code of Federal Regulations.[8] Ingredients are listed in prescribed concentration ranges under specific product type categories. Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, the value reported by the cosmetic formulator may not necessarily reflect the actual concentration found in the finished product; the actual concentration in such a case would be a fraction of that reported to the FDA. The fact that data are only submitted within the framework of preset concentration ranges also provides the opportunity for estimation 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 entered at the highest end of that range, thus introducing the possibility of a 2- to 10-fold error in the assumed ingredient concentration. DMDM Hydantoin is present in approximately 115 product formulations, ranging in concentration from ≤ 0.1 to 1% (Table 3).

Surfaces to Which Applied

Cosmetic products containing DMDM Hydantoin are applied to the skin and hair and may come in contact with the eyes, nasal mucosa, and other parts of the body.

Frequency and Duration of Application

Product formulations containing DMDM Hydantoin may be applied as often as several times daily. Many of the products may be expected to remain in contact with the skin for as briefly as 15 to 30 min and may be used repeatedly over a period of several years.

Noncosmetic Use

Noncosmetic uses of Hydantoins include herbicides, polymers, and antiarrhythmic and anticonvulsant agents.[10]

TABLE 3. Product Formulation Data[9]

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (%)	
			>0.1-1	≤0.1
Bubble baths	475	5	4	1
Other bath preparations	132	1	1	–
Eye lotion	13	1	1	–
Hair conditioners	478	19	16	3
Hair rinses (noncoloring)	158	7	7	–
Hair shampoos (noncoloring)	909	35	32	3
Tonics, dressings, and other hair grooming aids	290	4	4	–
Hair shampoos (coloring)	16	1	1	–
Makeup foundations	740	8	8	–
Makeup bases	831	5	5	–
Bath soaps and detergents	148	6	6	–
Other personal cleanliness products	227	1	1	–
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	3	3	–
Face, body, and hand skin care preparations (excluding shaving preparations)	832	9	9	–
Moisturizing skin care preparations	747	6	6	–
Night skin care preparations	219	2	2	–
Other skin care preparations	349	2	2	–
1981 TOTALS		115	108	7

BIOLOGICAL PROPERTIES

Absorption, Distribution, and Excretion

A 0.1 ml aqueous solution containing 0.1 mCi of 1,3-dihydroxymethyl-5,5′-dimethylhydantion-5-^{14}C was applied to the middorsal area of young adult male Sprague-Dawley rats. After 72 h, more than 90% of the applied dose was recovered; more than 98% of the recovered activity was confined to the dose site. At the time of killing, less than 1% of the radioactivity was distributed in all body tissues. The higher counts of radioactivity were reported for the gastrointestinal tract, liver, and bone marrow. For most tissue samples, there was no evidence of accumulation of DMDM Hydantoin or its metabolites. DMDM Hydantoin and its metabolites are excreted primarily via the urine. The amount of ^{14}C activity in the urine decreased approximately 6 times over a 72-h period; radioactivity in the feces remained approximately constant and significantly below that of the urine.[11]

TOXICOLOGY

Acute Inhalation Toxicity

Ten young adult Sprague-Dawley rats (male) were exposed for 1 h to 55% DMDM Hydantoin at a concentration of 204 mg/L in an air chamber (Table 4). Ten rats of the same sex and strain served as controls. The animals were killed after a 14-day holding period. Observations during exposure included immediate signs of discomforts, such as gasping for breath and eyes closed throughout the exposure period. There were no remarkable gross or microscopic tissue alterations in the control and test groups.[12]

Four groups of 10 Sprague-Dawley rats (5 males and 5 females per group) were exposed to a liquid droplet aerosol comprising a 55% DMDM Hydantoin solution for 4 h (Table 4). Each of the 4 groups was exposed to concentrations of 0.0, 13.7, 126.8, and 377.8 mg/L, respectively, in a 309-L inhalation chamber. The animals were observed for signs of toxicity during the 4-h exposure period and for 14 days thereafter; all animals survived. Dry pigmented material was noted around the noses of 3 middose (126.8 mg/L) and 5 high dose (377.8 mg/L) animals on the second day of exposure; no nasal discharge was apparent on the third day. The authors stated that these observations were indicative of irritated nasal membranes and capillaries. At microscopic examinations of tissues from the nasal passages, trachea, bronchi, lungs, liver, and kidneys, no remarkable dose-related effects were found.[13]

Acute Oral Toxicity

An acute oral toxicity study of 55% DMDM Hydantoin was conducted with albino rats (number of animals not specified). The acute oral LD_{50} reported was 3.72 ± 0.5 g/kg (Table 5). Results from postmortem examinations of animals that died included distended stomachs (2 animals), subdermal abdominal hemorrhages (1 animal), and a pale area on the liver (1 animal); gastrointestinal hemorrhages and pale kidneys were also noted (number of animals not specified).

TABLE 4. Inhalation Toxicity of DMDM Hydantoin Formulations

Animals tested	*Test substance*	*Methodology*	*Results*	*Reference*
10 Sprague-Dawley rats	55% DMDM Hydantoin product formulation	Administered in air stream for 1 h; exposure level = 204 mg/L of air	No remarkable tissue alterations	12
30 Sprague-Dawley rats (3 groups of 10 each)	55% DMDM Hydantoin product formulation	Each of the 3 groups was exposed to 13.7, 126.8, and 377.8 mg/L of air, respectively, for 4 h	No test substance-related lesions were reported	13

TABLE 5. Oral Toxicity of DMDM Hydantoin Formulations

Type of study	*Animals tested*	*Test substance*	*Methodology*	*Results*	*Reference*
Acute oral toxicity	Albino rats (no. not specified)	55% DMDM Hydantoin product formulation	–	Acute oral median lethal dose (LD_{50}) = 3.72 ± 0.535 g/kg; postmortem examinations of animals that died revealed gastrointestinal hemorrhages, pale kidneys, distended stomachs, and subdermal abdominal hemorrhages, all test substance-related; no gross pathological alterations in survivors	14
Acute oral toxicity	80 Sprague-Dawley rats (8 groups of 10 each)	55% DMDM Hydantoin product formulation	Doses of 2, 3, 5, and 10 g/kg administered to the 8 groups	LD_{50} (females) = 3–5 g/kg LD_{50} (males) = 2.0–3.65 g/kg Slightly toxic formulation	15
Acute oral toxicity	10 fasted Sprague-Dawley rats	0.4% DMDM Hydantoin mascara product	Single dose of 5.16 ml/kg	No deaths	16
Acute oral toxicity	White rats (10 or more)	0.10% DMDM Hydantoin product formulation	–	LD_{50} = 5 g/kg Practically nontoxic formulation	17
Subchronic oral toxicity	180 Sprague-Dawley rats (3 groups of 60 each)	55% DMDM Hydantoin product formulation	The 3 groups received 0.1, 0.2, and 0.4 g/kg/day, respectively, for 13 consecutive weeks	Test substance did not cause any significant toxic effects	18

The authors stated that all lesions appeared to have resulted from oral administration of the DMDM Hydantoin solution. Gross pathological alterations were not observed in survivors.[14]

Doses of 2, 3, 5, and 10 g/kg of 55% DMDM Hydantoin were administered orally to four groups of male (10 per group) and four groups of female (10 per group) Sprague-Dawley rats (Table 5). The reported LD_{50}s were between 3 and 5 g/kg (males) and 2.0 and 3.65 g/kg (females).[19]

The acute oral toxicity of a mascara containing 0.4% DMDM Hydantoin was evaluated in 10 fasted Sprague-Dawley rats (5 males, 5 females), ranging in weight from 200 to 265 g. Each animal was given a single dose (5.16 ml/kg) of the product via oral intubation. Observations for mortality were made for a period of 14 days; none of the animals died during the study, and no gross lesions were observed at necropsy.[16]

