BUFF

RE-REVIEWS

Glutaral

HC Red No. 1

CIR EXPERT PANEL MEETING
JUNE 27-28, 2011
Memorandum

To: CIR Expert Panel Members and Liaisons
From: Christina L. Burnett
Scientific Writer/Analyst
Date: May 17, 2011
Subject: Re-review of Glutaral

In 1996, the CIR Final Report on the safety of glutaral was published with the conclusion that this ingredient is “safe for use at concentrations up to 0.5% in rinse-off products. There is insufficient data to determine the safety of glutaral in leave-on products. Glutaral should not be used in aerosolized products.” A copy of the Final Report is included with this re-review.

Current uses of glutaral can be found in Table 2. The number of uses for glutaral has decreased from 60 to 13, and an industry survey reported that glutaral is currently being used at a concentration of $6 \times 10^{-6} \%$. Most of the reported uses are in non-coloring hair products.

In the discussion of the original report, the Panel sought a 2-year dermal carcinogenicity study from NTP. Such a study has not been published, but a 2-year inhalation study has. Numerous other studies have been published since the original report was issued, with a good majority focusing on the health effects from occupational exposure to glutaral. The pertinent studies have been captured in this re-review.

The task for the Panel at this meeting is to determine whether the conclusion on glutaral is still valid. If it is not, an amendment should be initiated. If the conclusion is still valid, the Panel may reaffirm the original conclusion.
Glutaral History

**Original Report:** In 1996, the Expert Panel determined that glutaral was safe for use at concentrations up to 0.5% in rinse-off products. At the time, there was insufficient data to determine the safety of glutaral in leave-on products. The Panel advised that glutaral not be used in aerosolized products.

**June 2011:** the RR of glutaral was presented to the Panel.
### Search Strategy for Glutaral

March 31, 2011: SCIFINDER search for CAS No. 111-30-8

Limited search to references published since 1994; 13,723 references came back.
Refined search to books, journals, preprints, reports, and reviews; 8741 references came back.
Further refined search to include the term “toxicity”; 506 references came back.
Further refined search to remove the term “cross link”; 359 references came back.

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**89 References Ordered/Downloaded Total**
Ms. Fise stated her preference for a conclusion on Ethyl Methacrylate that is very explicit. Specifically, it should be stated in the conclusion that this ingredient is safe for use only in products that are sold to and applied by trained professionals.

With the exception of one abstention (Dr. Belsito), the Panel approved the following conclusion on Ethyl Methacrylate: Based on the available data on the formulation of nail products containing Ethyl Methacrylate, the CIR Expert Panel concludes that this ingredient is safe as used by trained individuals. Skin contact should be avoided because of the sensitizing potential of Ethyl Methacrylate.

Dr. Bergfeld noted that the report on Ethyl Methacrylate would be submitted to the Panel for a mail review. The Panel needs to review the report discussion, the substance of which was mentioned during the Panel’s deliberations.

GLUTARAL

Dr. Belsito noted that there are two studies concerning airway sensitization, not included with the inhalation toxicity studies on Glutaral that had been reviewed by the Panel, that should be included in the CIR Tentative Report. Dr. Belsito’s Team recommended that these studies should be referred to in the report discussion. Based on COLIPA’s summary of these studies, Glutaral should not be used in aerosolized products. Furthermore, the discussion should be revised to include the 1% no effect level for Glutaral in skin irritation studies involving rabbits. Dr. Belsito’s Team established concentration limits of 0.5% for Glutaral in leave-on products and 0.1% for products that are used around the eye.

Dr. Belsito also noted that the UV spectral analysis of Glutaral was done using
UVB light, which did not represent the wavelength of light that was used in photosensitization studies. However, photosensitization was not viewed as a problem by his Team, based on the chemistry of Glutaral and historical data. Initial concerns about the large granular lymphocytic leukemia noted in rats were alleviated, based on the fact that the incidence of leukemia fell within the range of historical controls and on the poor dermal absorption of Glutaral.

Dr. Schroeter said that his Team concluded that Glutaral is safe for use in rinse-off products only at concentrations up to 0.5%, having taken into consideration the large granulocytic leukemia noted in female rats. Furthermore, a two-year dermal carcinogenicity study is needed in order to evaluate the safety of Glutaral in leave-on products.

Dr. Slaga noted that if one considers the reactivity of Glutaral, that there are more positive mutagenicity studies than negative in the CIR report, and that there appears to be a trend with respect to the observation of large granulocytic leukemia in at least one study, then there is reason for concern about the carcinogenic potential of Glutaral.

Dr. Carlton asserted that, very commonly, the incidence of large granulocytic leukemia increases under a number of circumstances and in response to a number of different types of chemicals. Furthermore, in his opinion, most individuals don't take much credence in variations in the incidence of mononuclear cell leukemia; it is a high incidence background and highly variable from one study to another.

Dr. Shank noted that the Panel had reviewed a battery of mutagenicity tests on Glutaral, many of which are positive, and that these results should not be overlooked.
Furthermore, he said that use of Glutaral in rinse-off products is not a concern, however, it cannot be concluded that the available data support the safety of this ingredient in leave-on products.

Similarly, Dr. Slaga said that because Glutaral does not penetrate the skin very easily, its use in rinse-off products may not be considered problematic. However, for leave-on products, the probability of skin penetration would be increased over longer periods of contact.

Dr. Klaassen noted that even though there was no dose response, there was a statistically significant increase in the incidence of large granulocytic leukemia in female rats, and this cannot be overlooked. He also noted that this is one of the more variable leukemias that one sees, and that NTP is experiencing tremendous problems; rats aren't surviving two-year studies because of the occurrence of this very lethal tumor. In fact, the incidence is increasing dramatically in this country for reasons that are yet to be understood. Dr. Klaassen also expressed concern over the carcinogenic potential of Glutaral, given the reactivity of this chemical and its mutagenic effects.

Dr. McEwen said that given the data that are available on Glutaral and large granulocytic leukemia and potential genotoxic effects of this ingredient, there is still an adequate margin of safety that would support declaring Glutaral safe as used in leave-on products. Furthermore, if a quantitative risk assessment were done, an acceptable level of risk would be determined.

Dr. Bailey noted that a safety factor of 70 or 80 x exposure doesn't yield a lot of comfort. Normally, one would think in terms of a safety factor of at least 100 x. Dr. Bailey also said that given the reactivity of Glutaral, the fact that positive mutagenicity
data exist, etc., this ingredient may be a potential human carcinogen under certain conditions.

Dr. Shank said that if the oral carcinogenicity study were negative, he would tend to agree with Dr. McEwen's assessment. However, the oral carcinogenicity study was not negative; difficulty was experienced in interpreting these data. He also noted that Glutaral is highly reactive and that mutagenicity data were positive in several, but not all, of the test systems. Furthermore, Glutaral was poorly absorbed in in vitro studies, and was not poorly absorbed in in vivo studies. Dr. Shank concluded that, in his opinion, these data do not support the safety of an ingredient in leave-on products.

The Panel concluded that Glutaral is safe as used in rinse-off products and that there is insufficient data to confirm its safety in leave-on products. With the exception of Dr. Carlton, all Panel members voted in favor of this conclusion.

Dr. Bergfeld stated that the report discussion would have to be revised such that the Panel's concerns regarding the absorption, reactivity, and genotoxicity of Glutaral, and the need for an NTP 2-year dermal carcinogenicity study on this ingredient are detailed.

Dr. Carlton noted that it should be stated in the discussion that the 2-year dermal carcinogenicity study should not be conducted using Fisher-344 rats.

**DISPERSE BLUE 1**

Dr. Schroeter noted that Disperse Blue 1 is used as a hair colorant in hair dyes, and that cutaneous absorption data and any assessment of carcinogenic potential were requested. Furthermore, it was determined that if these data are not considered
**Quaternium-22**

Dr. Bergfeld noted that comments on this ingredient were not received during the 90-day comment period for the Tentative Report. The Panel unanimously approved the issuance of a Final Report on Quaternium-22.

**Stearyl Heptanoate**

Dr. Bergfeld noted that the Panel now has confirmation of the concentration range for use of this ingredient in cosmetics, and that this information has been added to the discussion section of the report. Frequency of use information on Stearyl Heptanoate has also been updated and incorporated. Additionally, Dr. Bergfeld noted that comments were not received during the 90-day comment period for the Tentative Report.

The Panel unanimously approved the issuance of a Final Report on Stearyl Heptanoate with the editorial changes that were requested.

**Glutaral**

Dr. Bergfeld noted that the NTP inhalation studies that were completed recently, as well as a few other studies (from Union Carbide), have been added to the report on Glutaral.

Dr. Andersen added that the Panel reviewed the additional data that were received from Union Carbide. The data submission, received as a comment to the Tentative Report on Glutaral, is actually additional confirmatory data of the same type.
that are already included in the report. He also noted that there is ample precedent
that comments received during the final 90-day comment period in the review process
can be incorporated into the Tentative Report, which can then become a Final Report,
provided that the comments incorporated do not change the conclusion.

The Panel unanimously approved the issuance of a Final Report on Glutaral.

**CETRIMONIUM CHLORIDE**

Dr. Schroeter said that during the Panel’s discussion of the Tentative Report on
Cetrimonium Chloride, the concentration limit for this ingredient in leave-on products
was changed from 0.5% to 0.25%. So, this is the concentration limit (0.25%) that
should be stated in the conclusion.

Dr. Andersen noted that the minutes clearly capture that the Panel approved a
change in the concentration limit from 0.5% to 0.1%, with the understanding that this
change represents the correction of a calculation error in determining the actual test
concentration. The 0.1% concentration limit is based on the results of clinical skin
irritation and sensitization tests.

Dr. Belsito recalled that the Panel’s 0.1% concentration limit for leave-on products
was based on negative results in an ocular irritation study. Furthermore, the
concentration limit was based on ocular irritation data because Cetrimonium Chloride
is used in many hair grooming products.

After reviewing minutes on the Panel’s previous deliberations on Cetrimonium
Chloride, Dr. Andersen acknowledged that the 0.1% concentration limit for leave-on
products was, in fact, based on ocular irritation data, not clinical skin irritation and
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INTRODUCTION

Glutaral, the International Nomenclature of Cosmetic Ingredients (INCI) name for glutaraldehyde when used in cosmetic formulations, has previously been reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel. In 1996, the safety assessment was published with the conclusion that this ingredient is “safe for use at concentrations up to 0.5% in rinse-off products. There is insufficient data to determine the safety of glutaral in leave-on products. Glutaral should not be used in aerosolized products.”

Since the original review, numerous additional published studies on inhalation effects, genotoxicity, and sensitization have become available. These are included in this re-review document. Additionally, several other reviews of the effects of glutaral on human health have been published. These reviews have incorporated data from much of the same studies that the original report and this re-review have incorporated, and their conclusions focus mostly on the health effects from occupational exposure.

CHEMISTRY

DEFINITION AND STRUCTURE

Glutaral (CAS No. 111-30-8) is a 5-carbon dialdehyde. The molecular formula is C₅H₈O₂. The structure is shown in Figure 1. Synonyms and trade names are listed in Table 1.

PHYSICAL AND CHEMICAL PROPERTIES

Physical and chemical properties of glutaral can be found in the original safety assessment of glutaral.

USE

Cosmetic

Table 2 presents the historical and current product formulation data for glutaral. According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP), glutaral was used in a total of 60 non-coloring hair formulations at the time of the first safety assessment. An industry survey reported use concentrations of ≤ 1%. Currently, VCRP data indicate that glutaral is used in 13 cosmetic formulations, about half of which are in non-coloring hair formulations. In a survey of current use concentrations conducted by the Personal Care Products Council, glutaral is used at a concentration of 6 x 10^-6% in non-coloring hair products.

The European Commission has listed glutaral as an approved preservative in cosmetics at a maximum concentration of 0.1% and with the restriction that is should not be used in aerosols.

Non Cosmetic

Glutaral is a FDA class I medical device. The FDA has cleared its use as a high level disinfectant.

Numerous studies have been published regarding the use of cross-linked glutaral in several biomedical applications, including skin grafts, drug delivery systems, and bovine and porcine heart valves.

TOXICOLOGICAL STUDIES

Acute Dose Toxicity

Oral – Non-Human

The acute toxicity of acidic unbuffered glutaral (pH 3.1-4.5) and alkaline buffered glutaral (pH 7.8-8.2) in male and female Sprague Dawley albino rats were compared. The rats (5 males and 5 females in each dose group) received 2.2% glutaral at dosages of 2, 2.83, or 4 g/kg in unbuffered solution or 1, 2, 4, or 8 g/kg in buffered solution via gastric intubation. The animals were observed twice daily for toxic effects for 14 days and then killed for necropsy. The animals were weighed on the day of dosing and at days 7 and 14 post-treatment. In the unbuffered dose groups, signs of toxicity included
sluggishness, lacrimation, piloerection, slow breathing, pale extremities, diarrhea, perinasal and periocular red encrustation, and urine stain. Trace to moderate hematuria was observed in a few rats. Survivors recovered 2-7 days post-treatment. The animals in the buffered dose groups had similar clinical signs with the addition of slow and/or labored breathing and prostration. Survivors recovered in 4-7 days. In animals that died from both solution types, necropsy revealed red to bright red lungs, mottled dark maroon livers, red streaking of the glandular portion of the stomachs, red to dark red small intestines, and blood in the urine. A few rats in the unbuffered group had visceral congestion or hemorrhage. The LD$_{50}$ of the unbuffered glutaral solution was 3.45 g/kg while the LD$_{50}$ of the buffered glutaral solution was 4.16.

In an acute oral study, the LD$_{50}$ values for concentrations of glutaral ranging from 5-50% were 0.88-3.25 ml/kg in Hilltop-Wistar rats.$^{17}$

**Percutaneous – Non-Human**

In the study of buffered and unbuffered glutaral solutions, New Zealand white rabbits (5 of each sex) received a single undiluted dosage of 16.0 g/kg unbuffered or buffered glutaral on dorsal trunk skin.$^{16}$ The application site was then occluded for 24 h. The animals were observed twice daily for toxic effects for 14 days and then killed for necropsy. The animals were weighed on the day of dosing and at days 7 and 14 post-treatment. No animals died as a result of the treatment. No clinical signs were observed in the unbuffered group. Five animals (sex not specified) in the buffered group had unsteady gait, sluggishness, and rapid breathing 30 min to 3 h after treatment. The animals recovered in 1-4 days. Mean body weights were slightly reduced on day 7 in the males of the unbuffered group. In the unbuffered group, erythema, edema, ecchymoses, and a few possible necrosis were observed on day 1; punctate necrosis, erythema, and desquamation were observed on day 7; and punctate necrosis, desquamation, alopecia, and scab formation was observed on day 14. The buffered group had less marked reactions with erythema and edema on day 1, desquamation on day 7, and desquamation and alopecia on day 14. One female of the buffered group had punctate necrosis on day 1. Necropsy showed bright red lungs in 3 animals (sex not specified) of the unbuffered group and 1 animal in the buffered group had red consolidated areas in the lung.

In an acute percutaneous study in New Zealand white rabbits, the LD$_{50}$ values for 45%-50% glutaral were 1.59-2.71 ml/kg.$^{17}$ Reactions observed included erythema, edema, necrosis, scab formation, and desquamation that lasted from 2-14 days.

**Inhalation – Non-Human**

In Swiss OF1 male mice, the level of a 60 min exposure to glutaral which led to a 50% decrease in respiratory rate (RD$_{50}$) was calculated to be 2.6 ppm.$^{18}$

Groups of 4 male ND4 Swiss Webster mice were exposed to glutaral vapor concentrations ranging from 1.60-36.7 ppm.$^{19}$ The mice were exposed head-only for 30 min and then allowed to recover for 7 days. Animals were observed for signs of toxic effects during and after the exposure. Body weights were measured prior to treatment and 7 days post-treatment. No clinical signs of toxicity were observed during treatment. After treatment, 1 mouse in the 1.6 ppm group had corneal opacity in the left eye that lasted 7 days. All but the 4.65 ppm group had increased mean body weight. No body weight gain was measured in this group. Decreased respiratory rates were observed almost immediately on exposure to glutaral vapor and leveled off within 5-10 minutes, lasting throughout the exposure period. Respiratory rates increased again after treatment ceased, but did not return to control values. The RD$_{50}$ was calculated to be 13.86 ppm. The authors concluded that glutaral is a moderately potent peripheral sensory irritant.

In an inhalation study with unbuffered and buffered glutaral (2.2%), 5 male and 5 female Sprague Dawley albino rats were exposed to glutaral vapor for 6 h.$^{16}$ The animals were observed twice daily for toxic effects for 14 days and then killed for necropsy. The animals were weighed on the day of dosing and at days 7 and 14 post-treatment. No mortalities or
clinical signs of toxicity were observed during the study. Mean body weights increased in the buffered group and the unbuffered group males, but the unbuffered group females did not gain weight. No gross lesions were observed at necropsy.