The protocol outlined in Title 16 part 1500.3 (b)(6)(i)(A) of the Code of Federal Regulations[20] was used to assess the acute oral toxicity of a moisturizing lotion containing 0.10% DMDM Hydantoin. According to this protocol, a group of 10 or more laboratory white rats, each weighing between 200 and 300 g, was given a single dose of 50 mg/kg of the test substance (Table 5). An LD_{50} of 5 g/kg of body weight of the formulation was reported.[17]

Subchronic Oral Toxicity

A 55% DMDM Hydantoin solution was administered via gastric intubation to 240 Sprague-Dawley rats (6 weeks old) for 13 consecutive weeks (Table 5). Four groups of the rats (30 males, 30 females/group) received 0, 0.1, 0.2, and 0.4 g/kg/day, respectively. The animals were observed daily for mortality and signs of toxicity. During weeks 2 through 7, multiple small skin sores were observed on the head, shoulders, and neck in all treated groups. The sores healed quickly, without scarring; the etiology of these lesions was not determined. Enlarged salivary glands were observed in 2 middose (0.2 g/kg) males during the second week; the swelling had subsided by the third week. The authors stated that this condition occurs occasionally in young rats as a result of a viral infection. At gross and microscopic examination of the liver, heart, skeletal muscle, brain, and kidneys, no treatment-related lesions were found. It was concluded that administration of the 55% DMDM Hydantoin solution did not cause any significant toxic effects in the test animals.[18]

Acute Dermal Toxicity

The procedures outlined in Title 16 parts 1500.3 (c)(1)(ii)(c) and 1500.40 of the Code of Federal Regulations[21] were used to assess the acute dermal toxicity of a moisturizing lotion containing 0.10% DMDM Hydantoin. According to these procedures, the test substance was held in contact with either the clipped skin (5 rabbits) or clipped and abraded skin (5 rabbits) by means of an "impervious sleeve" and then removed after a 24-h period (Table 6). The LD_{50} was not achieved at a dose of 2 g/kg.[22]

Subchronic Dermal Toxicity

A dermal toxicity study of 55% DMDM Hydantoin was conducted with 12 young adult New Zealand albino rabbits (10–13 weeks old), ranging in weight from 2.46 to 3.06 kg (Table 6). One group of rabbits (3 males, 3 females) received a dose of 0.008 g/kg, and the other group (3 males, 3 females) received 0.8 g/kg. The 0.008 and 0.8 g/kg doses were applied in the form of 0.4 and 40% (w/v) aqueous solutions, respectively, to the clipped skin (2 animals per group) and clipped and abraded skin (4 animals per group) of the back. Doses were administered 5 days/week for 4 weeks (20 applications). A control group of rabbits (3 males, 3 females) received 20 dermal applications of tap water and were treated in a manner identical to that of test animals. None of the rabbits had any pharmacotoxic signs at any time during the study. All animals were killed, and tissues were subjected to gross and microscopic examinations at the end of the investigational period. Treatment-related lesions identified were limited to the skin of the test sites in the 0.8 g/kg dose group. It was concluded that the test material was practically nonirritating to the skins of rabbits receiving the 0.008 g/kg dose and mildly to moderately irritating to those receiving the 0.8 g/kg dose.[23]

Three groups of 30 New Zealand white rabbits (pregnant females) received daily doses of 0.0006, 0.0012, and 0.0024 g/kg/day (low, mid, and high doses, respectively) of 55% DMDM Hydantoin (Table 6).* A control group of rabbits (30) received deionized water. Applications were made on the dorsal shaved skin with a disposable syringe on days 7–18 of gestation. Animals were killed on day 29, and tissues were examined microscopically. Most (number not stated) of the treated rabbits had signs of dermal irritation at the site of application. Cutaneous erythema, edema, and desquamation were the most common observations. Cutaneous erythema and edema were noted on gestation days 8 and 12 in mid and high dose groups. The frequency and severity of these reactions were comparable in both groups from day 13 of gestation until the time of killing (day 29). The frequency of cutaneous erythema and edema was consistently less in the low dose group than in the other 2 groups. These reactions were not noted in the low dose group until day 13 or 14 of gestation. Cutaneous desquamation was noted in low and mid dose groups on day 19 of gestation and in the high dose group on day 16. The frequency and severity of desquamation were greater in mid and high dose groups than in the low dose group. Hyperkeratosis was observed in a few animals (number not stated) in each treatment group and occurred in a dose-related pattern on days 12 through 29 of gestation. Cutaneous fissuring, eschar formation, exfoliation, and atonia were noted in a few high dose rabbits (number not stated) during the latter half of gestation. There were no remarkable dermal changes noted for any of the control animals or for 4 low dose animals.[24]

Thirty-two New Zealand white rabbits (weight, 2.0–3.0 kg) were selected for a dermal toxicity study of 55% DMDM Hydantoin (Table 6). The animals were randomly distributed into two groups (8 males, 8 females/group), experimental and control groups, respectively. The test substance (dose = 0.0012 g/kg) was

*Teratogenic effects noted in this study are discussed in the section, Teratogenicity.

TABLE 6. Dermal Toxicity of DMDM Hydantoin Formulations

Type of study	*Animals tested*	*Test substance*	*Methodology*	*Results*	*Reference*
Acute dermal toxicity	5 rabbits	0.10% DMDM Hydantoin product formulation	Test substance applied by means of an "impervious sleeve" for 24 h	LD_{50} – >2 g/kg	22
Subchronic dermal toxicity	12 New Zealand albino rabbits (2 groups of 6 each)	55% DMDM Hydantoin product formulation as 0.22 and 22% aqueous solutions (effective DMDM Hydantoin conc. of 0.12 and 21.0%, respectively	0.008 and 0.8 g/kg of the 0.22 and 22% solutions, respectively, applied to clipped and abraded skin	The 0.008 g/kg dose was practically nonirritating; 0.8 g/kg dose was mildly to moderately irritating	23
Subchronic dermal toxicity	90 New Zealand white rabbits (3 groups of 30 each)	55% DMDM Hydantoin product formulation	The 3 groups received doses of 0.0006, 0.0012, and 0.0024 g/kg/day, respectively, for 12 days	Most of the treated rabbits had signs of dermal irritation at the site of application; erythema, edema, and desquamation were the most common observations	24

Chronic dermal toxicity	32 New Zealand white rabbits (2 groups of 6 each)	55% DMDM Hydantoin product formulation	Applied to clipped unabraded skin once a day and 5 days/week, for 28–91 days; dose, 0.0012 g	Erythema, desquamation, eschar formation, acute epidermal necrosis, and epidermal acanthosis all observed at the application site; these observations were test substance related	25
Skin irritation	Albino rabbits (minimum of 6)	Moisturizing lotion containing 0.1% DMDM Hydantoin	Administered to both abraded and intact clipped skin via patches made of surgical gauze; patches remained for 24 h	Test substance was nonirritating to abraded and intact skin	26
Skin irritation	6 New Zealand rabbits	0.4% DMDM Hydantoin mascara product	0.5 ml applied to abraded and intact skin for 5 consecutive days (8-h exposure)	Moderate irritation	27
Mucous membrane irritation	9 New Zealand white rabbits (3 groups of 3)	Liquid soap product containing 0.2% DMDM Hydantoin	Applied to vaginal mucosa at concentrations of 0.007, 0.037, and 0.066% daily for 10 days	Moderate reactions of leukocyte infiltration, edema, and vascular injection predominated	28

applied to the clipped unabraded dorsal surfaces of experimental animals once a day and 5 days/week for a period of 28–91 days. The control group was governed by the same treatment schedule, receiving 1 ml/kg of distilled water during each application. Six experimental and 6 control rabbits were killed after 28 days of treatment. The remaining 10 rabbits in each group completed the 91-day study. Interim (after 28 days) and terminal (after 91 days) gross and microscopic examinations were conducted. No pharmacological or toxicological signs were observed during the application period that were considered to be induced by the test substance. Grossly observable skin changes, seen only at the application sites of experimental animals (number of animals not specified), were noted during interim necropsies. The skin changes included erythema, desquamation, eschar formation, scattered raised areas, and rough surfaces. Microscopic skin changes included acute epidermal necrosis and acanthosis. At terminal necropsy, the following gross dermal changes were noted in the experimental group (number of animals not specified): necrosis, ulceration with eschar formation, fissures, exudation, thickening, discoloration, and edema. Microscopic lesions were present in the skin (at the application site), lips, and tongue; moderate to moderately severe epidermal acanthosis was noted in 4 male and 3 female rabbits. Experimental animals generally had a higher incidence and severity than control animals of erythema, edema, and desquamation at all intervals. The same was true for experimental animals regarding eschar formation (from week 2 to term) and exfoliation (from approximately week 6 to term). The gross and microscopic findings for the skin (at application site), lips, and tongue were considered to be test substance related.[25]