In another acute inhalation study, Wistar rats exposed to 3.0-48.1 ppm glutaral for 4-8 h at ambient temperatures (17-25°C) had transient peripheral sensory irritant effects to the eyes and respiratory tract. In Fischer rats, however, exposure to 10.6-42.7 ppm glutaral for 4 h at temperatures of 60-65°C caused severe toxic effects, including mouth and audible breathing, excess lacrimation, pericircular and peroral wetness, peroral encrustation, excess salivation, slow righting reflex, and mortality (LC50 range at 4 h was 23.5-40.1 ppm). Rats that died during the study had dose-dependent acute inflammation and necrosis in the nasal mucosa, larynx, trachea, and bronchi.

Repeted Dose Toxicity

Oral – Non-Human

The potential for esophageal injury from glutaral in male Sprague-Dawley rats was investigated. A total of rats were divided into 5 groups which each consisted of one control animals and 1 treated animal. The rats received either 1 ml of 3.2% glutaral or 1 ml of normal saline via daily gavage of the esophagus. Groups 1-4 were treated 1, 2, 3, or 4 days before they were killed, which was 10 h after the final gavage. Group 5 was treated for 4 days and then killed one week later. The esophagus of each rat was removed and fixed for histologic exam. The number of neutrophils in the submucosa of the gastroesophageal junction was quantified.

The treated rat in Group 5 died within 36 h of the final gavage. The rat was found to have extensive small bowel necrosis. In controls rats of Groups 1, 3, 4, and 5, mild neutrophilic infiltrates in the submucosa of the gastroesophageal junction were observed, while the treated rats in Groups 1, 2, and 4 all had marked neutrophilic infiltrates. The control rat in Group 2 had moderate neutrophilic infiltrate, which was likely due to trauma from the gavage. The treated rat in Group 3 had mild neutrophilic infiltrates. The treated rats in Groups 2-4 had segmental areas of vascular congestion and necrosis, which indicated vasculitis. The treated rat in Group 2 had severe inflammation in the mucus-secreting gland in the upper esophagus. Myositis and myonecrosis were observed in treated rats in Groups 2-4, which was also observed in the control rats of these groups. Muscle regeneration was also observed in the rat from Group 4. It was felt that because myositis and muscle regeneration was observed in both the controls and treated animals, the gavage itself may have caused trauma to the esophagus. The authors concluded that glutaral has a toxic effect on the rat esophagus.

Inhalation – Non-Human

In a nasal and pulmonary toxicity study of glutaral, groups of 10 male Swiss OF1 mice were exposed to glutaral vapors at concentrations of 0, 0.3, 1.0, or 2.6 ppm for 6 h/day, 5 days/week for 4, 9, or 14 days. The mice were killed at the end of the exposure period and underwent histopathological investigation. Another group of mice was exposed to 1.0 ppm for 14 days and killed after 1, 2, or 4 weeks of recovery. In the 2.6 ppm group, some mice that were dying on the third day and surviving mice that were showing signs of high toxicity (respiratory difficulty including gasping, lung rales, and mouth breathing) were killed after 5 days. The mice in the 1.0 ppm group had a 20% decrease in body weight after 14 days of exposure and showed marked excitation, abdominal swelling, rougher hair, and had a general unhealthy look. No clinical signs of toxicity were observed in the 0.3 ppm group. After 4 days of exposure, all exposure groups had lesions in the respiratory epithelium of the septum and in the naso- and maxilloturbinates. There was some involvement in the lateral wall but not the olfactory wall. Lesion severity increased from 0.3 ppm to 1.0 ppm but remained constant between 1.0 and 2.6 ppm. The respiratory epithelium changes were observed 2 weeks after the end of exposure to 1.0 ppm glutaral, but the lesions were not so significant after 4 weeks of recovery. No treatment-related histological abnormalities were detected in the trachea and lungs. The authors concluded that repeated exposure to glutaral at doses as low as 0.3 ppm caused upper respiratory tract damage in mice.
Another repeated dose inhalation study of glutaral in B6C3F1 mice was conducted.21 The mice were exposed whole-body to 100 ppb (400 µg/m³) for 6 h/day, 5 days/week for 52 (50 mice per sex) or 78 weeks (30 mice per sex). The animals were examined before each exposure. Animals that became ill were not exposed further and were killed. Once a week the animals were checked for nasal discharge, swelling of the nose and/or adjacent tissues, respiratory discomfort, dyspnea, and nasal prurit. The animals were also weighed weekly. All animals were killed and underwent a thorough necropsy.

No treatment-related mortalities occurred. Body weight gains were significantly decreased in female mice but significantly increased in male mice when compared to controls. No nasal discharge, swelling of the nose or adjacent tissues, dyspnea, or nose pruritus was observed during the weekly checks. At necropsy, hyperplasia of the squamous epithelium lining the dorsal wall and lateral aspect of the atrioturbinate was observed in females and confined to the nasal vestibule. Additionally, epidermal erosion and ulceration along with squamous and inflammatory exfoliation were noted in the nasal cavity.21

In a 4-week inhalation study to replicate occupational exposure in humans, male Wistar rats (number not reported) were exposed to 0, 0.025, or 0.1 ppm glutaral for 6 h/day, 5 days/week.22 At 24 h, 48 h, or 7 days after the final exposure, the animals were killed. The trachea was cannulated and bronchoalveolar lavage was performed. Clara-cell protein was measured and the lungs were studied microscopically. In the group examined 24 h post-treatment, exposure to glutaral at 0.1 ppm caused a decrease in lung weight. Numerous vacuoles and dilated spaces in epithelial cells in the bronchioles were also observed in the 0.1 ppm rats that were examined at 24 h post-treatment. Lipid vacuoles were observed in the 0.1 ppm dose group at 48 h post-treatment, in Clara cells of the bronchial epithelium, and in the endothelial cells of the alveolar capillaries. At 7 days post-treatment, many foci of collagen fibers were observed. The study concluded that glutaral in rats causes fibrotic effects under exposures similar to occupational threshold limits.

In a 2-year study, groups of 100 F344/N rats and 100 B6C3F1 mice (50 males and 50 females per dose group) received glutaral via whole-body inhalation exposure.23,24 Rats were exposed to concentrations of 0, 250, 500, or 750 ppb for 6 h/day, 5 days/week, for 104 weeks while mice were exposed to concentrations of 0, 62.5, 125, or 250 ppb for the same exposure periods. The concentrations were based on the results of an early 13-week toxicity study. Rats and mice were housed separately and received water and feed ad libitum during non-exposure periods. Animals were observed twice daily. Body weights and clinical observations were recorded prior to treatment, every 4 weeks from week 5-89, and then every 2 weeks from week 92 (in rats) or week 93 (in mice) until study end. All animals underwent necropsy an microscopic examination.

In rats, the survival of 500 and 750 ppb female dose groups was significantly less than that of the control animals. In the 750 dose group, 8 males and 5 females were removed in moribund condition between weeks 13 and 21 after experiencing breathing problems likely related to nasal lesions. The mean body weights of all dose groups of males and 500 and 750 female dose groups were generally less than those of the controls throughout the study. No treatment-related neoplasms were observed at any dose level. Hyperplasia and inflammations of the squamous epithelium; hyperplasia, including goblet cell hyperplasia, inflammation, and squamous metaplasia of the respiratory epithelium; and hyaline degeneration of the olfactory epithelium were observed. Incidences of fibroadenoma in female mammary glands occurred in a negative trend, with the incidence of this combined with carcinoma significantly decreased in the 750 ppb dose group. The incidence of adenoma of the pituitary gland was significantly decreased in the 500 ppb female dose group.

In mice, survival of both the males and females of all dose groups were comparable to the controls. The mean body weights of the 250 ppb dose group females were generally less than those of the controls throughout the study; there were no significant changes to the mean body weights in any of the other female dose groups or in any of the male dose groups. No
treatment-related increases in neoplasms were observed at any dose level in either sex. The occurrence of nasal lesions in the mice was qualitatively similar to those in rats. Both males and females had squamous metaplasia of the respiratory epithelium. In addition, females had inflammation and hyaline degeneration of the respiratory epithelium. Incidences of hepatocellular adenoma were decreased in the 62.5 and 250 ppb male mice and in the 250 ppb female mice. The authors of this 2 year study concluded that treatment with glutaral in rats and mice resulted in increased incidences of non-neoplastic lesions of the nose and not cause an increase in neoplastic lesions.23,24

The respiratory sensitizing potential of glutaral vapor was studied in 8 male Hartley guinea pigs.19 The animals were exposed whole body to ~14 ppm glutaral vapor 1 h per day for 5 consecutive days. Challenge exposures were performed 1 h with 4.4 ppm at 14, 21, and 35 days after the final induction exposure. No signs of toxicity were observed during the study. There was a slight decrease in body weight prior to the first challenge in the treated animals when compared to controls. A decrease in respiratory rate was observed in the majority of the exposures, but there were a few isolated instances of small increases. There was no indication that pulmonary sensitization had occurred during challenge. The authors concluded that glutaral did not produce respiratory sensitization in guinea pigs.

**GENOTOXICITY**

**In Vitro**

In an Ames test to study the mutagenic potential of the components of different dental bonding systems, a glutaral-containing compound induced strong mutagenic effects in *Salmonella typhimurium* strain TA102 with and without S9 metabolic activation.25 A weak mutagenic effect was observed in the other strains tested: TA97a, TA98, and TA100.

The mutagenicity of glutaral was investigated in a study that was determining the effectiveness of *S. typhimurium* strains TA 100, TA102, and TA104 in detecting mutagenicity in several aldehydes and peroxides.26 The study followed the Ames protocol and the concentration range for glutaral was 25-300 µg/plate, with and without S9 metabolic activation. Glutaral was mutagenic in all strains with and without metabolic activation (an equivocal response was noted in TA102 without S9).

A reverse mutation assay was used to investigate the mutagenic activity of glutaral using *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538, with and without S9.27 Concentrations tested ranged from 0.002-0.15 mg/plate. Water was the solvent control and 4-nitro-o-phenylenediamine, 2-aminoanthracene, sodium azide, and 9-aminoanthracene were the positive controls. The controls yielded expected results. Glutaral was observed to be highly cytotoxic. A weak, dose-dependent mutagenic response was observed in TA100. No mutagenic activity was observed in the other strains with or without S9.

In a Chinese hamster ovary (CHO) forward gene mutation assay at the HGPRT locus, glutaral was tested at concentrations of 0.00005-0.02 mg/ml, with and without S9.27 The vehicle control was water and the positive controls were ethylmethane sulfonate (without S9) and dimethylnitrosamine (with S9). The cells were incubated with glutaral for 24 h and then rinsed with PBS. After treatment, the cells were treated with trypsin, fixed, stained, and counted. The controls yielded expected results. No consistent, statistically significant, dose-related increases in the frequencies of 6-TG-resistant CHO cells with or without S9 were observed.

The authors of the above 2 studies investigated the potential of glutaral to induce sister chromatid exchanges (SCE) in CHO cells.28 The cells were incubated for 4 h with concentrations of glutaral ranging from 0.000005-0.002 mg/ml with S9 and 0.00002-0.002 mg/ml without S9. Colchicine was added to the cultures 2-3 h before harvest. The cells were then treated with trypsin, fixed, and stained. Small but statistically significant increases in SCE/chromosome were observed at 0.00002 and 0.00005 mg/ml glutaral without S9, but there was no increase at the highest concentration tested. The increases observed...
were thus determined to not be treatment-related. In the cells that received S9, a statistically significant increase in SCE/chromosome was observed in one culture of the low dose and one culture of the high dose, but these occurrences could not be replicated and there was no dose-response relationship. The study concluded that there were no reproducible or dose-related increases in SCE from exposure to glutaral.

In a CHO chromosomal aberration assay, glutaral was tested at concentrations ranging from 0.00003-0.03 mg/ml with and without S9. The number of aberrant cells in the cultures tested with glutaral without S9 was comparable to controls while there was a slightly greater number of aberrations in the 0.001 mg/ml and 0.01 mg/ml cultures when compared to controls. The differences were not statistically different nor were they dose dependent. The controls yielded expected results.

The cell-transforming activity of glutaral in Syrian hamster embryo (SHE) cells was reported in a study of 14 chemical agents used in dentistry. Glutaral was tested at concentrations of 0, 3, 10, and 30 µM with and without metabolic activation. The SHE cells were incubated for 48 h with the test materials. After the cultures were treated with trypsin, 2000 cells were replated and incubated for 7 days to allow colony formation. Morphological transformation was determined from the total number of colonies with an altered morphology relative to the total number of surviving colonies x 100. Glutaral did not increase morphological transformation with and without metabolic activation in this study.

The same laboratory tested the potential for glutaral to induce chromosomal aberrations in Syrian hamster embryo (SHE) cells. Glutaral was tested at concentrations of 0, 3, 10, and 30 µM with and without metabolic activation. The SHE cells were incubated for 24 h with the test materials. Three hours before harvest, the cells were treated with 0.2 µg/ml colchicine to arrest cells in metaphase. One hundred metaphases were scored per experimental group. Glutaral did not increase the level of chromosomal aberrations with and without metabolic activation in this study.

The potential for glutaral (24.8% w/v diluted to 300 mM) to induce SCE in SHE cells along with 12 other chemicals used in dentistry was studied. The cells were incubated for 24 h with glutaral at concentrations of 0, 1, 3, or 10 µM in medium containing 10 µg/ml 5-bromodeoxyuridine. Colcemid was added for the last 3 h of culture in order to arrest cells in metaphase. Cells were fixed and stained. A dose-related and significant increase in SCE in SHE cells was observed after exposure with glutaral. It was concluded that glutaral induced SCE in this study.

In another SCE study, glutaral was tested at concentrations of 2-20 µM in Chinese hamster V79 cells. Increases in the SCE frequencies were small, but reproducible and statistically significant. A significant reduction in the proliferation index was observed in cells treated with 10 µM glutaral, and no second-division metaphases with clear sister chromatid differentiation were observed in cells treated with 20 µM glutaral. It was concluded that glutaral induced SCE in a dose-related manner.

Glutaral was used as a reference compound of a validation test of the single cell gel assay (also known as a comet assay). The test material was incubated at concentrations ranging from 5-1000 µM for 4 h with cultured peripheral human leukocytes. Metabolic activation was not used. The cells were then prepared on slides for electrophoresis and assessed for DNA migration. Glutaral at doses of 20 µM and lower induced a significant increase in comet length over controls, with a maximum effect observed at 10 µM. A significant dose-related decrease in DNA migration was observed starting at 250 µM. At doses of 250 µM and greater, a significant reduction in head diameter was observed. The findings were similar to those of formaldehyde and the authors suggested that the same mechanism of action, DNA-protein crosslinking, was at work.

The genotoxic potential of glutaral was investigated in an alkaline comet assay using Chinese hamster V79 cells. Concentrations studied ranged from 0.5-20 µM under standard conditions and 0.5-50 µM in cultures that were irradiated with 2 Gy gamma radiation to induce DNA-protein crosslinks. Cells were incubated with the test material for 1 h. Under standard
conditions, there was no statistically significant, dose-related effect on DNA migration from exposure to glutaral. A trend of reduced DNA migration was observed at higher concentrations. In the irradiated cells, glutaral reduced DNA migration in a dose-related fashion, with a statistically significant decrease in DNA migration with treatment of 10 µM glutaral and greater. These results demonstrate the ability of glutaral to induce DNA-protein crosslinks.

This study also investigated the genotoxic potential of glutaral in a micronucleus study using V79 cells. Glutaral was tested at concentrations ranging from 2-20 µM. At concentrations of 10 µM and above, glutaral strongly inhibited cell growth. Small but statistically significant, dose-dependent increases in micronuclei were observed. According to FISH analysis, the majority of the treatment-related micronuclei were due to chromosome breaks. The study concluded that glutaral induced micronuclei in V79 cells in a dose-dependent manner.

**In Vivo**

The potential for glutaral to produce clastogenic effects in a mouse peripheral blood micronucleus test was studied. Groups of 5 male and 5 female Swiss-Webster mice received 40 or 80 mg/kg while 8 male and 8 female mice received 125 mg/kg. The mice were dosed by gastric intubation with 50.3% glutaral (w/w aq. solution). Water was the vehicle control and a single intraperitoneal (i.p.) injection of 0.3 mg/kg TEM was the positive control. Blood was collected from the tail vein at 30, 48, and 72 h post treatment. Blood smears were prepared and stained. The proportion of polychromatophilic erythrocytes (PCEs) per 1000 red cells per animal (PCE/NCE ratio) was determined. A minimum of 1000 PCEs per animal were evaluated for micronuclei.

There were no signs of toxicity at any dose level in females. One male in the 40 mg/kg dose group was found dead on day 4 while one male in the 125 mg/kg dose group died within 1 h of treatment. A male in the 80 dose group and another in the 125 dose group had to be killed. There were no significant differences between the treated groups and the vehicle control group for PCE/NCE ratios. There were no statistically significant or dose-dependent increases in the frequencies of micronucleated PCE in glutaral treated mice at any of the sampling times. The positive control yielded expected results. The authors concluded that glutaral was not clastogenic in this micronucleus test.