Skin Irritation

The skin irritation potential of a mascara containing 0.4% DMDM Hydantoin was evaluated in 6 New Zealand rabbits (3 males, 3 females), ranging in weight from 1.70 to 2.25 kg. Five-tenths milliliter of the product was rubbed onto abraded and intact skin sites (two sites) on the trunk of each animal during 5 consecutive days. Each site was covered with a patch made of surgical gauze. Patches were removed after an 8-h (± 30 min) contact period each day, after which sites were scored for erythema and edema. The grading scale for erythema was 0 to 4 (severe erythema to slight eschar formation) and the scale for edema was 0 to 4 (severe edema). Sites were again scored 24 h (± 30 min) later. The overall mean scores for erythema and edema were 0.86 (very slight) and 2.62 (slight to moderate), respectively. The overall dermal irritation score was 3.48 (moderate). This score was determined by summing all of the erythema and edema mean scores and dividing by 20 (the total of five 8-h and five 24-h observations for intact skin plus five 8-h and five 24-h observations for abraded skin).[27]

The protocol outlined in Title 16 parts 1500.3 (c)(4) and 1500.41 of the Code of Federal Regulations[29] was used to assess the primary irritation potential of a moisturizing lotion containing 0.10% DMDM Hydantoin. According to this protocol, the test substance (volume, 0.5 ml) was applied to both abraded and intact clipped skin of albino rabbits (a minimum of 6) via a square patch

made of surgical gauze (Table 6). The patches remained intact for 24 h, after which any reactions were evaluated. Subsequent evaluations were made 48 h after the initial ones. The moisturizing lotion was nonirritating to both abraded and intact skin.[26]

Mucous Membrane Irritation

A liquid soap product containing 0.2% DMDM Hydantoin was applied to the vaginal mucosae of New Zealand white rabbits (three groups of 3, <4 months old) at concentrations of 3.3% (low dose group), 18.5% (middose group), and 33% (high dose group), effective DMDM Hydantoin concentrations of 0.007%, 0.037%, and 0.066%, respectively (Table 6). The product control (a liquid soap product) and the vehicle control (water) were each applied to a group of rabbits (two separate groups of 3). All animals were treated for a period of 10 days, and, on day 10, representative samples of the lower, middle, and cervical vagina were excised and examined microscopically. All animals in the mid and high dose test groups had severe to very severe ulceration of the vagina. In the low dose group, moderate, severe, and very severe ulcerations were observed in 1 animal each. Moderate reactions of leukocyte infiltration, edema, and congestion predominated in all test groups. Group mean values for microscopic findings did not differ between product control and test groups but were greater in test groups than in the vehicle control group by factors of approximately 3 to 5. Mucous membrane irritation possibly may have been due to ingredients in the product other than DMDM Hydantoin.[28]

Ocular Irritation

The irritation potential of a moisturizing solution containing 0.10% DMDM Hydantoin was determined according to the procedures outlined in Title 16 parts 1500.3(c)(4) and 1500.42 of the Code of Federal Regulations[30] (Table 7). One-tenth milliliter of the test substance was placed in one eye of each of 6 albino rabbits. The untreated eyes served as controls. Grading for keratitis, iritis, and conjunctival redness was performed 24, 48, and 72 h after application. Positive reactions were not noted and it was concluded that the moisturizing lotion was nonirritating under the conditions of testing.[31]

A modification of the above test procedure was used in an ocular irritation study of 55% DMDM Hydantoin (Table 7). A 1% (w/v) solution of the test substance in distilled water (effective DMDM Hydantoin concentration, 0.55%) was applied in 0.1 ml volumes to one eye of each of 9 New Zealand white rabbits. The contralateral eye served as the control. Fifteen seconds after administration of the solution, the treated eyes of 3 rabbits were rinsed with 30 ml of tap water. Reactions in rinsed and unrinsed eyes were graded for irritation on days 1, 2, and 3 after administration of the test substance according to the method of Draize et al.[32] No signs of irritation were noted for either rinsed or unrinsed eyes.[33]

One-tenth milliliter of a 0.5% DMDM Hydantoin solution was instilled into the conjunctival sac of each of 9 young adult albino rabbits (6 males, 3 females)

TABLE 7. Ocular Toxicity of DMDM Hydantoin Formulations

Animals tested	*Test substance*	*Methodology*	*Results*	*Reference*
6 Albino rabbits	Moisturizing lotion containing 0.10% DMDM Hydantoin	0.1 ml of test substance placed in each eye; ocular reactions graded at 24, 48, and 72 h after application	Nonirritating	31
9 Albino rabbits	55% DMDM Hydantoin product formulation	0.1 ml of 0.55% aqueous solution placed in each eye; eyes of 3 rabbits rinsed after treatment; reactions in rinsed and unrinsed groups graded on days 1, 2, and 3 posttreatment	No signs of irritation in rinsed or unrinsed groups	33
9 Albino rabbits	0.5% DMDM Hydantoin product formulation	0.1 ml of test substance placed in eye; eyes of 3 rabbits rinsed after administration; rinsed and unrinsed eyes graded for irritation at 1, 2, 3, 4, and 7 days after treatment	Transient, minimal irritation in rinsed and unrinsed groups	34
9 New Zealand rabbits	0.4% DMDM Hydantoin mascara	100 mg instilled into one eye; eyes of 3 animals rinsed	No positive reactions	35

(Table 7). Thirty seconds after administration, the treated eyes of 3 animals were washed with 20 ml of deionized water. Rinsed and unrinsed eyes were graded for irritation at 1, 2, 3, 4, and 7 days after treatment. In the unrinsed group (6 animals), minimal irritation was noted for 6 and 2 rabbits 1 and 2 days after treatment, respectively. No signs of irritation were noted in this group at 3 days posttreatment or subsequently. In the rinsed group (3 animals), minimal irritation was noted in 2 rabbits only at 1 day posttreatment.[34]

The ocular irritation potential of a mascara containing 0.4% DMDM Hydantoin was evaluated in 9 New Zealand rabbits. One hundred milligrams of the test substance were instilled into one eye of each animal. The eyes of 3 animals were rinsed with 20 ml of deionized water 30 sec after instillation. Ocular reactions were scored at 1, 2, 3, 4, and 7 days postinstillation (Irritation Scale, 0–10). None of the animals had positive reactions, and the product was classified as a nonirritant.[35] In another study (same protocol), 100 mg of a different mascara product was instilled into the eyes of 9 New Zealand rabbits. None of the animals had positive reactions, and this product also was classified as a nonirritant.[36]

Teratogenicity

A 55% DMDM Hydantoin solution was administered orally via gavage to three groups of 30 (90) New Zealand white rabbits (Table 8). The three groups

TABLE 8. Teratogenicity of DMDM Hydantoin Formulations

Animals tested	*Test substance*	*Methodology*	*Results*	*Reference*
90 New Zealand rabbits (3 groups of 30 each)	55% DMDM Hydantoin product formulation	The 3 groups received oral doses of 0.150, 0.375, and 0.750 g/kg, respectively (days 6–18 of gestation)	No significant differences in control (positive and negative) and experimental groups in incidence of necropsy findings; test substance not teratogenic	37
90 New Zealand white rabbits	55% DMDM Hydantoin product formulation	The 3 groups received oral doses of 0.0006, 0.0012, 0.0024 g/kg/day, respectively (days 7–18 of gestation)	In comparison with control group, no increase in number of malformed fetuses in 3 experimental groups; test substance did not induce teratogenic effects	24

received doses of 0.150, 0.375, and 0.750 g/kg during days 6–18 of gestation. Deionized water (negative control) was administered to a control group of 30 New Zealand white rabbits at a dose of 0.750 g/kg, and 6-aminonicotinamide (positive control) was administered to another group of 30 rabbits at a dose of 2.5 mg/kg. On day 29 of gestation, necropsies of the dams and external and skeletal examinations of the fetuses were conducted. Only one test substance-related death was reported; it was in the 0.750 g/kg dose group. At postmortem examination of this animal, irritation and ulceration were observed in the stomach. There were no significant differences between control (positive and negative) and experimental groups concerning the incidence of necropsy findings. The results of external and skeletal fetal examinations were not significantly different between negative control and experimental groups. It was concluded that the 55% DMDM Hydantoin solution was not teratogenic when administered in oral doses of 0.150, 0.375, and 0.750 g/kg.[37]

The teratogenic potential of 55% DMDM Hydantoin was determined in pregnant New Zealand white rabbits. Three groups of 30 rabbits each received daily doses (undiluted) of 0.0006, 0.0012, and 0.0024 g/kg/day (low, mid, and high doses, respectively), applied to the dorsal skin on days 7–18 of gestation. A control group of 30 rabbits received deionized water (0.0024 g/kg/day). The fetuses were delivered via cesarean section and subjected to teratological evaluations. In comparison with the control group, there was no increase in the number of malformed fetuses in any of the three experimental groups. It was concluded that dermal application of the 55% DMDM Hydantoin solution did not induce teratogenic effects at doses of 0.0024 g/kg/day or less.[24]

Mutagenicity

The available mutagenic test data on DMDM Hydantoin are presented in this section. A comparison of results from mutagenic studies on DMDM Hydantoin, DM hydantoin, and formaldehyde is included.

The mutagenic potential of 55% DMDM Hydantoin was determined by means of the Ames *Salmonella*/microsome Plate Assay,[38,39] with and without metabolic activation (Table 9). A series of the in vitro assays was conducted with *Salmonella typhimurium* strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100 and *Saccharomyces cerevisiae* strain D4. Approximately 10^8 cells from an overnight culture of each indicator strain were added to the respective incubation plates, and the test solution was evaluated at concentrations ranging from 0.001 μl to 5 μl per plate. *Salmonella* cultures (with and without activation) were incubated for 48 h at 37°C. The *Saccharomyces* culture was incubated for 3–5 days at 30°C (without activation) and 37°C (with activation). The results were presented as revertants (or convertants for D4) per plate for each indicator strain used in the assay. Negative test results for the DMDM Hydantoin solution were reported in both the presence and absence of a metabolic activation system. It was concluded that the test substance was not mutagenic under the conditions of testing.[40]

A 55% DMDM Hydantoin solution was tested in the *Salmonella*/mammalian-microsome preincubation mutagenicity assay according to the procedures of Ames et al.[38] and Yahagi et al.[41] (Table 9). The following strains of *S. typhimurium* were tested with and without metabolic activation: TA-98, TA-100, TA-1535, TA-1537, and TA-1538. The test substance was solubilized and serially diluted before its use in the assay; five doses (each volume, 50 μl) were incubated with all of the strains (with and without activation) for 48 h at 37°C. The concentrations of the five doses used were not indicated. However, it was stated that a maximum concentration of 10 mg/incubation plate was acceptable, provided no toxic effects were noted at this concentration in a preliminary toxicity assay. In the mutagenicity assay, a positive test response was based on the test article causing at least a doubling in the average number of revertants per plate, accompanied by a dose response to increasing concentrations. For those cases in which the average number of revertants per plate was less than threefold, the response must have been reproducible. A positive response (3.2-fold increase in average number of revertants) was noted for strain TA-98, without metabolic activation; with metabolic activation, a 1.9-fold increase was noted. Additionally, increases of 2.2-fold and 1.7-fold were noted for strain TA-100 with and without activation, respectively.[43] In a similar study involving the same mutant strains, 55% DMDM Hydantoin was tested for its mutagenic potential (Table 9). Results of the preliminary toxicity assay indicated that the maximum dose to be tested in the mutagenicity assay was 2.0 μl/plate. The test substance did not cause a positive response in the average number of revertants per plate in any of the strains tested.[42]

A 55% DMDM Hydantoin solution was tested in the L5178Y TK+/− mouse lymphoma assay, with and without metabolic activation. The protocol was based on that of Clive and Spector[44] (Table 9). In the first mutagenicity assay, the test substance was evaluated at concentrations of 0.036, 0.023, and 0.010 μl/ml with six nonactivated cultures and 0.1 μl/ml with an activated culture. Three

TABLE 9. Mutagenicity of 55% DMDM Hydantoin Formulations*

Assay type	*Methodology*	*Results*	*Reference*
Ames *Salmonella*/microsome plate assay[38,39]	*Salmonella typhimurium* strains TA-1535, TA-1537, TA-1538, *Saccharomyces cerevisiae* strain D4. (+ S9 and − S9) Product concentration range: 0.001 to 5 μl/plate	−	40
Salmonella/mammalian-microsome preincubation mutagenicity assay[38,39,41]	*Salmonella typhimurium* strains TA-98, TA-100, TA-1535, TA-1537, and TA-1538. (+ S9 and − S9) Maximum product concentration tested: 2.0 μl/plate	−	42
Salmonella/mammalian-microsome preincubation mutagenicity assay[38,39,41]	*Salmonella typhimurium* strains TA-100, TA-1535, TA-1537, and TA-1538. (+ S9 and − S9) *Salmonella typhimurium* strain TA-98 (+ S9 and − S9)	− +	43
L5178Y TK+/− mouse lymphoma assay[44]	L5178Y mouse lymphoma cells Assay no. 1: product concentration ranges: 0.010–0.10 μl/ml (6 cultures, − S9) and 0.10–1.0 μl/ml (1 culture, + S9) 3 cultures (− S9) 3 cultures (− S9) 1 culture (+ S9) Assay no. 2: product concentration ranges: 0.006–0.060 μl/ml (7 cultures, − S9) and 0.020–0.20 μl/ml (10 cultures, + S9) 7 cultures (− S9) 6 cultures (+ S9) 4 cultures (+ S9)	 ++ − ++ ++ ++ −	45
Chromosome aberrations assay	Chinese hamster ovary cells (in vitro) Maximum product concentration tested: 0.3 μl/ml (− S9) Maximum product concentration tested: 0.9 μl/ml (+ S9)	 + +	46
Unscheduled DNA synthesis assay[47,48]	Rat hepatocyte suspensions; test article concentration range: 0.02–0.67 μl/ml	+	49
Unscheduled DNA synthesis assay	Rat hepatocyte suspension; test article concentration: 0.144 μl/ml	−	50
DNA strand breaks and crosslinks assays[51,52]	Rat testicular cellular suspensions	−	53

*Definition of abbreviations and symbols: + S9 = presence of S9 fraction in assay, − S9 = absence of S9 fraction, ++ = strongly mutagenic, + = mutagenic, − = not mutagenic.