A rat bone marrow chromosomal aberration test was conducted in Sprague Dawley rats. Groups of 15 male rats received 12.5, 30, or 60 mg/kg glutaral while groups of 15 female rats received 7.5, 20, or 40 mg/kg glutaral. An additional group of 15 rats of each sex received water as a vehicle control. A group of 5 male and 5 female rats received a 30 mg/kg i.p. injection of CP. At 12, 24, and 48 h post-treatment, 5 males and 5 females from each dose group were killed. Prior to being killed the rats received 4 mg/kg colchicine via i.p. injection. Bone marrow from the femurs was extracted and prepared for analysis. Five hundred cells per animal were scored to determine a mitotic index and 50 metaphase cells per animals were evaluated for frequency and nature of chromosomal damage. There were no statistically significant or dose-dependent increases in the incidence of chromosomal aberrations in any of the glutaral treated rats. The positive control yielded expected results. The authors concluded that glutaral did not induce chromosomal aberrations in this study.

**IRRITATION AND SENSITIZATION**

**Dermal Irritation – Non-Human**

New Zealand white rabbits (3 of each sex/treatment group) were used to determine the irritancy potential of unbuffered and buffered 2.2% glutaral. Approximately 0.5 ml of either test material was applied and occluded on shaved dorsal trunk skin. The test materials were left in place for 4 h before the patch was removed. Reactions were read and recorded at 1 h and 1, 2, 3, and 7 days after the patch removal. Minor erythema was observed within 1 h of patch removal.
and persisted for 2-7 days in both the buffered and unbuffered solutions. Mild edema that lasted less than 1 h was observed in 5 animals in the buffered group and 1 animal of the unbuffered group.

In a primary skin irritation study, New Zealand white rabbits received 0.5 ml of 1%-50% glutaral on the clipped dorsal trunk for 4 h. The patches were occluded. A dose-related effect was observed with threshold effect for irritancy observed at 1% and severe erythema and edema with alopecia, scab formation, and necrosis observed at 50%.

**Ocular Irritation – Non-Human**

In an ocular study, New Zealand white rabbits (3 of each sex/treatment group) had 0.1 ml of unbuffered or buffered 2.2% glutaral instilled into the inferior conjunctival sac or place on the surface of the cornea. The eyes were examined for injury or inflammation at 1 h and 1, 2, 3, 7, 10, 14, 17, and 21 days after treatment. Slightly more marked conjunctival reactions were observed in the eyes treated with 0.1 ml buffered glutaral. The buffered glutaral treated eyes also had marked and persistent corneal injury, which was only slight and transient with the unbuffered glutaral. No corneal injury was observed with treatment at 0.01 ml of solutions, but conjunctival reactions were more marked in the 0.01 ml unbuffered glutaral group.

A primary eye irritation study of glutaral was conducted on 6 New Zealand white rabbits. Volumes of 0.001-0.1 ml of 0.1-45.0% glutaral were instilled into the inferior conjunctival sac or place on the surface of the cornea. Eyes were examined for injury and inflammation at 1 h after treatment and then periodically for up to 3 weeks. Dose-related effects were observed with no effects at 0.1% and severe diffuse corneal injury, iritis, and severe chemosis at 45%. The threshold concentration for conjunctival inflammation was 0.2%

**Dermal Sensitization – Non-Human**

The potential of buffered and unbuffered glutaral to induce sensitization was evaluated in albino Dunkin-Hartley guinea pigs. The control groups and the treatment groups consisted of 5 males and 5 females and 10 males and 10 females, respectively. The animals first received 0.1 ml intradermal induction injections on the shoulder that consisted of 2 injections with 50% Freund’s complete adjuvant (FCA), 2 injections of 5% unbuffered or buffered glutaral in propylene glycol, and 2 injections of 5% unbuffered or buffered glutaral in 50% aqueous FCA. The positive control animals received 0.1% 2,4-dinitrochlorobenzene (DNCB) in propylene glycol and the vehicle controls animals received propylene glycol alone. One week later, the animals of the treatment group received topical applications (0.2 ml) of either 2.2% buffered or unbuffered glutaral on the shaved shoulder. The positive controls received 0.1% DNCB in 70% ethanol and the vehicle controls received 70% ethanol. The application sites were occluded for 48 h after each treatment. Test sites were cleaned with distilled water and gauze after patching. There was a 14 day rest period between induction and challenge. During the challenge phase, animals received a 10% dilution (v/v) of the original unbuffered or buffered glutaral solution. Application sites were occluded for 24 h. The skin was evaluated for reactions 24 and 48 h after patch removal. One guinea pig treated with unbuffered glutaral was found dead on day 8. Necropsy failed to determine a cause of death. In the challenge phases, 15 guinea pigs (sex not specified) had an erythematous response 24 h after treatment with unbuffered glutaral with a grade 1 response or greater in 13 of the animals. By 48 h, 9 guinea pigs still had an erythematous reaction with a grade 1 response or greater in 7 of the animals. In rechallenge, 12 animals had an erythematous reaction at 24 h with 6 having a score of 1, and at 48 h, only 2 animals had erythema with a score less than 1. The incidences of sensitization were lower in animals treated with buffered glutaral, with only 5 animals having a grade 1 or higher reaction in the challenge phase and 1 with a grade 2 reaction in the rechallenge. The sensitizing indices for unbuffered glutaral was 68% in challenge and 32% in rechallenge, and for buffered glutaral, the indices were 30% in challenge and 5% in rechallenge.
A local lymph node assay (LLNA) was used to estimate the relative skin sensitizing potency of glutaral in female CBA/CA mice. Glutaral at 50% in aqueous solution was applied in either acetone or dimethylformamide at concentrations of 0.1% (in either acetone only), 0.25%, 0.5%, 1%, 2.5%, or 5%. There were 4 mice in each of the dose groups. The mice received 25 µl of topical solution on the dorsum of each ear for 3 consecutive days. On day 6 of the study, the mice were injected with 20 µCi of 3H-methylthymidine and were killed 5 hours later. The auricular lymph nodes were removed and the lymph node cells were precipitated with 5% TCA. In acetone, the stimulation indices (SI) were 3 or greater at concentrations greater than 0.1% glutaral, with a SI of approximately 18 observed at 5% glutaral. In dimethylformamide, concentrations of 0.25% and greater induced SI greater than 3, with a SI of approximately 19 observed at 5% glutaral. The EC₃₅ were calculated to be 0.006 mol/L in acetone and 0.002 mol/L in dimethylformamide. It was concluded that glutaral is a potent skin sensitizer.

In a novel approach to the LLNA method in which a non-radioisotopic endpoint was used, several known sensitizers were tested to compare the results of the new approach to the standard method. Glutaral at a concentration of 2% in 2% 2,4-dinitrochlorobenzene was found to have a stimulation index (SI) close to 15. The authors classified glutaral as a human class 2 (moderate) sensitizer, which was comparable to the findings of previous standard LLNA studies.

Groups of 10 female BALB/c mice were tested to evaluate several chemicals, including glutaral (25% in water), for their potential to induce an IgE antibody response. Groups of mice received a 50 µl aliquot of 1.17-18.75mg glutaral in water:acetone (50:50, v/v; the 18.75 mg dose group was in water only) on a shaved flank on day 1. Seven days later, animals received 25µl of the different concentrations of glutaral to the dorsum of each ear. Mouse serum was collected 14 days after the initial treatment and the total IgE antibody content was evaluated by an enzyme-linked immunosorbent assay. Mice treated with glutaral had slightly higher concentrations of serum IgE antibodies than controls, but only 9.38 mg glutaral elicited a small but significant elevation in IgE. Incomplete retention may be the reason why an elevation in IgE was not observed in the higher dose group.

CLINICAL USE

Case Reports

Tonini et al. reported the case of a 45-year-old female employed as a nurse who developed vocal cord dysfunction after using sterilants, including glutaral, to disinfect endoscopy instruments. The patient was advised to avoid the exposure to the irritants.

Glutaral-contaminated endoscopic equipment was reported to have possibly caused a long esophageal stricture that initially presented as rapidly progressive dysphagia in a patient after a routine endoscopy.

Occupational Exposure

The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) for glutaral is 0.05 ppm. NIOSH has recommended an exposure limit of 0.2 ppm for glutaral.

Occurrence of allergic contact dermatitis in health care workers exposed to 1% glutaral was examined in a 5 year study. A total of 468 patients were patch tested to glutaral in addition to other common allergens, 51 of which were health care workers. In the non-health care workers, 1.9% (8/417) had a positive patch test to glutaral whereas 17.6% (9/51) of the health care workers had a positive patch test. The study also noted a higher than expected co-reactivity between glutaral and formaldehyde in patients with and without a career in the health industry that could not be explained by concomitant exposure.

A further study of occupationally-related allergic contact dermatitis was performed. Out of 1434 patients that underwent patch testing with North American Contact Dermatitis Group (NACDG) standardized allergen series, 100 patients
were classified as health care workers. The majority of these patients were female (84%) and had reported incidences of dermatitis mainly on the hands. From the patch testing, glutaral (1%, pet.) was one of the most common relevant occupational and non-occupational allergen among health care workers with an allergic contact dermatitis prevalence of 13.2% (n=53) when compared to non-health care workers that had a prevalence of 0.88% (n=685, p<0.001). Of the allergens tested, the study found that the glutaral was one of the most common allergens in health care workers, with a prevalence of occupationally related allergic contact dermatitis to glutaral (1% pet.) of 22.6% (n=31). Workers in the dental field had an especially high prevalence of glutaral allergy of 56% (n=9) when compared to other health care workers (prevalence = 5%, p<0.01).

The prevalence of occupation-relevant allergic contact dermatitis was studied in health care workers patch-tested from 1998 to 2004 by the NACDG. Glutaral (1%) elicited positive patch tests in 4.96% (62/1249) health care workers and 5.25% (60/1143) health care workers with patient contact. In non-health care workers tested, 0.13% (18/13,508) had positive responses to glutaral. Nurses and dental workers had the highest rate of occupation-related reactions to glutaral.

In an exposure assessment of health care workers in five hospitals in Canada, air samples found detectable concentrations of glutaral between 0.005-0.15 ppm. Symptoms of exposure in the workers included headache; burning, itchy, and/or watery eyes; itchy and/or runny nose, cough, and sneezing.

Workers exposed to glutaral while making bioprosthetic heart valves were reported to have an increased risk of occupational asthma.
Table 1. Synonyms and trade name for glutaral.

<table>
<thead>
<tr>
<th>Synonyms</th>
<th>Trade Names</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutaraldehyde</td>
<td>Glutaraldehyde 25%</td>
</tr>
<tr>
<td>Glutaric Dialdehyde</td>
<td>Glutaraldehyde 50%</td>
</tr>
<tr>
<td>Pentanediol</td>
<td>Surcide G-50</td>
</tr>
<tr>
<td>1,5-Pentanediol</td>
<td>Ucarcide Preservatives</td>
</tr>
</tbody>
</table>

Table 2. Historic and current uses and concentrations of glutaral.

<table>
<thead>
<tr>
<th></th>
<th># of Uses</th>
<th>Conc. of Use (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1996</td>
<td>2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>60</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>&lt;1*</td>
<td>6 x 10^-6</td>
</tr>
</tbody>
</table>

**Duration of Use**

|                               |            |                  |
| Leaf-On                       | 18         | 8                |
|                               |            | 6 x 10^-6        |
| Rinse Off                     | 42         | 5                |
|                               |            | 6 x 10^-6        |

**Exposure Type**

|                               |            |                  |
| Eye Area                      | 2          | NR               |
| Possible Ingestion            | NR         | NR               |
| Inhalation                    | 1          | 1                |
| Dermal Contact                | 19         | 7                |
| Deodorant (underarm)          | NR         | NR               |
| Hair - Non-Coloring           | 41         | 6                |
| Hair-Coloring                 | NR         | NR               |
| Nail                          | NR         | NR               |
| Mucous Membrane               | NR         | 1                |
| Bath Products                 | NR         | NR               |
| Baby Products                 | NR         | NR               |

*Breakdown is not available.

NR = Not Reported; Totals = Rinse-off + Leave-on Product Uses.

Note: Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure type uses may not equal the sum total uses.
References


TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: John Bailey, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: January 7, 2011

SUBJECT: Concentration of Use by FDA Product Category: Glutaral
Concentration of Use by FDA Product Category
Glutaral

<table>
<thead>
<tr>
<th>Product Category</th>
<th>Concentration of Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hair conditioners</td>
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</tr>
<tr>
<td>Tonics, dressings and other hair grooming aids</td>
<td>0.000006%</td>
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Information collected in 2010
Table prepared January 6, 2011
### FDA 2011 VCRP Data

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<th>Ingredient</th>
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<th>Description</th>
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</thead>
<tbody>
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<td>04E - Other Fragrance Preparation</td>
</tr>
<tr>
<td>GLUTARALDEHYDE</td>
<td>2</td>
<td>05A - Hair Conditioner</td>
</tr>
<tr>
<td>GLUTARALDEHYDE</td>
<td>1</td>
<td>05C - Hair Straighteners</td>
</tr>
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<td>GLUTARALDEHYDE</td>
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<td>05G - Tonics, Dressings, and Other Hair Grooming Aids</td>
</tr>
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<td>GLUTARALDEHYDE</td>
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<td>05H - Wave Sets</td>
</tr>
<tr>
<td>GLUTARALDEHYDE</td>
<td>1</td>
<td>10A - Bath Soaps and Detergents</td>
</tr>
<tr>
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<td>12F - Moisturizing</td>
</tr>
<tr>
<td>GLUTARALDEHYDE</td>
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<td>12J - Other Skin Care Preps</td>
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Final Report on the Safety Assessment of Glutaral

Abstract: The dialdehyde Glutaral (also commonly called glutaraldehyde) is used in a wide variety of cosmetics as a preservative. In vitro dermal penetration studies of Glutaral indicate low penetration through animal skin and even less through human skin. The oral LD₅₀ of Glutaral for rats ranged from 66 mg/kg up to 733 mg/kg. A 28-day dermal toxicity study of Glutaral produced skin irritation and slight effects on weight and blood chemistry with concentrations as low as 50 mg/kg/day. Animal skin irritation was dose-dependant, with a no-effect concentration of 1%. Ocular exposure to Glutaral caused severe irritation in rabbits at concentrations >1%, with a no-effect level of 0.1%. Glutaral was not embryotoxic, fetotoxic, or teratogenic at concentrations that did not cause severe maternal toxicity. The no observable adverse effects level for reproduction toxicity was >1,000 ppm. Bacterial mutagenesis tests produced mixed results, as would be expected for a preservative. In most mammalian system mutagenesis tests, Glutaral was not genotoxic. In a 2-year drinking water study in rats, there was an increase in large granular lymphocytic leukemia (LGLL), but only in females administered 50–1,000 ppm Glutaral. The response was not dose dependent. Clinical studies report some evidence of dermal irritation and sensitization, but no photosensitization. Occupational data and animal studies indicate that inhalation of Glutaral can cause respiratory irritation, in addition to skin effects. Evaluation of the increased incidence of LGLL in the 2-year drinking water study indicated that the incidence was within the historical control levels for this spontaneously occurring neoplasm. These data, however, were not considered sufficient to base a finding of safety of Glutaral in products intended for prolonged use. It was concluded that a 2-year dermal carcinogenicity study following National Toxicology Program (NTP) procedures was needed to complete the safety assessment of Glutaral for use in leave-on products. For rinse-off products, it was concluded that the ocular and dermal irritancy of Glutaral could be substantially avoided if the concentration did not exceed 0.5% and exposure was only brief and discontinuous. Because it can cause respiratory irritation, it was concluded that Glutaral should not be used in aerosolized cosmetic products. Key Words: Glutaral—Glutaraldehyde—Dermal penetration study—Skin irritation—Respiratory irritation.

Glutaral is a broad-spectrum antimicrobial used as a preservative in cosmetics. The safety of such use is addressed in this report.

1 Reviewed by the Cosmetic Ingredient Review Expert Panel.
Address correspondence and reprint requests to Dr. F. A. Andersen at Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, D.C. 20036, U.S.A.
CHEMISTRY

Definition and Structure

Glutaral (CAS No. 111-30-8) is a saturated 5-carbon dialdehyde that conforms to the formula:

\[ \text{OHC(CH}_2\text{)}_2\text{CHO} \]

Other names for Glutaral include glutaraldehyde, glutaric dialdehyde, pentanodial, 1,5-pentanodial, and 1,3-diformylpropane (Estrin et al., 1982; Russell and Hopwood, 1976; Weast, 1982; Windholz, 1983).