of the nonactivated cultures, duplicates at 0.036 μl/ml and one at 0.023 μl/ml, had mutant frequencies of approximately 27.3, 21.7, and 9.0 times the average mutant frequency for the solvent controls, respectively. The activated culture had a mutant frequency of 4.5 times. The remaining cultures had mutant frequencies similar to those of solvent controls. In the second mutagenicity assay, the test substance was evaluated at concentrations of 0.045, 0.037, 0.029, and 0.021 μl/ml with seven nonactivated cultures and 0.15, 0.12, 0.097, 0.071, and 0.046 μl/ml with ten activated cultures. The seven nonactivated cultures had mutant frequencies ranging from 4.6 to 33.4 times the average mutant frequency of solvent controls. Duplicate activated cultures at each of the following doses: 0.15, 0.12, and 0.097 μl/ml, had mutant frequencies of 7.0 and 5.8, 5.2 and 3.8, and 3.0 and 2.6, respectively. The remaining cultures had mutant frequencies similar to those of solvent controls. Under the conditions of testing, the test substance caused a significant dose-dependent increase in the mutant frequency of cultures treated in both the presence and absence of metabolic activation.[45]

The mutagenic potential of 55% DMDM Hydantoin was evaluated in Chinese hamster ovary cells in vitro (Table 9). The chromosome aberrations assay was performed at eight decreasing doses from 0.3 μl/ml in nonactivated cultures and from 0.9 μl/ml in activated cultures. Details about the methodology involved in this study were not included. Cell populations treated with the test substance had a significant increase in the frequency of cells with chromosomal aberrations in both the activated and nonactivated systems in comparison with the frequency noted in the negative controls. A significant increase in the number of cells with chromosomal aberrations also was noted in cultures exposed to positive control articles.[46]

A 55% DMDM Hydantoin solution was examined for its potential to cause unscheduled DNA synthesis in primary cultures of rat hepatocytes according to a protocol derived from that of Williams[47,48] (Table 9). Doses of 0.67, 0.44, 0.30, 0.20, 0.13, 0.09, 0.06, 0.04, 0.03, and 0.02 μl/ml were chosen for the assay. Unscheduled DNA synthesis was monitored via the incorporation of ^{3}H-thymidine into hepatocyte nuclei. The test substance induced a dose-related increase in the mean net number of grains per nucleus, a positive response for the induction of unscheduled DNA synthesis.[49] In a similar study employing the same protocol, 55% DMDM Hydantoin was tested in the unscheduled DNA synthesis assay at a concentration of 0.144 μl/ml (Table 9). The test substance did not induce unscheduled DNA synthesis.[50]

The potential for 55% DMDM Hydantoin to induce strand breaks and crosslinks in testicular DNA was investigated according to modifications of procedures by Kohn et al.[51] and Bradley and Erickson[52] (Table 9). The test substance was administered via gavage to male Sprague-Dawley rats (strand breaks assay animals) in doses of 1700, 567, and 170 mg/kg (acute exposures) and 850, 283, and 85 mg/kg (repeated exposures). Doses for repeated exposures were administered daily for 5 days. Animals designated for the crosslinks assay received the intermediate and maximum doses (acute and repeated exposures) specified for strand breaks assay animals. Suspensions of testicular cells were prepared by mechanical disruption of the semineferous tubules, and cells from 3 animals per treatment group were pooled. The presence of strand breaks and crosslinks was

monitored by measurement of the elution rate of testicular DNA through 2 μm pore membrane filters. It was concluded that the test substance did not induce strand breaks or crosslinks in rat testicular DNA.[53]

Because 55% DMDM Hydantoin solution was mutagenic in the following assays: cytogenetics assay (Chinese hamster ovary cells), L5178y TK+/− mouse lymphoma assay, *Salmonella*/mammalian-microsome preincubation mutagenicity assay, and the unscheduled DNA synthesis assay, further studies were conducted to determine whether mutagenicity was due to the dimethylhydantoin or formaldehyde content of this solution. In the in vitro cytogenesis assay, DM hydantoin (no formaldehyde content) was not mutagenic; results were positive when a 37% formaldehyde solution was tested. DM hydantoin was not mutagenic in the L5178y TK+/− mouse lymphoma assay, whereas 37% formaldehyde was. In the *Salmonella*/mammalian-microsome preincubation assay (Ames assay), 37% formaldehyde was mutagenic to *S. typhimurium* strains TA-98 and TA-100. DM hydantoin was not mutagenic in this assay. Neither DM hydantoin nor formaldehyde induced unscheduled DNA synthesis.[54]

CLINICAL ASSESSMENT OF SAFETY

Skin Irritation

A moisturizing lotion containing 0.10% DMDM Hydantoin was applied to the facial skin of 53 panelists at a concentration of 100% over a period of 4 weeks (Table 10). Applications were made with closed patches according to the standard 48-h method by Fregert.[55] No skin reactions to the product were reported.[56]

A liquid soap product containing 0.11% DMDM Hydantoin was applied at a concentration of 2.5% in water (effective DMDM Hydantoin concentration, 0.003%) to 14 male and female subjects (Table 10). Individual applications were made via occlusive patches to the medial aspect of the upper arm of each subject; each patch contained 0.3 ml of the test substance. A total of five applications (5 consecutive days) per subject were made according to the repeated insult occlusive patch test by Smiles and Pollack.[57] The grading scale for signs of irritation ranged from 0 (no reaction) to 8 (erythema, dryness, and strong edema).[58] Three subjects had a grade of 1 (slight erythema or scaling), the highest score reported in the study.[59] In a similar study employing the same protocol (Table 10), a liquid soap product containing 0.20% DMDM Hydantoin was applied to 12 subjects (male and female) at a concentration of 8% in water (effective DMDM Hydantoin concentration, 0.16%); each occlusive patch contained 0.2 ml of the test substance. The grading scale for erythema ranged from 1 (slight redness) to 5 (extreme redness, edema, vesicles), and a mean erythema score of 0.30 was reported for the 12 subjects.[60] In another study (same protocol), a liquid soap product containing 0.20% DMDM Hydantoin was applied at a concentration of 8% in water to 9 subjects (male and female) (Table 10). Each occlusive patch contained 0.2 ml of the test substance. Erythema was graded on a scale of 1 (slight erythema) to 4 (severe erythema/edema), and dryness of the skin was graded on a scale of 1 (scaling) to 3 (fissures); mean scores of 0.60 and 0.53 were reported for erythema and dryness, respectively.[61]

TABLE 10. Clinical Assessment of Safety

Type of study	*No. of subjects*	*Test substance*	*Methodology*	*Results*	*Reference*
Skin irritation	53	Moisturizing lotion containing 0.10% DMDM Hydantoin	Closed patches applied according to the standard 48-h method by Fregert[55]	No skin reactions to the product noted	56
Skin irritation	14	Liquid soap product containing 0.11% DMDM Hydantoin	Test substance applied as 0.003% aqueous solution by means of occlusive patches, 5 applications over a 5-day period	3 subjects had slight erythema and scaling at the test site, the most severe reactions observed	59
Skin irritation	12	Liquid soap product containing 0.20% DMDM Hydantoin	Test substance applied as 0.016% aqueous solution by means of occlusive patches, 5 applications over a 5-day period	Mean erythema score = 0.30, scale 1–5	60
Skin irritation	9	Liquid soap product containing 0.20% DMDM Hydantoin	Test substance applied as 0.016 aqueous solution with occlusive patches, 5 applications over 5-day period	Mean scores of 0.60 and 0.53 reported for erythema and dryness, respectively; erythema scale 1–4; dryness scale 1–3	61
Skin irritation	14	4 liquid soap products, each containing 0.20% DMDM Hydantoin	Test substance applied as 0.016 aqueous solution with Duhring chamber containing occlusive patch; 5 applications made over 5-day period	Mean erythema and dryness scores of 0.56, 0.58, 0.75, and and 0.42, respectively, for	63