Chemical and Physical Properties

The molecular weight of Glutaral is 100.1 and its specific gravity is 0.72. Glutaral boils and decomposes at 187–189°C (760 mm Hg), and has boiling points of 106–108°C (50 mm Hg) and 71–72°C (10 mm Hg). It is soluble in water, alcohol, and benzene. The index of refraction for Glutaral for sodium light at 25°C is 1.4338 (Weast, 1982; Windholz, 1983).

Glutaral is commonly available as 2.25, or 50% aqueous solutions with acidic pH. The pure chemical is a colorless liquid, but commercial samples often have an amber tint and an odor similar to spoiled fruit (Russell and Hopwood, 1976).

United States Pharmacopeia (USP) grade of Glutaral concentrate is described as a solution of Glutaral in "purified" water. The solution contains 49.0–51.0% \( \text{C}_5\text{H}_8\text{O}_2 \) by weight. It has a specific gravity between 1.125 and 1.133 at 20°C, and a pH between 3.7 and 4.5. The Glutaral solution contains \(<0.4\% (\text{w/w})\) acetic acid, and contains 0.001% heavy metals (USP, 1980).

Polymerization of Glutaral occurs under both acidic and alkaline conditions (Figs. 1 and 2). At acid pH, the biocidal activity of Glutaral solutions is relatively slow but constant. At an alkaline pH of 7.5–8.0, Glutaral solutions are excellent chemosterilizers; the solutions destroy all forms of microbial life including bacterial and fungal spores, tubercle bacilli, and viruses. When the pH is >9.0, the solutions are less stable. In addition to pH, time and temperature influence the biocidal activity of Glutaral solutions. The activity of alkaline solutions lasts about 2 weeks. Heat and ultrasound increase the biocidal activity of acid Glutaral solutions. However, heat decreases the stability of alkaline solutions. Biocidal activity appears to depend on the presence of free aldehyde groups. The presence of free aldehyde groups is decreased by the polymerization of Glutaral. The extent of polymerization is time and temperature dependent and is more rapid as the pH of the aqueous solution is increased (Gorman and Scott, 1980; Gorman et al., 1980).

Commercially available alkaline Glutaral solutions have been prepared with a variety of different buffers in attempts to lengthen shelf life. Such preparations may be active for up to 28 days (Pepper, 1980). The addition of a variety of agents can increase the antimicrobial activity and the shelf life of Glutaral solutions (Gorman and Scott, 1980).

Glutaral will polymerize in the presence of water, but it can be stabilized by the
addition of ethanol. Solutions of Glutaral can be inactivated by ammonia, ammonium compounds, and primary amines at neutral pH. Glutaral will cross-link proteins, but retains its antimicrobial activity in the presence of low concentrations of organic matter (Gorman and Scott, 1980; Hunting, 1983; Kabara, 1984).

The two aldehyde groups of Glutaral may react singly or together under the appropriate conditions to form bisulfite complexes, acetals, cyanohydrins, oximes, and hydrazones. These reactions are typical of aldehydes (Russell and Hopwood, 1976; Stonehill et al., 1963).

**Ultraviolet Light Absorbance**

Pure Glutaral has a single maximal ultraviolet absorbance at 280 nm. The ultraviolet absorption spectrum of commercial Glutaral contains two broad peaks: the 280-nm peak is due to the Glutaral monomer and a 235-nm peak appears to be due to a polymer (Gorman and Scott, 1980; Ranly, 1984; Jones, 1974).

**Methods of Manufacture**

Glutaral is synthesized in a two-step process: ethyl or methyl vinyl ether is reacted with acrolein to produce an ethoxy or methoxy dihydropyran, respec-

---

GLUTARAL

\[
\text{CHO(CH}_2\text{)}_3\text{CHO} + \text{CHO} \rightarrow \text{CHO(CH}_2\text{)}_3\text{CHO}
\]

FIG. 2. Scheme for Glutaral polymerization in aqueous alkaline media. Aldol-type polymer (a) progression to higher polymeric form (b) with time and increased pH. (Modified from Gorman, et al., 1980 and Gorman and Scott, 1980.)

Intermediates

\[
\text{CHO(CH}_2\text{)}_3\text{CH} = \text{CCH}_2\text{C} = \text{CH(CH}_2\text{)}_3\text{CHO}
\]

respectively, which is then hydrolyzed to produce Glutaral and ethanol or methanol, respectively (Russell and Hopwood, 1976; Windholz, 1983; Haley, 1981).

Impurities

Glutaral solutions contain both the monomer and polymers of Glutaral, and may be distilled to yield high concentrations of monomeric Glutaral (Dijk et al., 1985). Proposed impurities in Glutaral solutions, such as acrolein, glutamic acid, and glutaraldoxime, have not been found, although chromatographic evidence suggests the presence of converted molecular species other than polymerized Glutaral (Ranly, 1984).

Analytical Methods

Qualitative and quantitative determinations of Glutaral are made by titrimetric (USP, 1980; Gorman and Scott, 1980; Lyman et al., 1978), iodometric (Miner et al., 1977), and osmometric methods (Gorman and Scott, 1980). Thin-layer chromatography (Lyman et al., 1978), thin-layer chromatography followed by bioautographic visualization (Hartog and Liem, 1977), gas chromatography (Lyman et al., 1978), nuclear magnetic resonance (Holloway and Dean, 1975; Korn et al., 1972), and spectrophotometry are also used (Gorman and Scott, 1980; Haley, 1981; Ranly, 1984; Jones, 1974).
Glutaral is a highly effective broad-spectrum antimicrobial. It is used in cosmetics as a preservative (Gorman and Scott, 1980; Hunting, 1983), and is a recommended disinfectant for cosmetic production equipment (Janik et al., 1977). Glutaral will react with coloring components in some cosmetic formulations, and finished products will be discolored (Meltzer and Henkin, 1977).

As shown in Table 1, the product formulation data submitted to the Food and Drug Administration (FDA) in 1994 reported that Glutaral was used in a total of 60 cosmetic product formulations (FDA, 1994).

Concentration-of-use values are no longer reported to the FDA by the cosmetic industry (Federal Register, 1992). However, product formulation data submitted to the FDA in 1984 stated that Glutaral was used at concentrations up to 1% (FDA, 1984).

Cosmetic products containing Glutaral may be applied to or come in contact with the skin, eyes, hair, nails, and mucous membranes. Product formulations containing Glutaral may be applied as often as several times a day and may remain in contact with the skin for variable periods following application. Daily or occasional use may extend over many years.

International

Glutaral is approved for use by members of the European Union as a preservative for cosmetic products at a maximum concentration of 0.1%. Cosmetics must be labelled as containing Glutaral when the concentration of Glutaral in the

<table>
<thead>
<tr>
<th>Product category</th>
<th>Total formulations in category</th>
<th>Total formulations containing glutaral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other eye make-up preparations</td>
<td>132</td>
<td>2</td>
</tr>
<tr>
<td>Other fragrance preparations</td>
<td>136</td>
<td>1</td>
</tr>
<tr>
<td>Hair conditioners</td>
<td>614</td>
<td>26</td>
</tr>
<tr>
<td>Permanent waves</td>
<td>387</td>
<td>1</td>
</tr>
<tr>
<td>Rinses (noncoloring)</td>
<td>58</td>
<td>2</td>
</tr>
<tr>
<td>Tonics, dressings, and other hair grooming aids</td>
<td>563</td>
<td>1</td>
</tr>
<tr>
<td>Wave sets</td>
<td>98</td>
<td>7</td>
</tr>
<tr>
<td>Other hair preparations</td>
<td>376</td>
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<td>Foundations</td>
<td>345</td>
<td>3</td>
</tr>
<tr>
<td>Make-up bases</td>
<td>157</td>
<td>2</td>
</tr>
<tr>
<td>Cleansing preparations</td>
<td>746</td>
<td>6</td>
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<tr>
<td>Moisturizing products</td>
<td>839</td>
<td>3</td>
</tr>
<tr>
<td>Night preparations</td>
<td>208</td>
<td>1</td>
</tr>
<tr>
<td>Skin fresheners</td>
<td>222</td>
<td>1</td>
</tr>
<tr>
<td>1994 Total</td>
<td>746</td>
<td>60</td>
</tr>
</tbody>
</table>
finished product exceeds 0.05%. Glutaral is prohibited in aerosol products (European Economic Community, 1993).

Noncosmetic

Glutaral is a food additive permitted for direct addition to food for human consumption. Glutaral may be used as a cross-linking agent for insolubilizing a coacervate of gum arabic and gelatin used as a component of microcapsules for flavoring substances (21 Code of Federal Regulations (CFR) 172.230). Glutaral is also a secondary direct food additive permitted in food for human consumption. It may be used as a fixing material in the immobilization of glucose isomerase enzyme preparations for use in the manufacture of high-fructose corn syrup (21 CFR 173.357). Glutaral is permitted as an indirect food additive, and may be used in adhesives that are used as components of articles intended for use in packaging, transporting, or holding food (21 CFR 175.105). It may be used as an antimicrobial agent in pigment and filler slurries used in the manufacture of paper and paperboard at concentrations not >300 ppm by weight of the slurry solids. Glutaral may also be used as a component of uncoated or coated food contact surfaces of paper and paperboard in contact with aqueous, fatty, and dry foods and may be used in products for producing, manufacturing, packaging, preparing, treating, packing, transporting, or holding food (21 CFR 176.170, 176.180) (Rothschild, 1991; Federal Register, 1985). Glutaral has been used as a disinfectant in poultry processing (Gorman and Scott, 1980).

Glutaral has been employed in the cold disinfection or sterilization of surgical, endoscopic, anesthetic, and dental instruments. It is present in the emulsion of most "rapid process" x-ray films and also has been an ingredient in antiseptic soaps for hand disinfection. Glutaral has been used for the topical treatment of dermatological disorders, including hyperhidrosis, onychomycosis, pitted keratolysis, warts, herpes viral diseases, epidermolysis bullosa, and pseudomonas infections; in endodontic therapy; and in the treatment of allografts and xenografts (Gorman and Scott, 1980; Ballantyne and Berman, 1984; Fisher, 1981; Slesinski et al., 1983).

Glutaral has been used widely to attach enzymes or markers to proteins. Cross-linked proteins and enzymes may retain the immunological activities of the proteins and the activities of the enzymes, respectively. Glutaral can be used to prepare vaccines. Preparations made from bacterial toxins, bacterial cells, viruses, venoms, allergens, and tumor cells are generally antigenic but not infectious. Glutaral-treated tumor cells have induced immunity in certain experimental tumor systems (Russell and Hopwood, 1976; Relyveld and Ben-Efraim, 1983).

Due to its antimicrobial properties and its abilities to cross-link proteins and reduce the water solubilities of various materials, Glutaral has been widely used in industries other than the food and medical industries. Glutaral is used as a chemical intermediate and in the manufacture of adhesives, sealants, and electrical products. It is used as a preservative in household cleaning products, and may also be used as an embalming fluid. It is used in the paper industry, for tanning leather, and as a fixative for biological specimens for light and electron micros-

Glutaral's threshold limit value ceiling, set by the American Conference of Governmental Industrial Hygienists (ACGIH), is 0.2 ppm (ACGIH, 1980).

**BIOLOGICAL PROPERTIES**

**Absorption and Metabolism**

**In Vitro**

Permeability chambers were used to estimate human percutaneous absorption of a 10% aqueous solution of Glutaral. Glutaral was placed for 1 h on samples of isolated thin stratum corneum from the chest and the abdomen, on isolated abdominal epidermis, and on thick stratum corneum from blister tops on the soles of the feet. Twelve percent of the applied dose of Glutaral penetrated the one chest sample, and 3.3% and 13.8% penetrated each of two samples of the abdomen stratum corneum. In the three samples of abdominal epidermis, 2.8–4.4% of the applied Glutaral penetrated within 1 h. No Glutaral penetrated the six samples of thick stratum corneum (Reinfenrath et al., 1985).

Frantz et al. (1993) used a flow-through skin penetration chamber design to determine the in vitro skin penetration over a 6-h exposure period of 0.75 and 7.5% [1,5-[14C]-Glutaral on excised skin from Fischer 344 rats, CD-1 mice, Hartley guinea pigs, New Zealand white rabbits, and human beings (mammoplasty patients). Total recoveries of radioactivity for all the species ranged from 75–92% for both concentrations. Overall, <0.5% of 0.75% [1,5-[14C]-Glutaral and <0.7% of the 7.5% solution was absorbed through the skin of the animals. For human beings, approximately 0.2% of the applied radioactivity penetrated the skin for both doses tested, largely owing to binding to the skin. The investigators concluded that only a minimal amount of Glutaral was available for systemic uptake and distribution following cutaneous exposure, and that the potential for absorption may be less for human beings than for commonly used laboratory species.

**In Vivo**

Material balance studies and pharmacokinetic studies were conducted with groups of Fischer 344 rats (four of each sex) and New Zealand white rabbits (two of each sex) using both intravenous (i.v.) and dermal routes of exposure. The dose volumes for both exposures were 0.2 ml for rats and 2.5 ml for rabbits. In both the material balance and the pharmacokinetic studies, rats and rabbits received an i.v. dose of either 0.075 or 0.75% Glutaral in the tail vein or ear vein, respectively. In dermal material balance studies with rabbits and pharmacokinetic studies with both species, concentrations of 0.75 and 7.5% Glutaral were applied to the skin under a 24-h occlusive dressing. Dermal material balance studies with rats were conducted with 0.075, 0.75, and 7.5% Glutaral with [14C] in the 1- and 5- positions.

Glutaral was rapidly distributed and eliminated when administered i.v. to rats and rabbits. When 0.075% Glutaral was administered, 75–80% of the dose in the
rat and 66–71% in the rabbit was recovered as $^{14}$CO$_2$ during the first 24 h following administration; 80% of this $^{14}$CO$_2$ was recovered during the first 4 h. When 0.75% Glutaral was tested, the proportion of i.v.-administered Glutaral recovered as $^{14}$CO$_2$ decreased, and the amount of radioactivity recovered in the urine, tissues, and carcass increased. The average plasma concentration of radioactivity increased tenfold in rats and rabbits with a tenfold increase in dose, but the tissue concentration increased by a greater amount. It was suggested that the mechanisms involved in the disposition of Glutaral were saturated and resulted in a shift in the elimination pathway.

Following dermal application of Glutaral, 41–61% of the dose in the rat and 31–45% in the rabbit were recovered from the treated skin. Treatment with 0.75% Glutaral resulted in superficial mild dermatitis and necrosis, and treatment with 7.5% Glutaral resulted in moderate dermal edema, dermatitis, and superficial dermal necrosis. The calculated dermally absorbed doses ranged from 4.1–8.7% in the rat and 33–53% in the rabbit.

The investigators concluded that Glutaral does have the potential to penetrate human skin. However, they believe that only a small proportion of an applied dose will be absorbed. They noted that more concentrated solutions of Glutaral are irritating to the skin and would thus limit exposure. Although absorption was observed at nonirritating doses, the proportion absorbed was lower and the absolute amount of Glutaral absorbed was significantly less. Because Glutaral was rapidly distributed and eliminated, the potential for cumulative toxicity was considered low. Additionally, there was no preferential distribution of Glutaral in any of the specific organ systems (McKelvey et al., 1992).

Although the metabolism of Glutaral has not been studied in detail, it was suggested that Glutaral might be oxidized first to a mono- or di-carboxylic acid by aldehyde dehydrogenase (Hjelle and Petersen, 1983; Weiner, 1980) and then further oxidized through an acidic intermediate to CO$_2$ (McKelvey et al., 1992).

Myers et al. (1986) investigated the systemic absorption and distribution of Glutaral from pulpotomy (excision of dental pulp) sites in dogs. Five dogs were anesthetized and pulpotomies were performed on the 16 maxillary and mandibular anterior teeth of each. One cotton pellet containing 20 μl of 2.5% $^{14}$C-Glutaral with an activity of $6.25 \times 10^5$ dpm/μl (specific activity: 2.28 mCi/mol) was placed in each pulpotomy site for 5 minutes. Blood and urine samples were taken via catheters and expired air was captured for up to 90 minutes. All of the dogs were killed and the liver, kidneys, lungs, heart, and diaphragm were analyzed for radioactivity. Bile samples were also analyzed.

The total systemic absorption was $2.91 \pm 0.94\%$ of the applied dose, or $9.32 \pm 3.02$ μl/h. The mean plasma concentration peaked at 45 minutes then gradually declined. Radioactivity in the urine increased throughout the study period but was always approximately 10 times greater than that of the plasma. Approximately 8% of the absorbed dose was found in the urine, 3.6% in expired air, and 0.6% in the bile. The tissue-to-plasma (T/P) ratio for $^{14}$C-Glutaral was greatest in the red blood cells ($2.21 \pm 0.92$). However, the other tissues had T/P ratios that were not statistically different from 1.0, which the investigators suggested indicated that Glutaral did not have a high binding capacity.
Cytotoxicity

St. Clair et al. (1991) evaluated the cytotoxic effects of Glutaral in vitro using the human TK6 lymphoblast cell line. Cultures of TK6 lymphoblasts were exposed to micromolar concentrations of Glutaral for 30 minutes. A concentration of 25 μM Glutaral killed all the cells, and 5 μM had no effect on the rate of growth.