				the 4 products; erythema scale 0–5; dryness scale 0–3	
Skin irritation	12	Liquid soap product containing 0.20% DMDM Hydantoin	Test substance applied as 0.016 aqueous solution with Duhring chamber containing occlusive patch; 5 applications made over 5-day period	Mean erythema score = 0.88, erythema scale 1–4	64
Skin irritation	12	0.4% DMDM Hydantoin mascara	Product applied to back via 23-h closed patch for 21 consecutive days	Slight potential for very mild cumulative irritation	65
Skin irritation	10	Product formulation containing 0.5% DMDM Hydantoin	Test substance applied (no patch cover) for a total of 21 consecutive applications/subject; each application remained for 23 h	No evidence of cumulative irritation in any subjects	66
Skin irritation	10	Product formulation containing 0.5% DMDM Hydantoin	Test substance applied (no patch cover) for a total of 21 consecutive applications/subject; each application remained for 23 h	Evidence of a moderate potential for mild cumulative irritation	67
Skin irritation and sensitization	50	55% DMDM Hydantoin product formulation	Product tested at concentration of 4000 ppm in tap water in repeated insult patch test (occlusive patches)	None of the subjects had erythema or edema during the induction phase; no evidence of skin sensitization after challenge applications	15

TABLE 10. (Continued)

Type of study	*No. of subjects*	*Test substance*	*Methodology*	*Results*	*Reference*
Skin irritation and sensiti-	202	Product formulation containing 1% DMDM Hydantoin	Product tested in repeated insult patch test (occlusive patches)	2 subjects had erythema and edema during induction phase; during challenge phase, erythema and edema noted in 3 subjects; the product may have potential to produce mild sensitization	68
Skin irritation and sensitization	41	Liquid soap product containing 0.20% DMDM Hydantoin	Product tested as 0.002% aqueous solution in repeated insult patch test (occlusive patches)	On last day of induction, 20 subjects had mild erythema and 8 subjects had intense erythema; 2 subjects had mild erythema 48 h after challenge; 1 subject had mild erythema 96 h after challenge	69
Skin irritation and sensitization	23 (group 1) 18 (group 2)	2 liquid soap products, each containing 0.20% DMDM Hydantoin (products 1 and 2 tested in both groups)	Each product tested as 0.002% aqueous solution in repeated insult patch test (occlusive patches)	Mild erythema, intense erythema and intense erythema and edema noted during induction phase	70

				for each product in both groups; erythema was noted 48 and 96 h after challenge	
Skin sensitization	109	Product formulation containing 55% DMDM Hydantoin	Product tested as 0.275% aqueous solution in repeated insult patch test (occlusive patches)	No evidence of skin sensitization	79
Skin sensitization	25	Product formulation containing 0.5% DMDM Hydantoin	Product tested in repeated insult patch test (occlusive patches)	No evidence of skin sensitization	71
Skin irritation	25	0.4% DMDM Hydantoin mascara	Modification of procedure by Kligman and Epstein[72]	No evidence of sensitization	73
Skin photosensitization	25	Product formulation containing 55% DMDM Hydantoin	Product tested at concentration of 4000 ppm in tap water using occlusive patches; irradiation accomplished with UV light ranging in wavelength from 250 to 300 nm at intensity of 75 $\mu W/cm^2$	No phototoxic or photoallergic reactions noted	74
Photoallergenicity	30	Skin lotion containing 0.25% DMDM Hydantoin	Test substance applied with occlusive patches, followed by irradiation for 15 min with UV-A light dosage of approximately 4400 $\mu W/cm^2$	Only transient reactions of erythema noted; no photoallergic reactions induced	75
Photoallergenicity	30	Skin lotion containing 0.25% DMDM Hydantoin	Test substance applied with occlusive patches, followed by irradiation for 15 min with UV-A light dosage of approximately 4400 $\mu W/cm^2$	Only transient reactions of erythema noted; no photoallergic reactions induced	76

Four liquid soap products containing 0.20% DMDM Hydantoin were each applied at a concentration of 8% in water (effective DMDM Hydantoin concentration, 0.16%) to 14 subjects (male and female), all older than 17 years (Table 10). Exposures were made with a Duhring chamber according to the protocol of Frosh and Kligman[62] for a period of 5 weekdays. The chamber contained an occlusive patch moistened with 0.2 ml of the test substance and was sealed to the ventral skin of the forearm of each subject. The first application remained for 24 h, and fresh solutions were applied to the same site, remaining for 6 h daily during the next 4 days. The last four exposures were followed by 18-h nontreatment periods. The sites were examined for erythema and dryness on the Monday morning after the removal of chambers (on Friday afternoon). Erythema was graded on a scale of 0 (no reaction) to 5 (extreme erythema, vesiculation), and dryness was graded on a scale of 0 (no dryness) to 3 (fissuring). Mean erythema/dryness scores (14 subjects) were 0.56, 0.58, 0.75, and 0.42 for the four products.[63] Another liquid soap product containing 0.20% DMDM Hydantoin was applied to 12 subjects at a concentration of 8% in water according to the aforementioned protocol (Table 10). All subjects (male and female) were over 17 years of age. The grading scale for erythema ranged from 1 (slight) to 4 (fiery/edema), and a mean erythema score of 0.88 was reported for the 12 subjects.[64]

The skin irritation potential of a mascara containing 0.4% DMDM Hydantoin was evaluated in 12 male and female subjects (18–>60 years old). A closed patch containing the product (amount sufficient to cover patch) was applied to the back of each subject. Patches were removed 23 h after application, and sites were bathed immediately. Reactions were scored 1 h after patch removals. Skin irritation was not observed in any of the subjects after removal of the first patch. The product was applied to the same site for a total of 21 consecutive days. Cumulative irritation was graded on a scale ranging from 0 to 756 (primary irritation). The total irritation score (12 subjects) was 60, interpreted as evidence of a slight potential for very mild cumulative irritation under the conditions of testing.[65]

The cumulative irritation potential of a 0.5% DMDM Hydantion product formulation was evaluated in 10 subjects (9 females, 1 male) according to the procedures of Lanman[77] and Phillips et al.[78] (Table 10). The test substance was applied (no patch cover) to the paraspinal region of each subject. A total of 21 consecutive applications were made, and each remained for 23 h. Scoring for cumulative irritation was done 24 h after application of the test substance; reapplication at the original site was done immediately afterward. The authors concluded that there was no evidence of cumulative irritation in any of the subjects during the study.[66] Another product formulation containing 0.5% DMDM Hydantoin was tested in 10 subjects according to the protocol previously described (Table 10). There was evidence of a moderate potential for mild cumulative irritation under the conditions of testing.[67]

Skin Irritation and Sensitization

The skin irritation and sensitization potentials of 55% DMDM Hydantoin were evaluated in 50 human subjects by means of a repeated insult patch test (Table 10). The solution was tested at a concentration of 4000 ppm in tap water.

A series of nine occlusive patches, each containing approximately 0.05 ml of the solution, was applied to each subject at a common induction site. Patches were applied on Mondays, Wednesdays, and Thursdays throughout the induction period, remaining for 24 h. Grading for signs of irritation was done after the removal of a patch from the induction site. A 12-day nontreatment period interposed the placement of the ninth induction patch and placement of the challenge patch. The challenge patch was applied to a site adjacent to the induction site and remained for 24 h, immediately after which any sensitization reactions were noted. Subsequent observations were made 48 and 72 after patch removal to detect any delayed reactions. None of the subjects had erythema or edema during the series of induction patch applications. Also, there was no evidence of skin sensitization in any of the subjects after challenge applications of the test substance.[15]

A 1% DMDM Hydantoin solution in water was evaluated for its irritation and sensitization potentials in a total of 202 subjects (31 males, 171 females) ranging in age from 15 to 72 years, using a repeated insult patch test (Table 10). Occlusive patches, each containing approximately 0.2 ml of the test substance, were applied to the medial aspect of the arm (or between scapulae) and to the waist of each subject and removed after 24 h on Tuesdays, Thursdays, and Saturdays. Removals on Tuesdays and Thursdays were followed by 24 h nontreatment periods; Saturday removals were followed by 48 h nontreatment periods. Observations for signs of irritation were made at the termination of each nontreatment period. This procedure was repeated until 10 induction exposures had been made. Challenge patches were applied to the original contact sites and to adjacent sites 10 to 21 days after application of the last induction patch. Observations for sensitization reactions were made 24 and 48 h after application. Two subjects had erythema and edema during the induction phase. During the challenge phase, erythema and edema were noted in 3 subjects. One subject had these reactions at the original site at 24 and 48 h after application of the challenge patch. The reactions were observed at the original site at 24 and 48 h and at the adjacent site at 48 h in another subject. The other subject had erythema and edema at the adjacent site at 24 h. According to the authors, the product may have the potential to produce a mild sensitization in a small number of subjects tested with occlusive patches. In their estimation, actual use conditions would, in all probability, induce sensitization in a smaller percentage of the population.[68]

A liquid soap product containing 0.20% DMDM Hydantoin was applied at a concentration of 1% in water (effective DMDM Hydantoin concentration, 0.002%) to 41 male and female subjects, all older than 17 years; each occlusive patch contained 0.2 ml of the test substance (Table 10). A patch was applied to the arm of each panelist on Mondays, Wednesdays, and Fridays during a 3-week induction period. Each induction patch was removed after 24 h, and the sites were then graded for signs of irritation. After a 2-week nontreatment period, duplicate challenge patches were applied to the original site and an alternate site, respectively, and were removed after 24 h. Test sites were graded for signs of irritation after removal of each induction patch and 48 and 96 h after application of the challenge patch. Mild erythema was first observed in subjects on Wednesday during the first week of induction, and observations continued

throughout the 3-week induction period. The first observations involved 4 subjects, compared to 20 with mild erythema on the last day of induction. Intense erythema was first observed on Wednesday during the second week of induction in 1 subject; 8 subjects had this reaction on the last day of induction. Intense erythema and edema were observed in 4 subjects only on the last day of induction. Forty-eight hours after application of the challenge patches, 2 subjects had mild erythema at the original and alternate sites, and 1 subject had intense erythema at the original site. Ninety-six hours after challenge applications, 1 subject had mild erythema at the original and alternate sites.[69]

In another study, the sensitization and irritation potential of two liquid soap products (product 1, product 2), each containing 0.20% DMDM Hydantoin, was evaluated in subjects at a concentration of 1.0% according to the same protocol (Table 10). One experimental group consisted of 23 subjects (male and female) and the other of 18 subjects (male and female); all subjects were more than 17 years of age. Two occlusive patches were applied to each subject per group during each day of the induction phase and during the challenge applications. One patch contained 0.2 ml of product 1, and the other contained 0.2 ml of product 2. On the last day of induction, subjects in group 1 treated with product 1 had mild erythema (8 subjects), intense erythema (7 subjects), and intense erythema and edema (2 subjects); treatment with product 2 on the last day induced erythema (6 subjects), intense erythema (10 subjects), and intense erythema and edema (2 subjects). The two products did not induce signs of irritation on the first day of the 3-week induction period in any of the group 1 subjects. Forty-eight hours after challenge applications of product 1 to group 1 subjects, mild erythema (1 subject) and intense erythema (1 subject) were noted at the original site. In the same group, mild erythema was noted in 2 subjects (original site) and in 2 subjects (alternate site) at 48 h after challenge with product 2; mild erythema was also observed at 96 h in 2 subjects (alternate site) and in 1 subject (alternate and original sites). Results for subjects in group 2 exposed to both products were similar to those for subjects in group 1, the exception being that no reactions were noted in group 2 subjects 96 h after challenge applications.[70]

Skin Sensitization

The skin sensitization potential of 55% DMDM Hydantoin was determined in 109 subjects (18 males, 91 females), ranging in age from 18 to 65 years, by means of a repeated insult patch test (Table 10). Each occlusive patch contained 0.3 ml of a 0.5% w/v aqueous solution of the test substance (effective concentration of DMDM Hydantoin, 0.275%). Induction and challenge patches remained in contact with the skin for 24 h. Induction applications were made at the same site, and an alternate site was chosen for the challenge applications. Further details concerning the protocol were not included. There was no evidence of skin sensitization in any of the treated subjects.[79]

The skin sensitization potential of a solution containing 0.5% DMDM Hydantoin was evaluated in 25 adults according to the protocol of Kligman and Epstein[72] (Table 10). Occlusive patches, each containing 0.3 g of the test sub-

stance, were applied to the volar aspect of the forearm and remained for 48 h. Each application of the test substance was preceded by the application of an occlusive patch containing a 2% aqueous solution of sodium lauryl sulfate (SLS); the SLS patch remained for 24 h. The preceding sequence was repeated for a total of five exposure periods. After a 10-day nontreatment period, an occlusive patch containing a 10% aqueous solution of SLS was applied to the challenge site (new test site) and removed 1 h later. A challenge patch containing the test substance was then immediately applied and remained for 48 h. Observations for sensitization reactions were made immediately after challenge patch removal and 24 h thereafter. The test substance did not induce sensitization reactions.[71]

The sensitization potential of a mascara containing 0.4% DMDM Hydantoin was evaluated in 25 female subjects (17–69 years old) according to a modification of the procedure by Kligman (1966). At the beginning of induction, 1.0 ml of 5% aqueous SLS was applied to the forearm of each subject via an open patch; patches were removed after 24 h of contact. An open patch containing the test substance was applied to the same site on each subject and removed after 48 h. Sites were graded after patch removals according to the scale 0 to 4 (erythema and vesiculation). The challenge phase was initiated following an 11-day nontreatment period. An open patch containing SLS was first applied to each subject and removed 1 h later. After a 48-h nontreatment period, the product was applied (closed patch) during 3 consecutive days: 15-min exposure (first day), 24-h exposure (second day), and 48-h exposure (third day). Sites were scored according to the same grading scale after each challenge. During induction, reactions to the test substance ranged from none to erythema and papules. The only reaction reported during the challenge phase was erythema (1 subject). Since reactions to the product during the challenge phase were not any worse than those observed during induction, the authors stated that the reactions should be classified as hyperreactive rather than sensitization.[73]

Skin Photosensitization

A 55% DMDM Hydantoin solution was tested for its photosensitization potential at a concentration of 4000 ppm (in tap water) in a study involving 25 human subjects (12 males, 13 females) (Table 10). The protocol used was that of Curwen and Jillson.[80] Before the photosensitization test, the minimal erythema dose (MED) was determined for each subject by irradiating separate sites on the back for graded exposure periods of 2 to 10 sec. The MED is defined in this study as that period of irradiation on normal skin required for the production of a faintly perceptible erythema 24 h following irradiation. Irradiation was accomplished with ultraviolet light ranging in wavelength from 250 to 300 nm at an intensity of 75 $\mu W/cm^2$. The lamp was positioned at a distance of 3 inches from the back of each subject. A 2-week induction period was initiated after MEDs had been determined and comprised the application of three occlusive patches to the back of each subject on days Monday through Friday per week. Each patch contained approximately 0.3 ml of the test substance. Patches applied on Mondays, Wednesdays, and Fridays remained for 24 h, whereas those applied

on Tuesdays and Thursdays remained for 30 min. The test sites were cleansed and then exposed to twice the MED of ultraviolet light. The induction period was followed by a 14-day nontreatment period, after which the sensitization phase was begun. During the first day of the 4-day test period (sensitization phase), three occlusive patches, each containing approximately 0.3 ml of the test substance, were applied to the three sites on each subject treated during the induction phase. Two of the patches were removed after a 24-h contact period, and one of the sites was irradiated for 0.5 sec less than the observed MED. The other site was irradiated with the delayed erythema dose (DED), defined as 8 times the MED of ultraviolet light. The third patch site served as the control. A sharply demarcated erythema at the MED irradiated site, usually more marked at 24 h after irradiation, denoted a positive phototoxic reaction. An eczematous or papular response at the DED irradiated site, appearing 48 h after irradiation, denoted a positive photoallergic reaction. The test substance did not induce phototoxic or photoallergic reactions in any of the subjects during the study.[74]