ANIMAL TOXICOLOGY

Acute Toxicity

Oral

Smyth et al. (1962) reported the oral LD₅₀ of 25% Glutaral for Carworth-Wistar rats was 2380 mg/kg.

In another study (Miner et al., 1977), the acute oral LD₅₀ of a 2% alkaline Glutaral solution was determined by administering increasing volumes to rats by gavage. The solution contained 2% Glutaral buffered to an alkaline pH of 7.5 to 8.0, surfactants, sodium nitrite, a peppermint oil odorant, and yellow and blue FD and C dyes. The oral LD₅₀ of the 2% Glutaral solution was 17.5 ml/kg. Most of the deaths occurred within 24 to 48 h after administration.

After preliminary dose range-finding tests, doses of 2% alkaline Glutaral in saline were administered by stomach tube to groups of 10 albino rats and mice. The LD₅₀ of 2% Glutaral for the rats was 252 mg/kg, and the LD₅₀ for the mice was 352 mg/kg. Irritation of the gastrointestinal tract, with hemorrhagic irritation at larger doses, was observed at necropsy. Congestion of the lungs and of the abdominal viscera was observed in many animals (Stonehill et al., 1963).

Groups of 10 male and 10 female Sprague-Dawley rats and 10 male and 10 female ICR mice were given oral doses of Glutaral and were observed for 7 days. The LD₅₀ of Glutaral was 134 mg/kg for male rats, 165 mg/kg for female rats, 100 mg/kg for male mice, and 110 mg/kg for female mice. Following the same procedures, Cidex, a detergent containing 2% Glutaral, sodium formaldehyde monohydrate, trisodium phosphate monohydrate, sodium bicarbonate, and green no. 204, also was administered orally to rats and mice. The LD₅₀ of Cidex was 96.1 mg/kg for male rats, 113 mg/kg for female rats, 122 mg/kg for male mice, and 209 mg/kg for female mice (Uemitsu et al., 1976).

The acute oral LD₅₀ of 0.5-50% (w/w) solutions of Glutaral ranged from 99 to 733 mg/kg, and were generally larger at greater concentrations of Glutaral. This may have been a result of different rates of absorption due to injuries in the alimentary tract with greater concentrations, or a result of differences in the mechanism of acute toxicity. Most of the rats given concentrations of 5-50% died within 3 days of compound administration. At necropsy, the rats that died had the following changes: gastric distension; congestion and hemorrhagic areas in the gastric wall; thickening of the pylorus; distended and congested small intestine; variable congestion of the adrenal glands, kidneys, liver, spleen, and lungs; and scattered hemorrhagic areas in the lungs. Piloerection, red pericocular and perinasal encrustation, sluggish movement, rapid breathing, and diarrhea were observed prior to death. Rats that survived the 14-day observation period recovered from...
these effects within 5 days of Glutaral administration. These animals usually had no gross lesions at necropsy except for mild thickening of the wall of the pyloric area of the stomach. Solutions <5% Glutaral were significantly less toxic. A 1% Glutaral solution was only slightly toxic and an oral dose of 16 ml/kg of a 0.5% Glutaral solution did not result in any deaths or signs of toxicity in rats (Union Carbide Corporation, 1986). Acute oral LD₅₀'s may vary due to different degrees of polymerization of Glutaral solutions (Gorman and Scott, 1980).

**Dermal**

Smyth et al. (1962) applied 25% Glutaral under occlusive patches to the clipped abdomens of four New Zealand white rabbits for 24 h. After a 14-day observation period, they reported the dermal LD₅₀ for 25% Glutaral was 2,560 mg/kg.

No evidence of systemic effects was observed in rats and rabbits that received a single dermal application of Glutaral as a 2% aqueous solution (dose unspecified) (Stonehill et al., 1963).

Increasing volumes of a stabilized alkaline 2% Glutaral solution were applied under an occlusive wrap to the shaved skin of rabbits for 48 h. The method used was described in the regulations of the Hazardous Substances Labeling Act. There were no deaths when 50 ml/kg 2% Glutaral, the largest practical achievable dose, was applied (Miner et al., 1977).

The acute dermal LD₅₀ of Glutaral was determined in New Zealand white rabbits. Concentrations of 5-50% Glutaral (w/w) in water and doses of 0.5–16.0 ml/kg were applied under occlusive dressings for 24 h to groups of four to five male and female rabbits, and the rabbits were observed for 14 days. The acute dermal LD₅₀ of 25–50% Glutaral solutions ranged from 897–3,045 mg/kg. The 45 and 50% solutions were of moderate acute lethal toxicity and the 25% Glutaral solution was only slightly toxic. One of 10 rabbits treated with 16 ml/kg of a 15% solution died. No deaths were observed at doses of 16 ml/kg for 5% and 10% Glutaral solutions. Animals that died usually did so within 3 days of Glutaral administration and, at necropsy, congestion of the liver, lungs, kidneys, and spleen was observed. The thoracic and abdominal viscera appeared normal in the 14-day survivors. Edema, necrosis, and scab formation were observed at the treatment site following application of 10% or greater solutions of Glutaral. The 5% Glutaral solution produced mild to moderate local inflammation (Union Carbide Corporation, 1986).

**Subcutaneous**

Groups of 10 male and 10 female Sprague-Dawley rats and ICR mice were treated subcutaneously (s.c.) with Glutaral and observed for 7 days. The LD₅₀ of Glutaral was 2.390 mg/kg for male rats, 4.860 mg/kg for female rats, 1.430 mg/kg for male mice, and 2630 mg/kg for female mice (Uemitsu et al., 1976).

**Inhalation**

No deaths occurred when 10 rats were exposed to air nebulized with 400 mg alkaline 2% Glutaral solution per liter of air per hour. The LC₅₀ was greater than
1,500 mg/L/h; therefore, Glutaral was considered nontoxic to rats (Miner et al., 1977).

Groups of 10 male and 10 female Sprague-Dawley rats and ICR mice were exposed to 0.02 ml/l Glutaral, and the length of exposure that resulted in the death of half of the animals was measured. This time was 60 minutes for male rats, 86 minutes for female rats, 51 minutes for male mice, and 94 minutes for female mice (Uemitsu et al., 1976).

An alkaline 2% aqueous Glutaral solution was allowed to evaporate freely at room temperature in a closed system, and rats and mice were exposed to Glutaral for 4 h. No gross effects were observed in animals killed immediately after exposure or those killed at varying intervals after exposure. Twelve rats exposed to 1.5 ml of the Glutaral solution per liter of air were slightly more restless than the control rats, but all of the rats were alive 7 days after exposure. An initial weight loss was observed in 5 of the 12 rats, but the weight was regained within 1 or 2 days. Pneumonitis, also seen in the control rats, was observed in 10 rats. None of the five mice exposed to Glutaral died. Two mice had an initial weight loss, which was subsequently regained; and pneumonitis, also seen in control mice, was observed in two experimental mice. Higher concentrations of Glutaral in the evaporating solutions produced more signs of respiratory tract irritation in the animals (Stonehill et al., 1963).

An alkaline 2% aqueous solution of Glutaral was allowed to evaporate to produce concentrations of 33 and 133 mg of Glutaral per liter of air, and groups of 10 male NMRI mice were exposed for 24 h. There were 20 control mice. Half of the mice underwent necropsy immediately after exposure and the other half 1 day later. The lungs, liver, and kidneys were evaluated histopathologically. No remarkable gross changes were observed in the lungs or kidneys. None of the control mice died, and one mouse had bronchopneumonia. One mouse exposed to 33 μg of Glutaral per liter of air died, and one mouse had a local inflammation of the liver, possibly not of toxic origin. No mice exposed to 133 μg of Glutaral per liter of air died, but six mice had toxic hepatitis. The hepatitis was present to a "somewhat lesser degree" in mice killed 1 day after the end of exposure than in the mice killed immediately after exposure; the authors speculated that the hepatitis may have been reversible. The use of a 25% Glutaral solution did not cause death immediately among exposed mice or during a 7-day observation period following exposure (Varpela et al., 1971).

Groups of six rats were exposed to saturated vapor atmospheres of Glutaral generated from 25, 45, and 50% aqueous Glutaral solutions for 8 h. The vapor was generated either statically, overnight equilibration of the chamber atmosphere against Glutaral solution before exposure, or dynamically, air stream bubbled through Glutaral solution and then passed into the exposure chamber. No deaths occurred during the exposure or during the 14-day postexposure observation period. Signs of toxicity were observed during exposure and up to 2 days after exposure. Periocular and perinasal wetness and encrustation, mouth breathing, audible respiration, and a few cases of slight transient weight loss were observed. At necropsy, no lesions were observed (Union Carbide Corporation, 1986).

Groups of six male and six female Fischer 344 rats were exposed for 4 h to
10.6–42.7 ppmv of Glutaral (dynamically generated), and control rats were exposed to room air only. The LC50 for Glutaral was 23.5 ppmv for male rats and 40.1 ppmv for female rats. There were no deaths at 10.6 ppmv Glutaral. Audible breathing, mouth breathing, excess lacrimation and salivation, and decreased feed and water consumption and body weight gain were observed in all groups. Decreased breathing rates, slow righting reflex, and abdominal breathing were observed at the greatest Glutaral concentration. Most of these signs subsided during the second postexposure week (Union Carbide Corporation, 1986).

The toxicity of Glutaral to the nasal epithelium was also studied. Male Fischer 344 rats had 40 μl of 10, 20, or 40 mM Glutaral instilled into one of their nasal passages. The rats were killed 72 h later, and their nasal passages evaluated histopathologically. Lesions appeared on the nasal epithelium of the rats treated with 20 and 40 mM Glutaral, and the passages were inflamed, marked by neutrophilic infiltrates, epithelial erosion, and respiratory epithelial hyperplasia and squamous metaplasia. These changes were more severe and extensive in the high-dose group. Increased cell proliferation in the nasal epithelium also occurred. The rats treated with 10 mM Glutaral did not have nasal lesions or signs of irritation (St. Clair et al., 1990).

**Short-Term Toxicity**

**Oral**

Groups of three rats were given drinking water containing 0.05, 0.1, or 0.25% Glutaral for 11 weeks. A control group of rats was given regular water. All of the animals had a "largely normal" rate of weight gain and appeared normal clinically. The rats were killed at the end of the experiment, and nervous system tissue was examined microscopically. No signs of adverse effects were found (Spencer et al., 1978).

Drinking water containing 10, 100, and 1,000 ppm Glutaral (w/w) was given to groups of male and female Fischer 344 rats (number not specified) for 14 days. A control group of animals was administered treated water. Decreased feed and water consumption in males and decreased water consumption in the females were observed in the high-dose group. No other clinical signs of toxicity, alterations in serum chemistry and hematology, effects on organ weights, or microscopic changes were observed (Union Carbide Corporation, 1993).

Groups of five male and five female Harlow-Wistar albino rats were fed diets containing 0.1, 0.48, 1.02, and 1.63 g/kg/day of Glutaral for 7 days. No animals died. At the largest Glutaral dose, body weight gain in males and females was decreased and the absolute kidney and liver weights in males were decreased. The no-effect level for Glutaral was 1.0 g/kg/day (Union Carbide Corporation, 1986).

**Dermal**

A 0.5-ml dose of a 2% alkaline Glutaral solution was applied daily for 6 weeks to the closely clipped skin of albino rabbits. The solution was spread with a brush.
and allowed to dry. No evidence of systemic toxicity was observed (Stonehill et al., 1963).

Doses of 50 µl of 0.05, 0.25, 0.5, 2.5, 5, 25, and 50% Glutaral (w/w) in water were applied to the clipped dorsal skin of groups of 10 male C3H/HeJ mice for a total of 10 applications (application schedule not specified). All mice that received 25% or 50% Glutaral died within nine applications. At necropsy, no consistent lesions were observed. Decreased body weights were observed in mice that received 5% Glutaral after four and six doses only. At concentrations <5% Glutaral, no signs of toxicity and no effects on body weight were observed (Union Carbide Corporation, 1986).

**Subcutaneous**

Groups of 10 male and 10 female Sprague-Dawley rats were treated s.c. with 1, 5, 25, or 125 mg/kg/day Glutaral for 35 days. At the 1-mg/kg dose, no signs of toxicity were observed. A slight decrease in body weight and slightly reduced feed consumption were observed in male rats given the 125-mg/kg/day dose. Inflammation and necrosis at the injection sites were noted in the 25- and 125-mg/kg/day groups. In addition, there were increased numbers of leukocytes and a decreased concentration of hemoglobin. Hypertrophy of white pulp of the thymus, atrophy of the thymus, atrophy of the glandular epithelium of the prostate, and degeneration of the renal tubules were observed at microscopic examination. No abnormal findings were observed in the urinalysis and blood chemistries, except for an increase in total protein in the urine and an increase in N-urea in the serum (Uemitsu et al., 1976).

**Inhalation**

Kari (1993) conducted a 2-week inhalation study of Glutaral with F344/N rats and B6C3F1 mice. Groups of five males and five females of each species were exposed to vaporized Glutaral through whole-body exposure at concentrations of 0, 0.16, 0.5, 1.6, 5.0 and 16.0 ppm. Exposures were for 6 h and 20 minutes per day, 5 days a week, for a total of 12 exposure days. The animals were weighed on days 1 and 8, and observations were made regularly for mortality and morbidity and for clinical signs of toxicity. Complete necropsies were performed on all of the animals.

Respiratory difficulty and ocular and nasal discharge were observed immediately after exposure to 5.0 and 16.0 ppm Glutaral. All of the rats exposed to these concentrations died or were moribund before the end of the study. No deaths occurred in the lower exposure groups. Body weight gain was negligible for the male rats and decreased in the female rats of the 1.6-ppm group as compared with the controls, but was normal for both sexes of the 0.5- and 0.16-ppm groups. The following clinical observations were made of the rats of the 1.6- and 5.0-ppm groups: labored breathing, breathing through the mouth, ocular and nasal discharge, and rough haircoat. No clinical signs of toxicity were observed among the rats exposed to 0.5 and 0.16 ppm. At necropsy, gross lesions were observed only in the animals that died or were moribund. These lesions included crusted exudate...
at the anterior tip of the nares, gray and thickened laryngeal mucous, and exudate or crust on the surface of the tongue. Exposure-related histopathologic lesions were found in the nasal passages and larynx of the rats exposed to concentrations of 0.5 ppm Glutaral and greater. These lesions included necrosis and acute (neutrophilic) inflammation of the respiratory and olfactory epithelium and hyperplasia and squamous metaplasia of the respiratory epithelium. Minimal to mild inflammation of the trachea was observed in the 5.0 and 16.0 ppm exposure groups, and inflammation and squamous metaplasia of the lungs and bronchi and inflammation and/or erosion of the tongue were observed in the 16.0-ppm group. The investigator noted that mild to moderate thymic (lymphoid) atrophy was observed in 5 of 10 rats exposed to 1.6 ppm Glutaral, but attributed this observation to secondary stress.

In the study with mice, all of the animals exposed to concentrations of 1.6 ppm and greater died or were moribund before the end of the study. Marked respiratory difficulties, breathing through the mouth, ocular and nasal discharge, and cessation of eating and drinking were observed in these animals. All of the mice of the 0.5- and 0.16-ppm groups survived the study and body weight gain was comparable to that observed with control mice. No clinical signs of toxicity were observed in these groups. At necropsy, red crust at the anterior of the nares and gray, thickened laryngeal mucous were observed in the mice of the 16.0-ppm group. Exposure-related histologic lesions of the nasal passages were observed in mice exposed to 1.6 ppm Glutaral and greater, and included minimal to mild necrosis and acute (neutrophilic) inflammation of the respiratory and olfactory epithelium and squamous metaplasia of the respiratory epithelium. Necrosis of the mucosa and squamous metaplasia of the respiratory epithelium of the larynx were also observed. Lesions of the trachea, consisting of inflammation and necrosis of the respiratory mucosa, were observed only in the mice exposed to 16.0 ppm Glutaral.

In another study (Union Carbide Corporation, 1986), a statically generated Glutaral atmosphere was produced from a 50% (w/w) aqueous solution of Glutaral, and six female rats were exposed for 6 h and observed for 14 days. The Glutaral vapor concentration ranged from 2.0-11.0 ppmv and averaged 4.0 ppmv. No deaths occurred. Periocular wetness and labored breathing were observed during and immediately after exposure. At necropsy, no lesions were found.

Groups of 10 male and 10 female Fischer 344 rats were exposed to 0.2, 0.63, and 2.1 ppmv Glutaral vapor for 6 h a day for 9 days over an 11-day period. The vapor was produced dynamically. Control rats were exposed to unaltered air. Sixteen rats treated with 2.1 ppmv and one rat treated with 0.63 ppmv died between days 3 and 9. Excess lacrimation, salivation, and nasal discharge were observed in rats from all treatment groups. Labored breathing, loss of initial body weight, decreased feed consumption, and absolute decreases in organ weights were observed in the rats exposed to the two largest Glutaral concentrations (Union Carbide Corporation, 1986).