Photoallergenicity

The photoallergic potential of a skin lotion containing 0.25% DMDM Hydantoin was evaluated in 30 subjects (5 males, 25 females) ranging in age from 19 to 63 years (Table 10). Each occlusive patch, containing approximately 0.2 ml of the test substance, was applied to the medial aspect of the forearm and removed after 24 h. The sites were then graded for signs of irritation, and those not designated for irradiation served as controls. The sites designated for irradiation were exposed for 15 min at a distance of 10 cm with a UV-A light dosage of approximately 4400 $\mu W/cm^2$. Exposures were made on Mondays, Wednesdays, and Thursdays until a total of 10 applications/irradiations were completed. The test substance was reapplied to the original site throughout the induction period, provided there were no signs of irritation. After a 10- to 14-day nontreatment period, the challenge patch was applied to a site adjacent to the original one on each subject. Challenge sites were then examined for dermal reactions and subsequently irradiated. Examinations were conducted 40 and 72 h after application of the challenge patches. The test substance did not induce photoallergic reactions, and only slight transient reactions of erythema were noted during the study.[75] Identical results were reported in a similar study (Table 10) of a skin lotion containing 0.25% DMDM Hydantoin tested according to the aforementioned protocol.[76]

SUMMARY

DMDM Hydantoin (white crystalline solid) is a formaldehyde donor containing up to 2% of the free aldehyde in equilibrium with the hydantoin. It is produced by reacting 3 to 5 moles of formaldehyde (as the 37% by weight aqueous solution) with 1 mole of dimethyl hydantoin at 84°C and is stable over a wide range of pH and temperature conditions.

DMDM Hydantoin is a cosmetic preservative, present in approximately 115 product formulations at concentrations ranging from ≤0.1% to 1%. These products are applied to the skin and hair and may come in contact with the eyes, nasal mucosa, and other parts of the body. Noncosmetic uses of hydantoins include herbicides, polymers, and antiarrhythmic and anticonvulsant agents.

When ^{14}C-DMDM Hydantoin was administered to the middorsal area of Sprague-Dawley rats, more than 98% of the recovered radioactivity was confined to the dose sites after 72 h. The higher counts of remaining radioactivity were reported for the gastrointestinal tract, liver, and bone marrow. Radioactivity in the urine decreased approximately by a factor of 6 during the 72-h period after administration; radioactivity in the feces remained approximately constant and significantly lower than that of the urine.

Results from acute inhalation studies involving Sprague-Dawley rats indicated no alterations associated with the administration of formulations containing DMDM Hydantoin.

In acute oral toxicity studies in which DMDM Hydantoin formulations were administered to albino and Sprague-Dawley rats, LD_{50}s ranged from 2 g/kg to >5.16 ml/kg. No significant toxic effects were noted in Sprague-Dawley rats in a subchronic oral toxicity study.

An LD_{50} of greater than 2 g/kg was reported for a DMDM Hydantoin formulation in an acute dermal toxicity study involving 5 rabbits. Results from subchronic dermal toxicity studies using aqueous solutions of DMDM Hydantoin formulations indicated varying degrees of irritation at the application sites in New Zealand albino rabbits; in 1 study, erythema, edema, and desquamation were the most common observations. In a chronic dermal toxicity study, observations included erythema, desquamation, eschar formation, epidermal acanthosis, and acute necrotizing dermatitis, all at the application sites, in New Zealand albino rabbits that received applications of a DMDM Hydantoin formulation.

In a skin irritation study involving albino rabbits, a moisturizing lotion containing DMDM Hydantoin was nonirritating. In another study, a mascara containing DMDM Hydantoin induced moderate skin irritation in New Zealand rabbits. Moderate reactions of leukocyte infiltration and edema were noted in New Zealand white rabbits during a mucous membrane irritation study of a DMDM Hydantoin liquid soap product.

Transient minimal irritation was noted in albino rabbits treated with DMDM Hydantoin formulations in ocular irritation studies.

Test substance-related teratogenic effects were not demonstrated in litters during studies in which pregnant New Zealand white rabbits received oral doses of DMDM Hydantoin formulations.

In two studies, 55% DMDM Hydantoin formulations were not mutagenic in *Salmonella*/microsome assays, whereas, in another study, mutagenicity was demonstrated in strain TA-98. Both positive and negative mutagenic activities were noted when 55% DMDM Hydantoin was tested in the L5178y TK+/− mouse lymphoma assay. The formulation was also mutagenic in the chromosome aberrations and unscheduled DNA synthesis assays. In two other studies, 55% DMDM Hydantoin formulations were not mutagenic in unscheduled DNA synthesis and DNA strand breaks/crosslinks assays.

In clinical studies, skin irritation ranged from none to observations of intense erythema and edema when various formulations containing DMDM Hydantoin were applied. The more severe reactions were probably caused by substances other than DMDM Hydantoin in the formulations. DMDM Hydantoin formulations did not induce sensitization in some of the studies. In other sensitization studies, erythema was noted in subjects subsequent to challenge applications; whether or not these were regarded as sensitization reactions was not indicated. Results from photosensitization and photoallergenicity studies involving DMDM Hydantoin formulations indicated no phototoxic or photoallergic reactions in any of the subjects.

DISCUSSION

DMDM Hydantoin is a formaldehyde donor in aqueous media. A comparison of Ames test results from studies of a 55% DMDM Hydantoin product and formaldehyde indicates a similar number of revertants per formaldehyde equivalent. Furthermore, positive Ames test results were obtained for both substances with *Salmonella* strain TA-98 in these studies. Because of similar mutagenic potencies and the observation of positive results in the same bacterial strain, it is probable that the mutagenic activity of the product is attributable to formaldehyde release. This probability is further supported by comparable mutagenic potencies of formaldehyde and a 55% DMDM Hydantoin product in the mouse lymphoma assay and positive results for the two in the chromosome aberrations assay. The possibility that preparations may contain, in addition to formaldehyde, other genotoxic agents has not been ruled out.

Clinical studies revealed some observations of skin irritation subsequent to induction and challenge applications of DMDM Hydantoin formulations. Authors have suggested that such clinical findings are related to the release of formaldehyde from DMDM Hydantoin. The CIR Expert Panel has previously reviewed the safety of formaldehyde in cosmetic products and concluded*:

> Formaldehyde in cosmetic products is safe to the great majority of consumers. The Panel believes that because of skin sensitivity of some individuals to this agent, the formulation and manufacture of a cosmetic product should be such as to ensure use at the minimal effective concentration of formaldehyde, not to exceed 0.2% measured as free formaldehyde. It cannot be concluded that formaldehyde is safe in cosmetic products intended to be aerosolized.

Use of DMDM Hydantoin at its current concentration of use in cosmetic products would not expose the consumer to concentrations of formaldehyde above the limit previously stated.

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*Final report on the safety assessment of Formaldehyde (1984). J. Am. Coll. Toxicol. **3**(3), 157–79.

CONCLUSION

Based on the available data included in this report, the Expert Panel concludes that DMDM Hydantoin is safe as a cosmetic ingredient in the present practices of use.

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