In another study (Union Carbide Corporation, 1986), groups of 12 male and 12 female Fischer 344 rats were exposed to mean Glutaral concentrations of 0.3, 1.1, and 3.1 ppmv, generated dynamically. Over an 11-day period, the rats were ex-
posed for 6 h a day for 9 days. Thirteen rats in the 3.1 ppmv group died between days 6 and 9 of exposure. Rats from this group had audible breathing and periorcular and perinasal encrustation. Mouth and abdominal breathing were observed in 12 rats of the 1.1 ppmv group. Body weights, feed and water consumption, and organ weights decreased in the rats from the 1.1- and 3.1-ppmv groups. Hematological and clinical findings reflected disturbances due to reduced feed and water intake, and possibly early hepatic damage. Hepatocellular atrophy, rhinitis, and mild atrophy of the olfactory mucosa were observed in the rats from the 3.1-ppmv Glutaral group. Rhinitis and squamous metaplasia of the nasal mucosa were observed in the rats from the 1.1-ppmv Glutaral group. No signs of irritation, toxicity, or lesions were observed in the 0.3-ppmv–treated group, and there were no treatment-related abnormalities in the sciatic nerves of rats from the 0.3- and 1.1-ppmv Glutaral group.

Subchronic Toxicity

Oral

Male and female rats were given 50, 250, and 1,000 ppm Glutaral (w/w) in their drinking water for 13 weeks. This was 5, 25, and 100 mg/kg/day Glutaral for males and 7, 35, and 120 mg/kg/day for females. Control animals were given regular water. The rats were killed following treatment or were observed for 4 weeks further. Body weight, feed and water consumption, hematology, serum chemistry, urinalysis, and gross and microscopic evaluations were performed. No effects were observed in rats of the 50-ppm Glutaral group. In the 250-ppm Glutaral group, decreased water consumption, decreased urine volume with increased specific gravity, increased relative, and, in females only, absolute kidney weight, and increased blood urea nitrogen were observed. In the rats of the 1,000-ppm Glutaral treatment group, decreases were observed in feed and water consumption and absolute and relative body weights. There were decreases in urine volume with increased specific gravity, increases in relative, and, in females only, absolute kidney weight and an increase in serum urea nitrogen. The researchers stated that 1,000 ppm Glutaral in the drinking water of rats was minimally toxic, and that marginal physiological compensatory changes in response to aversion-induced decreased water consumption were observed when drinking water contained 250 and 1,000 ppm Glutaral (Union Carbide Corporation, 1986).

A subchronic toxicity study with albino CD-I mice was also performed (Bushy Run Research Center, 1989). Groups of 20 mice of each sex were administered 100, 250, and 1,000 ppm Glutaral in drinking water for 90 days. An additional 10 mice of each sex were similarly administered 1,000 ppm Glutaral, but were maintained on untreated water for 6 weeks following the 13-week treatment period. A control group of 30 mice was maintained on untreated water. Twenty of these were killed for necropsy after 13 weeks, and the remaining mice were maintained for an additional 6 weeks.

No clinical signs of toxicity or alterations were found in food consumption, absolute body weight, ophthalmic findings, hematology measurements, clinical chemistry measurements, necropsy findings, or histopathologic findings. Water
consumption was reduced in the high-dose group throughout the testing period. Urinalysis indicated decreased urine volume for males in the 250-ppm treatment group and for males and females in the 1,000-ppm group, as well as increases in urine osmolality for both sexes in the 1,000-ppm groups. The mean kidney weight relative to body weight was increased for females in the 1,000-ppm treatment group. All parameters that were altered during the study returned to control values during the 6-week recovery period. The investigators concluded that 1,000 ppm Glutaral was a minimal toxic dose and that 250 ppm Glutaral may have produced a marginal physiological response. The no observable-adverse effect level (NOAEL) for this ingredient was considered to be 100 ppm.

In a study with beagle dogs, groups of four dogs of each sex were administered 50, 150, or 250 ppm Glutaral in drinking water for 13 weeks. The mean daily uptake was calculated to be 3.3, 9.6, and 14.1 mg/kg/day for male dogs and 3.2, 9.9, and 15.1 mg/kg/day for female dogs. A control group of dogs was given untreated drinking water. The dogs were monitored for clinical signs of toxicity and measurements of various biological parameters were made. All of the dogs were killed for necropsy and histological examination of the major organs was performed.

Intermittent vomiting was observed among the dogs administered 150 and 250 ppm Glutaral. This reaction was considered to be related to the irritant properties of Glutaral to the gastric mucosa, but it was noted that there was no apparent gross or microscopic evidence of gastrointestinal injury at the end of the study. No other treatment-related changes were observed in terms of clinical signs of toxicity, ophthalmologic changes, food consumption, hematology, clinical chemistry measurements, urinalysis, or necropsy findings.

It was noted that water consumption was lower during the first part of the study in both the mid- and high-dose groups, but this was attributed to a slight aversion to the treated water. Body weights and cumulative weight gain were lower for all the groups of treated female dogs, but no dose-response was observed. The body weight gain for male dogs was normal. The kidney-to-body-weight ratio was increased for the female dogs of the high-dose group, but this was considered to be of minimal biological significance because urine analyses were normal and there was no biochemical or microscopic evidence of renal injury (Bushy Run Research Center, 1990).

Dermal

A 28-day dermal toxicity study was conducted using Fischer 344 rats. Occlusive patches containing 50 and 100 mg/kg were applied to the shaved backs of groups of 10 rats of each sex for 6 h. A total of 20 applications were made over the 28-day period. Groups of 15 rats of each sex were treated similarly with 150 mg/kg/day Glutaral or with distilled water to serve as the control. All of the rats from the 50- and 100-mg/kg/day Glutaral groups and 10 rats of each sex from the control and 150-mg/kg/day groups were killed for necropsy on day 29. The remaining 10 animals from the control and high-dose groups were held but not treated for an additional 4 weeks.
No treatment-related deaths occurred during the study. All clinical signs of toxicity were confined to the treatment sites. Slight, dose-related erythema was observed in all the treatment groups during the course of the dosing phase, but edema was rarely observed. There was also dose-related discoloration of the skin, desquamation or exfoliation, excoriation, scab formation, and skin necrosis. Some of these conditions were resolved during the second to fourth weeks of the treatment period, and most of these clinical findings were resolved during the 4-week recovery period.

Slight effects on body weight, body weight gain, food consumption, clinical chemistry (blood urea nitrogen in males), and hematology (platelet and reticulocyte counts in females) were observed during the study. Both gross and microscopic lesions were found in the skin, even at the lowest dose tested. The investigators concluded that the NOAEL had not been determined, even though effects on the rats treated with 50 mg/kg/day Glutaral were marginal (Bushy Run Research Center, 1994a).

Inhalation

In a subchronic inhalation study (Kari, 1993) using F344/N rats and B6C3F1 mice, groups of 10 males and 10 females of each species were exposed to vaporized Glutaral at concentrations of 0, 62.5, 125, 250, 500, and 1,000 ppb. Exposures were to the whole body for 6.5 h/day, 5 days a week for 13 weeks. The animals were observed regularly for clinical signs of toxicity and body weights were monitored weekly. Necropsy was performed on all of the animals. In the study with rats, supplemental groups of 10 rats were exposed to the same vapor concentrations of Glutaral. These animals were used to evaluate clinical chemistry parameters on days 4 and 24 of the study, and clinical chemistry data from the core study animals were taken at the end of the study.

No treatment-related deaths occurred in the study with rats. Mean final body weights and body weight gains were significantly lower for rats exposed to 1,000 ppb Glutaral as compared with control values. Lower mean body weight gain was also noted for female rats of the 500-ppb group, but the mean final body weight for this group was comparable to the control value. During the first 5 weeks of the study, dyspnea, ruffled fur, and emaciation were observed in the 1,000-ppb group. However, no clinical symptoms were observed in this group later in the study or at any time with the lower exposure groups. Hematology and clinical chemistry parameters were normal on day 4. At day 24, significant increases in total counts of segmented neutrophils and in activities of alanine aminotransferase and alkaline phosphatase were observed in the groups exposed to 250 ppb Glutaral and greater. Additionally, increases in total leukocyte count and urea nitrogen, and mild decreases in the concentrations of total protein and of albumin were observed. A few of these changes were also observed in the rats of the 125-ppb group. No other treatment-related changes were observed when these parameters were evaluated at the end of the study.

At necropsy, a few statistically significant changes in the relative weights of various organs were observed; however, the investigators noted that the differ-
ences were small and did not consider them to be toxicologically significant. Microscopic lesions of the nasal passages and/or turbinates, affecting the respiratory and olfactory epithelium, were observed at all exposure levels but were found predominantly in the groups exposed to 250 ppb Glutaral and greater. Hyperplasia and squamous metaplasia were present in the respiratory epithelium of the septum, as well as in the nasal turbinates and lateral wall. In the high-dose group, hyperplasia of goblet cells on the nasal septum and minimal inflammation (neutrophilic) were also observed. Other observations included: minimal to mild olfactory degeneration, minimal to mild squamous exfoliation in the nasal vestibule, and minimal focal erosion of the squamous mucosa.

In the study with mice, all of the mice exposed to 1,000 ppb Glutaral and two female mice exposed to 500 ppb Glutaral died before the end of the study. Mean final body weight was reduced in a concentration-dependent fashion, and was statistically significant in the 250- and 500-ppb groups. Clinical signs of toxicity observed in animals exposed to 500 and 1,000 ppb Glutaral included: dyspnea, emaciation, abnormal posture, hypoaactivity, ruffled fur, paraphimosis, and tachypnea. The investigators observed increases in organ-weight to body-weight ratios for the heart, kidneys, and lungs of both males and females, the liver of females, and the testes of male mice exposed to Glutaral. However, because no significant increases in absolute organ weights were observed, the investigators attributed these increases to typically higher relative organ weights in lighter weight animals. A few gross lesions were found in mice that died during the study, but the researchers attributed these lesions to secondary effects of dyspnea or generalized stress. Inflammation and squamous metaplasia of the respiratory epithelium and squamous exfoliation of the nasal vestibule and anterior nares was observed in mice exposed to 1,000 ppb Glutaral. These effects were also observed in some mice exposed to 250 and 500 ppb Glutaral, in addition to inflammation and erosion of the nasal vestibule. At the lower exposure concentrations, nasal inflammation and erosion were observed only in female mice. Squamous metaplasia and necrosis of the larynx were observed only in the 1,000-ppb Glutaral group.

In another study (Union Carbide Corporation, 1986), groups of 20 male and 20 female rats were exposed to mean Glutaral concentrations of 20.8, 49.3, and 194.2 ppbv for 6 h/day, 5 days a week, for 14 weeks. No deaths occurred. Rats from the 49.3- and 194.2-ppbv Glutaral groups had perinasal discharge and encrustation and reduced body weights. Rats from the 20.8-ppbv Glutaral group had no signs of irritation, and their weight loss was transient. There were no treatment-related effects on feed consumption, urinalysis, hematology, serum chemistry, and organ weights. No abnormalities were found during necropsy or microscopic examination.

**Immunology**

Two New Zealand rabbits were injected intramuscularly with 10 mg of rabbit serum albumin (RSA) treated with 2% Glutaral in Freund's complete adjuvant. At weeks 2, 3, and 4, the rabbits were given s.c. injections of 10 mg of antigen without adjuvant. Blood samples were taken at weeks 5, 6, and 7. The sera from
each rabbit were pooled and analyzed for elicited antibodies using enzyme-linked immunosorbent assay (ELISA) and horseradish peroxidase (HRP) assay. A weak immunologic response was observed. The average IgG concentration in response to Glutaral-treated RSA was 0.02 mg/ml serum (Ranly et al., 1985).

**Dermal Irritation**

The dermal irritation potential of Glutaral was studied using ICR/CD-1 mice. Denuded mice had 0.25 or 2% Glutaral (0.8 ml/spray) sprayed on their abdomens eight times a day for 2 days. Lesions with focal thickening and hardening, epithelial erosion, and collagen degeneration resulted from this treatment. Irritation was more severe with 2% Glutaral (Hess et al., 1989).

An alkaline 2% Glutaral solution was applied to the intact and abraded skin of rabbits for 24 h, and irritation was scored at 24 and 48 h. Glutaral was a moderate skin irritant; the primary irritation index was 2.125 (maximum possible score: 8.0) (Miner et al., 1977).

A 0.5-ml dose of a 2% aqueous alkaline solution of Glutaral was applied for 6 weeks to the clipped dorsal skin of 20 albino rabbits. The solution was spread with a brush and allowed to dry. The skin was examined daily. After the first application of Glutaral, the skin and hair was stained a faint yellow. The stain became more intense and turned golden brown during the 6 weeks, and it persisted for up to 35 days after the last application. Erythema was mild or questionable, but the discoloration of fur interfered with the estimation of erythema. A mild rash was observed following the first few applications of Glutaral but, in most cases, it disappeared despite continued application of Glutaral. A severe erythematous reaction with edema followed by necrosis and scarring was observed when 25% Glutaral was applied to the skin of rabbits (Stonehill et al., 1963).

Glutaral (of unknown pH) was applied to the skin of rabbits at concentrations of 0.2, 0.7, 2.0, 7.0, and 25%. No irritation was observed with Glutaral concentrations up to 7.0%. However, 25% Glutaral caused mild erythema and eschar formation, which cleared by day 13 (Uemitsu et al., 1976).

Moderate to marked erythema with edema was observed at 24 h when 0.01 ml droplets of 50% Glutaral were applied to shaven rabbit skin. A 25% concentration of Glutaral produced mild to moderate erythema at 24 h (Union Carbide Corporation, 1986).

A 0.5-ml dose of 1, 2, 5, 25, 45, and 50% Glutaral (w/w) solutions was applied under an occlusive dressing for 4 h to the shaven skin of groups of six rabbits, and the treatment sites were observed for 21 days. A dose-response relationship was observed for severity and duration of inflammatory effects. The 45 and 50% Glutaral solutions produced moderately severe and persistent inflammation, which appeared to be associated with, or bordered on, a local corrosive effect. Effects were less marked with 25% Glutaral, but irritation persisted for 14 days. Less persistent minor to moderate effects were observed with 5% Glutaral. The threshold for primary skin irritation was 2% Glutaral, and the no-effects concentration was 1% (Union Carbide Corporation, 1986).

Gad et al. (1986) used Glutaral as one of the known human sensitizers to vali-
date the mouse ear swelling test. In this test, 100 µl of 1% Glutaral was applied to the shaved and tape-stripped abdomens of 10 mice, and allowed to dry. This procedure was repeated for 3 consecutive additional days. After a 7-day nontreatment period, 20 µl of 10% Glutaral was applied to the left pinna of each animal. The right pinna was treated with 70% ethanol, the vehicle. The thickness of both pinnae was measured 24 and 48 h later. Sixty-seven percent of the animals were sensitized to Glutaral, and the degree of pinna swelling was 125%. After reviewing these data, the CIR Expert Panel decided the reported reactions were irritant in nature rather than sensitizing.

**Dermal Sensitization**

Stern et al. (1989) studied the contact hypersensitivity response to Glutaraldehyde using guinea pigs and mice. In preliminary studies, the authors determined that for both species the minimal irritation concentration and maximal nonirritating concentration of Glutaral were 10% and 3%, respectively. Groups of six female Hartley strain guinea pigs were treated with 0.3, 1, and 3% Glutaral (100 µl) on their shaved backs for 14 consecutive days. The guinea pigs were shaved 1 day prior to the first application, and on days 5 and 10. The skin was abraded on the first day of treatment, and then every other day during the induction period. After a 7- or 14-day nontreatment period, the guinea pigs were treated with 10% Glutaral at a previously untreated site. So that a radioisotopic assay could be conducted, the guinea pigs were administered 1.0 ml 5-fluorodeoxyuridine (FUDR) and 10 µCi (1.0 ml) 125I-iododeoxyuridine i.p. 1 day prior to and on the day of challenge. Visual examination was used to detect delayed-type contact hypersensitivity responses 24 and 48 h following the challenge application. Two groups of animals were treated with 1-fluoro-2,4-dinitrobenzene using the same procedures to serve as positive controls, and another group of animals received the vehicle alone to serve as an untreated control.

Cutaneous evidence of sensitization was observed in the animals treated with 3% Glutaral. A radioisotopic assay of the skin removed after 48 h supported this observation, and also indicated that 1% Glutaral stimulated a lesser but significant contact hypersensitivity response. The authors noted that radioisotopic assay of ear biopsies from 14-day rested guinea pigs treated with 3% Glutaral and challenged with 3% Glutaral was negative.

Female B6C3F1 mice were also used to test the sensitization potential of Glutaral. Using similar procedures, the skin on the ventral side of the mice was treated with 0.3, 1, and 3% Glutaral (20 µl) for 5 or 14 consecutive days. After a 4-day nontreatment period, the mice were challenged with 10% Glutaral on the dorsal surface of the left pinna. The mice were killed 24 h later and radioassays were conducted with the ear biopsies. As in the guinea pig study, positive and negative controls groups were tested concurrently. No evidence of contact hypersensitivity was detected in the animals treated with Glutaral. However, when the authors modified the study by using the upper dorsal area of the mice for the induction site, extending the nontreatment period to 7 days, and removing the biopsies after 48 h, contact hypersensitivity responses were detected in the animals treated with 1 and 3% Glutaral (Stern et al., 1989).
In a study by Cornacoff et al. (1988), the radioisotopic incorporation method was compared with the mouse ear swelling test for its ability to detect weak sensitizers. Twenty-four Balb/c mice had filter discs treated with 10% Glutaral (25 μl) attached to their shaved and tape-stripped abdomens. Glutaral was applied to the discs for 3 additional consecutive days. One day prior to challenge, the discs were removed and the mice were injected with 1 mg/kg FUdR, followed by 1 μCi [125I]-iododeoxyuridine 1 h later. On the following day, 25 μl of 2% Glutaral was applied to the left pinna of each animal; the vehicle was applied to the right pinna to serve as a control. The mice were killed 24-48 h later and the thickness of the pinnae was measured. Reactivity to Glutaral was not detected by the radioisotopic assay. However, in the ear swelling test, reactivity was minimally responsive and significant (ear swelling: 0.60 mm × 10^-2).

Descotes (1988) used the mouse ear sensitization assay to determine the sensitization potential of Glutaral. The right pinnae of 18 female Balb/c mice were topically treated on both sides with 1% Glutaral on days 0 and 2. A scapular subcutaneous injection of Freund’s complete adjuvant was also administered on day 2. On day 9, the thickness of the left pinnae was measured followed by topical application of 10% Glutaral. When ear thickness was measured 24 h later, there was a significant increase in thickness.

**Anhidrotic Activity**

Glutaral was dissolved in distilled water at concentrations of 780 to 25,000 mg/100 ml and was applied to the footpads of groups 10 to 20 Swiss Or1 mice. The anhidrotic effect of Glutaral was evaluated by measuring the reduction in palmar skin conductance following its application. Mice grasped the electrodes of a conductance meter by reflex action and skin conductance was measured 1, 4, and 7 h after treatment. The concentration of Glutaral that reduced sweating by 50% was calculated by regression analysis. At 4 h, the concentration of Glutaral that inhibited sweating by 50% was 4,390 mg/100 ml, and at 7 h, the concentration was 6,360 mg/100 ml. Washing the footpads of the mice did not modify the anhidrotic action of Glutaral (Marcy and Quermonne, 1976).

Glutaral also inhibited sweating in studies by Juhlin and Hansson (1968), Comaish (1973), Gordon and Maibach (1969), and Sato and Dobson (1969).

**Ocular Irritation**

A single drop of a 2% acid Glutaral solution was placed in one conjunctival sac of each of two Dutch belted rabbits, and the eyes were observed periodically for 72 h. The rabbits were killed at the end of the 72-h period, and the eyeballs were examined microscopically. Edema and swelling of the conjunctiva were observed 6 h after Glutaral administration. Swelling and exudate was moderate at 18 h. The ocular exudate was thick and purulent, and the cornea was cloudy at 24 h. At 72 h, the cornea remained cloudy, the conjunctiva was red and inflamed, and a slight purulent exudate was present. Edema of the cornea was diffuse, and most of the corneal epithelium was desquamated with thinning and flattening of the remainder of the corneal epithelium. Heterophils, particularly concentrated at the denuded
GLUTARAL

surface, diffusely infiltrated the cornea; conjunctivitis was diffuse, acute, purulent, and necrotizing. The 2% acid Glutaral produced severe and extensive conjunctival injury (Martin, 1978).

A 0.1-ml volume of an alkaline 2% Glutaral solution was placed in one conjunctival sac of the eye of each of 12 rabbits, and the eyes of 3 of the rabbits were rinsed 30 seconds later. Severe corneal opacity and irritation of the iris and conjunctiva were observed in unrinsed eyes during the 7-day observation period. Irritation of the conjunctiva, which was similar in rinsed eyes; also lasted 7 days. However, irritation of the iris and cornea was less in rinsed eyes than in unrinsed eyes, and recovery was partial. The alkaline 2% Glutaral solution was a severe ocular irritant (Miner et al., 1977).

A 0.1-ml volume of an alkaline 2% Glutaral solution was instilled into the conjunctival sac of one eye of each of five albino rabbits. The eyes were examined at 2 h and then daily. Inflammation, lacrimation, and edema were observed. The irritation score was 17 (maximum possible score: 110). Complete recovery occurred 7–8 days after instillation. The alkaline 2% glutaral solution caused severe eye irritation in rabbits (Stonehill et al., 1963).

Glutaral, at concentrations of 0.2, 2, and 25%, was instilled into the conjunctival sac of rabbits. Some of the eyes were rinsed 4 or 10 seconds after instillation. The 0.2% and 2% Glutaral solutions caused chemosis and discharge from both rinsed and unrinsed eyes. In addition to these signs, the 25% Glutaral caused opacity of the cornea and loss of reaction to light by the iris (Uemitsu et al., 1976).

A dose of 0.005 ml of 5–50% (w/w) Glutaral was instilled into the conjunctival sac of one eye of each of six rabbits for each concentration tested, and the eyes were examined after 24 h. Severe chemosis, necrotizing blepharitis, iritis, and severe keratitis were observed in all the rabbits (Union Carbide Corporation, 1986).

A dose of 0.5 ml of 0.25–2.5% Glutaral (w/w) was instilled into the conjunctival sac of the eyes of rabbits (unspecified number), and the eyes were examined after 24 h. Mild superficial corneal injury was observed with 0.25 and 0.5% Glutaral, moderate keratitis with 1.0% Glutaral, and severe keratitis with 1.25 and 2.5% Glutaral (Union Carbide Corporation, 1986).

Groups of six male albino rabbits had 0.005–0.1 ml of 0.1–5.0% Glutaral instilled into the conjunctival sac of their eyes, and the eyes were observed for 3 weeks. There was a dose-response relationship for conjunctival and corneal injury. The 1.0% Glutaral solution was the lowest concentration producing transient minor corneal injury, and 0.2% was the lowest concentration producing transient minimal conjunctival irritation. The no-effects concentration was 0.1% Glutaral (Union Carbide Corporation, 1986).

In an alternative to the Draize rabbit eye irritation test, Glutaral was cytotoxic to human corneal endothelial cell cultures (Douglas and Spilman, 1983).

Reproduction and Developmental Toxicity

Groups of 28 male and 28 female CD rats (F₀ generation) were given Glutaral in drinking water at concentrations of 0, 50, 250, and 1,000 ppm for 10 weeks. At the
end of 10 weeks, the rats were randomly paired within each dose group and allowed to mate. Treated water was administered throughout the mating, gestation, and lactation periods. The F₀ generation and its offspring (F₁ generation) were monitored for toxicity until the weaning of the litters at postnatal day 21. After weaning, the parental F₀ generation and 10 F₁ weanlings of each sex were killed and necropsy was performed. Groups of 28 rats of each sex were selected from the F₁ generation and were administered Glutaral for 10 weeks in drinking water at the same concentrations as their parents. These rats were paired and allowed to mate as described above to produce the F₂ generation. Treatment with Glutaral was continued with the dams and offspring through mating, gestation, and lactation. The F₁ males were killed for necropsy after delivery of the litters, and the F₁ females were killed and necropsied following weaning of the F₂ generation. Ten F₂ weanlings of each sex were also killed for necropsy.

No treatment-related deaths or clinical signs of toxicity were observed in the F₀ generation during the 10-week prebreed period. There was a consistent reduction in water consumption at concentrations of 250 and 1,000 ppm Glutaral, which was attributed to aversion to the taste of the treated water. In the high-dose group, there were transient reductions in body weight, body weight gains, and reductions in food consumption. There was also a slightly decreased average body weight gain in F₀ males of the low-dose group during the first week of treatment. During gestation and lactation, water consumption remained reduced in the dams of the mid- and high-dose groups. Reduced food consumption was also observed in the high-dose females (F₀ and F₁) near the end of gestation.

No effects on mating, fertility, or gestational factors were observed. There were no adverse effects on litter size or sex ratio in the F₁ or F₂ litters. The investigators noted that the pup body weights and weight gains were reduced in both the F₁ and F₂ 1,000 ppm Glutaral treatment groups. However, this did not affect the viability or survival of these pups. The investigators concluded that the NOAEL for adult and offspring toxicity was 50 ppm and 250 ppm Glutaral, respectively. The NOAEL for reproductive effects was >1,000 ppm Glutaral (Bushy Run Research Center, 1994b).

In the subchronic inhalation study by Kari (1993), described earlier in this report, no adverse effects on sperm morphology or vaginal cytology was observed in F344/N rats whole-body exposed to vaporized Glutaral at concentrations of 1,000 ppb and lower. However, in studies with B6C3F₁ mice, there were changes in the amount of time spent in estrous stages for females of the 250- and 500-ppb groups as compared with controls. More time was spent in estrus and diestrus and less time in metestrus and proestrus (500-ppb group only). No adverse effects were observed in any of the reproductive parameters studied in male mice.

Groups of 15 pregnant Himalayan rabbits were administered 5, 15, and 45 mg/kg of Glutaral by gavage on days 7-19 of gestation. A control group of rabbits was administered double distilled water. The animals were monitored for changes in feed consumption, body weight, and general behavior. On day 29 postinsemination, all of the surviving animals were killed, necropsy was performed, and the fetuses were examined.

Maternal toxicity was marked in the rabbits of the 45-mg/kg Glutaral treatment
GLUTARAL

A group. Five of the rabbits died and feed consumption and body weight gain were severely reduced. Blood was found in the bedding of these animals, and some rabbits had diarrhea or no defecation during treatment or posttreatment. There was increased postimplantation loss due to a large number of early resorptions, and live fetuses were found in only one rabbit. The four fetuses had significantly reduced mean body weights, but there was no evidence of teratogenic effects. In the 5- and 15-mg/kg Glutaral groups, there was no evidence of maternal or fetal toxicity, and no evidence of teratogenicity (BASF Corporation, 1991a).

Glutaral was also tested for prenatal toxicity using Wistar rats. Groups of 25 pregnant female rats were administered drinking water containing 50, 250, or 750 ppm Glutaral on days 6–16 of gestation. A control group of rats was treated with doubly distilled water. Food and water consumption and body weights were recorded regularly. On day 20 postcoitum, all the rats were killed, the fetuses were removed and examined, and necropsy was performed. Glutaral appeared to make the water unpalatable, as water consumption was dose-dependently reduced in both the 250- and 750-ppm Glutaral groups. There was no evidence of embryotoxicity, fetotoxicity, or teratogenicity (BASF Corporation, 1991b).

Groups of 18–48 albino CD-1 mice, of which 11–24 were pregnant, received 0.8, 1.0, 1.2, 2.0, 2.5, or 5.0 ml/kg/day of Sonacide on days 6–15 of gestation via gastric intubation. Sonacide contains 2% Glutaral, nonionic ethoxylates of isomeric linear alcohols, and, when necessary, orthophosphoric acid to adjust the pH to between 2.7 and 3.7. All of the mice were killed on day 18. A control group of 86 pregnant mice were administered distilled water. The general health and reproductive status of the mice were evaluated, and the external, visceral, and skeletal malformations of the fetuses were characterized.

Sonacide was highly toxic to mice at doses of 2.0 ml/kg/day and greater; 6 of 35 mice administered this dose died. Twelve of 48 mice that received 2.5 ml/kg/day died, and 19 of 35 mice that received 5.0 ml/kg/day died. A significant and dose-related reduction in the average weight gain was observed during pregnancy in mice that received 0.8, 1.2, and 5.0 ml/kg/day. Fetal weight was significantly reduced in the 0.8-ml/kg/day group. Fetal toxicity was apparent in the 5.0-ml/kg/day group, where the number of stunted fetuses was significantly increased. Stunted fetuses weighed 0.5 g or less than two-thirds of the mean weight of their larger littermates. The percentage of malformed fetuses in the 5.0-ml/kg/day group was increased. The authors speculated that this may have been a statistical anomaly. One of three dams with at least one malformed pup had only two fetuses, and both were malformed. Not including this litter, the results at the 5.0-ml/kg/day Sonacide dose (100 mg/kg/day Glutaral) were not significant. No indication of significant teratogenic effects was observed at doses that were not highly toxic maternally (Marks et al., 1980).

Groups of pregnant mice (number unspecified) were given oral doses of 3.3, 10, and 30 mg/kg/day Glutaral on days 7 to 12 of gestation and were killed on day 18. A slight decrease in maternal body weight was observed in the group given 30 mg/kg/day Glutaral. There was no evidence of embryotoxicity, fetotoxicity, or teratogenicity (Union Carbide Corporation, 1986).

In a pilot study, oral doses of 100, 300, and 1000 mg/kg/day of 25% Glutaral
killed pregnant rats. When oral doses of 25 and 50 mg/kg/day were given to pregnant albino rats on days 6 to 15 of gestation, a decrease in maternal body weight was observed. However, there was no evidence of fetotoxicity, or of external, visceral, or skeletal malformations (Union Carbide Corporation 1986).

**MUTAGENICITY**

A number of mutagenicity studies were conducted on Glutaral, producing both positive and negative results. These studies are summarized in Table 2.

**CARCINOGENICITY**

In a 2-year carcinogenicity study (Union Carbide Corporation, 1986), 100 male and 100 female Fischer 344 rats were administered 50, 250, and 1,000 ppm Glutaral in their drinking water. A control group of 100 male and 100 female rats were given regular water. The animals were observed for clinical signs of toxicity weekly, and palpating for masses began on week 27. Body weight and feed and water consumption were measured weekly, and analyses of the urine and blood were conducted regularly. Twenty rats (10 of each sex) were killed at 52 and 78 weeks for interim necropsy; necropsy was performed on the remaining rats either at the time of interim death or when they were killed at the end of the study.

None of the dosages tested had any effect on mortality or survival. Water and feed consumption were reduced, which probably contributed to the observed renal and urinary effects (renal weight changes, decreased urine volume, and increased osmolality). Tubular pigmentation and basophilia were found during microscopic examination of the kidneys, but the authors attributed these lesions to low-grade hemolytic anemia that accompanied the large granular lymphocytic leukemia (LGLL) found at necropsy. The cumulative incidence of LGLL in the spleen and liver is presented in Table 3.

All parameters examined and the results that were obtained are presented in Table 4.

Because the spleen is the site of origin of LGLL, statistical analyses were conducted on the data obtained from this organ. The incidence of LGLL was significantly increased in the female rats of all the dose groups, but there was no dose-response or statistical evidence of an increase in LGLL in male rats. Based on data from historical controls, the authors noted that LGLL is a commonly occurring spontaneous neoplasm in Fischer 344 rats, and that the incidence LGLL varies among studies in the same laboratory as well as among laboratories (Bushy Run Research Center, 1993). The incidence of LGLL in the untreated control group for this study was 43% for males and 24% for females, whereas the incidences were 22% for males and 66% for females in a previous study in the same laboratory. In light of this, the incidence of LGLL in the control females of the current study may have been low. Additionally, the female rats received higher doses of Glutaral than the males (See Table 3). Based on these considerations, the authors concluded: "The nature of the response [increased incidence of LGLL in female rats only], and the factors associated with it, suggest that this was not a direct chemical carcinogenic effect but resulted from a modifying influence on
# TABLE 2. Mutagenicity studies with Glutaral

<table>
<thead>
<tr>
<th>Test</th>
<th>Concentration</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ames test—TA1535, TA1537, TA1538, TA98, and TA100; ± metabolic activation</td>
<td>0.15-15.4 µg/plate</td>
<td>Negative</td>
<td>Slesinski et al., 1983</td>
</tr>
<tr>
<td>Ames test—TA98 and TA100; ± metabolic activation</td>
<td>0-20 µg/plate</td>
<td>Negative</td>
<td>Sakagami et al., 1988a</td>
</tr>
<tr>
<td>Ames test—TA98 and TA100; ± metabolic activation</td>
<td>Preincubation with 25% Glutaral</td>
<td>Negative</td>
<td>Sasaki and Endo, 1978</td>
</tr>
<tr>
<td>Ames test—TA1535, TA1537, TA98, and TA100; ± metabolic activation</td>
<td>3.3-3.333 µg/plate</td>
<td>Negative</td>
<td>Haworth et al., 1983</td>
</tr>
<tr>
<td>Ames test—TA102</td>
<td>25 µg/plate</td>
<td>Positive</td>
<td>Levin et al., 1982</td>
</tr>
<tr>
<td>t-arabinose forward mutation assay—S. typhimurium BA9 and BA13.</td>
<td>1-100 µg/ml</td>
<td>Positive at levels as low as 10 µg/ml ± metabolic activation</td>
<td>Sakagami et al., 1988b</td>
</tr>
<tr>
<td>conc. rec-assay—B. subtilis M-45(rec') and H-17(rec') ± metabolic activation</td>
<td>0-300 µg/ml</td>
<td>Positive at levels as low as 200 µg/ml both ± metabolic activation</td>
<td>Sakagami et al., 1988a</td>
</tr>
<tr>
<td>Mutation assay E. coli WP2 uvrA; no metabolic activation</td>
<td>20-10,000 µM</td>
<td>Negative</td>
<td>Hemminki et al., 1982</td>
</tr>
<tr>
<td>Forward mutation assay—E. coli WP2 uvrA/pKM 101; no metabolic action</td>
<td>5 µg/ml (SM'-5)</td>
<td>Positive dose-response effect</td>
<td>Kosako and Nishioka, 1982</td>
</tr>
<tr>
<td>Forward mutation assay—L5178Y tk+/tk mouse lymphoma cells: ± metabolic activation</td>
<td>0.5-8.0 µg/ml</td>
<td>Positive</td>
<td>McGregor et al., 1988</td>
</tr>
<tr>
<td>Human TK6 lymphoblast cell line mutation analysis</td>
<td>1-20 µM</td>
<td>Positive between 10-20 µM</td>
<td>St. Clair et al., 1991</td>
</tr>
<tr>
<td>CHO cell HGPRT assay: ± metabolic action</td>
<td>0.03-30.6 µM</td>
<td>Negative</td>
<td>Slesinski et al., 1983</td>
</tr>
<tr>
<td>SCE in CHO cells: ± metabolic activation</td>
<td>0.6-2.5 µM</td>
<td>Negative</td>
<td>Slesinski et al., 1983</td>
</tr>
<tr>
<td>SCE in BHK-21 baby hamster kidney cells</td>
<td>1-20 ppm</td>
<td>Negative</td>
<td>Torres, 1987</td>
</tr>
<tr>
<td>SCE in CHO cells</td>
<td>0.36-0.16 E + 02 µg/ml</td>
<td>Positive in 2 laboratories</td>
<td>Galloway et al., 1985</td>
</tr>
<tr>
<td>Chromosome aberrations in CHO cells</td>
<td>0.30-0.16E + 02 µg/ml</td>
<td>Positive in 1 laboratory:</td>
<td>Galloway et al., 1985</td>
</tr>
<tr>
<td>Unscheduled DNA synthesis in male F-344 rat hepatocytes</td>
<td>0.01-100 µM</td>
<td>Positive at 50 and 100 µM; induction significant only at 100 µM</td>
<td>St. Clair et al., 1991</td>
</tr>
<tr>
<td>Unscheduled DNA synthesis in rat hepatocytes</td>
<td>0.05-51.0 µM</td>
<td>Negative</td>
<td>Slesinski et al., 1983</td>
</tr>
<tr>
<td>Unscheduled DNA synthesis in male rat hepatocytes isolated</td>
<td>30-600 mg/kg</td>
<td>Negative</td>
<td>Mirsalis et al., 1989</td>
</tr>
<tr>
<td>following oral administration</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(continued)
TABLE 2. Continued

<table>
<thead>
<tr>
<th>Test</th>
<th>Concentration</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unscheduled DNA synthesis in male rat hepatocytes isolated following oral administration</td>
<td>Not stated</td>
<td>Negative</td>
<td>Mirsalis et al., 1985</td>
</tr>
<tr>
<td>D. melanogaster sex-linked recessive lethal mutation assay—Glutaral to larvae in feed</td>
<td>3.500 ppm</td>
<td>Negative</td>
<td>Zimmering et al., 1989</td>
</tr>
<tr>
<td>D. melanogaster sex-linked recessive lethal assay—in feed or by injection</td>
<td>feed: 7,500–10,000 ppm Inject: 3,000–4,000 ppm</td>
<td>Negative</td>
<td>Yoon et al., 1985</td>
</tr>
<tr>
<td>Dominant lethal index—given to female mice prior to mating</td>
<td>30–60 mg/kg</td>
<td>Negative</td>
<td>Tamada et al., 1978</td>
</tr>
<tr>
<td>Segmentation mitosis—Triturus helveticus, Pleurodeles waltii, Triturus marmoratus, and Bufo b u o eggs treated at various stages</td>
<td>0.00312–0.05 M</td>
<td>Arrested mitosis; spindle and astral fibers disappeared; chromosomes immobilized at the equatorial region; centromeres blocked</td>
<td>Sentein, 1975</td>
</tr>
</tbody>
</table>

determinants normally controlling the expression of this spontaneously occurring neoplasm" (Union Carbide Corporation, 1993).

CLINICAL ASSESSMENT OF SAFETY

Provocative Tests and Case Studies of Dermal Irritation

Case studies and reports of dermal irritation caused by Glutaral are detailed in Table 5. Mixed results were reported.

Dermal Sensitization

Glutaral was tested for sensitization in 102 male subjects. Ten occlusive induction patches containing 0.1% Glutaral in petrolatum were applied to the upper

TABLE 3. Incidence of large granular lymphocytic leukemia in the two-year drinking water studya

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Sex</th>
<th>Dosage group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 ppm</td>
</tr>
<tr>
<td>Spleen</td>
<td>M</td>
<td>43/100</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>24/100</td>
</tr>
<tr>
<td>Liver</td>
<td>M</td>
<td>37/100</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>23/100</td>
</tr>
</tbody>
</table>

a Union Carbide Corporation, 1993.
**GLUTARAL**

**TABLE 4. Observations during a 2-year drinking water study**

<table>
<thead>
<tr>
<th>Parameter measured</th>
<th>Dosage group (Glutaral in water)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 ppm</td>
</tr>
<tr>
<td>Average daily Glutaral consumption</td>
<td>Males: 3.6 ± 0.76</td>
</tr>
<tr>
<td>(mg/kg/day)</td>
<td>Females: 5.5 ± 0.90</td>
</tr>
<tr>
<td>Mortality,</td>
<td>All 3 endpoints: no effect</td>
</tr>
<tr>
<td>Mean survival time, and</td>
<td></td>
</tr>
<tr>
<td>Ophthalmology</td>
<td></td>
</tr>
<tr>
<td>Absolute body weights</td>
<td>No effect</td>
</tr>
<tr>
<td></td>
<td>Females: no effect</td>
</tr>
<tr>
<td>Body weight gains</td>
<td>No effect</td>
</tr>
<tr>
<td></td>
<td>Females: no effect</td>
</tr>
<tr>
<td>Water consumption</td>
<td>Males: slightly decreased</td>
</tr>
<tr>
<td></td>
<td>Females: slightly decreased</td>
</tr>
<tr>
<td>Feed consumption</td>
<td>No effect</td>
</tr>
<tr>
<td></td>
<td>Females: slight decrease</td>
</tr>
<tr>
<td>Hematology</td>
<td>No effect</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical chemistry</td>
<td>No effect</td>
</tr>
<tr>
<td>Urinalysis</td>
<td>No effect</td>
</tr>
<tr>
<td>Relative renal weight</td>
<td>Males: decrease at 104 wk</td>
</tr>
<tr>
<td></td>
<td>Females: no effect</td>
</tr>
<tr>
<td>Absolute renal weight</td>
<td>Males: decrease at 104 wk</td>
</tr>
<tr>
<td></td>
<td>Females: no effect</td>
</tr>
<tr>
<td>Gastric pathology</td>
<td>No effects</td>
</tr>
<tr>
<td>Renal pathology</td>
<td>No effects</td>
</tr>
<tr>
<td></td>
<td>Females: tubular pigmentation and basophilia at 104 wk</td>
</tr>
<tr>
<td>Tumors</td>
<td>Males: no effect</td>
</tr>
<tr>
<td></td>
<td>Females: increased incidence of large granular lymphocytic leukemia at 104 wk</td>
</tr>
</tbody>
</table>

* Union Carbide Corporation, 1993

* Statistically significant findings.

lateral portion of the arm for 48 to 72 h over 3 weeks. A nontreatment period of 2 weeks was followed by an occlusive challenge patch containing 0.5% Glutaral in petrolatum. Skin reactions were graded on a scale of 1 to 4, and a grade of 2 or greater was considered positive. No sensitization was observed among the 102 men. The experiment was repeated with 30 men and with 5.0% Glutaral induction
### TABLE 5. Provocative tests and case studies of dermal irritation

<table>
<thead>
<tr>
<th>Case study/testing</th>
<th>Findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>657 patients with eczematous dermatitis were patch-tested with 2% Glutaral</td>
<td>One (0.1%) positive reaction occurred</td>
<td>Angelini et al., 1985</td>
</tr>
<tr>
<td>160 patients were patch-tested with 1% Glutaral (pH 7.5)</td>
<td>No positive reactions occurred</td>
<td>Juhlin and Hansson, 1968</td>
</tr>
<tr>
<td>The chamber-scarification test was used to test the irritancy of 2% Glutaral on 5 subjects</td>
<td>‘Marked” irritancy was observed at 72 h</td>
<td>Frosch and Kligman, 1977</td>
</tr>
<tr>
<td>The chamber-scarification test was used to test the irritancy of 2% Glutaral on 5 subjects</td>
<td>‘Marked” irritancy was observed at 72 h</td>
<td>Frosch and Kligman, 1977</td>
</tr>
<tr>
<td>2 patients allergic to Glutaral were patch-tested with fabric treated with a fabric softener containing 550 and 5,500 ppm Glutaral</td>
<td>No adverse reactions occurred</td>
<td>Weaver and Mailbach, 1977</td>
</tr>
<tr>
<td>An “open” exaggerated use test was conducted on 2 subjects allergic to Glutaral using undiluted fabric softener containing 550 ppm Glutaral</td>
<td>No signs of cutaneous allergy were observed</td>
<td>Weaver and Maibach, 1977</td>
</tr>
<tr>
<td>14 Glutaral-sensitive subjects were cotton T-shirts treated with fabric softener containing 550 ppm Glutaral for 24 h for 14 days</td>
<td>No irritation occurred</td>
<td>Weaver and Maibach, 1977</td>
</tr>
<tr>
<td>13 patients were patch-tested with 1% Glutaral</td>
<td>9 subjects had irritant reactions</td>
<td>Hansen and Menne, 1990</td>
</tr>
<tr>
<td>884 patients were patch-tested with 0.1% Glutaral</td>
<td>2 subjects had allergic reactions, 1 of which had clinical relevance; no irritant reactions were observed</td>
<td>Hansen and Menne, 1990</td>
</tr>
<tr>
<td>A woman with allergic contact dermatitis to a hair conditioner containing &gt; 1% Glutaral was patch-tested with standard screening trays, the conditioner, and 0.05, 0.1, 0.5, and 1% Glutaral</td>
<td>The woman had positive reactions to all of the Glutaral concentrations except 0.05%</td>
<td>Jaworsky et al., 1987</td>
</tr>
<tr>
<td>2 patients with allergic contact dermatitis to Glutaral were patch-tested with 0.25 and 1% aq. Glutaral, moistened Glutaral-tanned leather, and formaldehyde</td>
<td>The patients had positive reactions to both concentrations of Glutaral and most of the leather samples; neither subject reacted to formaldehyde</td>
<td>Jordan et al., 1972</td>
</tr>
<tr>
<td>20 subjects suspected to be contact-sensitive to Glutaral were patch-tested with 1% Glutaral</td>
<td>All of the subjects had strong reactions</td>
<td>Maibach. 1975</td>
</tr>
<tr>
<td>9 subjects sensitized a year previously to Glutaral were patch-tested with Glutaral</td>
<td>6 subjects (66%) had positive reactions</td>
<td>Maibach and Prystowsky, 1977</td>
</tr>
<tr>
<td>Subjects who patch-tested positive to Glutaral were tested in a usage test with 25% Glutaral on soles of feet and antecubital fossae</td>
<td>6/6 subjects were negative on the soles; severe dermatitis on antecubital fossae occurred on 5/5 subjects</td>
<td>Maibach and Prystowsky, 1977</td>
</tr>
</tbody>
</table>

patches and a 0.5% Glutaral challenge patch. Seven (23.3%) of the men were sensitized (Marzulli and Maibach, 1974).

Patch tests were performed on the backs of 109 male and female subjects with 0.1, 0.2, and 0.5% aqueous Glutaral. Ten induction patches were applied over 3
weeks. The patches were held in place for 48 to 72 h with an occlusive dressing and tape. On removal of each patch, the sites were scored. Two weeks after removal of the final induction patch, challenge patches with the same three concentrations of Glutaral were applied under occlusive dressing for 48 h to new application sites. Reactions were scored 24 h after removal of the challenge patches. No reactions were observed in any of the 109 subjects during induction or after challenge with 0.1% Glutaral. Doubtful reactions were observed in two subjects with 0.2% Glutaral induction patches; one subject had a doubtful reaction after the third induction application and another reaction after the ninth application. The other subject developed an erythematous reaction after removal of the sixth induction patch. No reactions resulted from the 0.2% challenge patch. Reactions were doubtful in nine subjects, and definite erythema was observed in seven subjects treated with 0.5% Glutaral. These definite reactions were present after only one patch in five subjects and after two successively applied patches in the other two individuals. At challenge, 0.5% Glutaral caused a doubtful reaction in one subject, and local erythema and edema in another subject. Neither of these subjects reacted to the induction patches (Ballantyne and Berman, 1984).

The sensitization potential of a fabric softener containing 550 ppm Glutaral was evaluated. Repeat insult patch tests were performed on 706 volunteers who were not hypersensitive to Glutaral. Twenty-four hour occlusive patches of a 4% aqueous solution of the fabric softener (22 ppm Glutaral) were applied to the upper arm of 366 subjects, and patches of a 4% aqueous solution of the experimental formulation (220 ppm Glutaral) were applied to interscapular areas of 340 subjects 9-10 times over 3 weeks. The subjects received single 24- or 48-h challenge patches after a nontreatment period of 2 weeks. Each of these tests was duplicated. No evidence of irritation was observed at any time (Weaver and Maibach, 1977). The preceding studies are summarized in Table 6.

No cross sensitization between Glutaral and formaldehyde has been reported (Union Carbide Corporation, 1986).

Phototoxicity and Photoallergy

Fifty-two volunteers had 0.2 ml of 0.005, 0.01, 0.02, and 0.05% Glutaral applied under occlusive patches to duplicate sets of sites on the infrascapular region of their backs. The patches were removed after 24 h and the sites graded. One set of sites was irradiated with 24 J/cm² of UVA (320-400 nm) using a xenon arc solar simulator (150 W). A UVB absorbing filter was used to eliminate eryhemogenic wavelengths (below 320 nm). The control site, which was not treated with Glutaral, was also irradiated. The other set of treated sites was not exposed to irradiation. All the sites were examined 24 and 48 h after irradiation. Eight subjects had erythema at their treated-irradiated sites at the 24- and 48-h reading for one or more of the concentrations. The authors discovered minimal leakage of UVB irradiation around the filter, and speculated that the irritation may have been the result of exposure to both UVA and UVB. The eight subjects and another subject, who had irritation at both a treated-irradiated site and the control site, were
TABLE 6. Human dermal sensitization studies with Glutaral

<table>
<thead>
<tr>
<th>Test</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>102 male subjects, induction with 0.1% and challenge with 0.5%</td>
<td>No sensitization was observed</td>
<td>Marzulli and Maibach, 1974</td>
</tr>
<tr>
<td>30 male subjects, induction with 5.0% and challenge with 0.5%</td>
<td>7 (23.3%) positive for sensitization</td>
<td>Marzulli and Maibach, 1974</td>
</tr>
<tr>
<td>109 male and female subjects, induction and challenge with 0.1%</td>
<td>No irritation or sensitization was observed</td>
<td>Ballantyne and Berman, 1984</td>
</tr>
<tr>
<td>109 male and female subjects, induction and challenge with 0.2%</td>
<td>2 doubtful reactions during induction; no reactions at challenge</td>
<td>Ballantyne and Berman, 1984</td>
</tr>
<tr>
<td>109 male and female subjects, induction and challenge with 0.5%</td>
<td>9 doubtful and 7 definite erythematous reaction during induction; 1 doubtful and 1 local erythematous reaction with edema during challenge; both failed to react to induction patch</td>
<td>Ballantyne and Berman, 1984</td>
</tr>
<tr>
<td>RIPT on 706 individuals using a 4% aq. solution containing either 22 or 220 ppm Glutaral</td>
<td>No signs of irritation or sensitization were observed</td>
<td>Weaver and Maibach, 1977</td>
</tr>
</tbody>
</table>

Glutaral was also tested for the induction of photoallergic responses at concentrations of 0.005, 0.01, 0.02, and 0.05%. Ninety-nine volunteers had 0.2 ml of Glutaral applied under occlusive patches to duplicate sets of sites on the right and left lumbar areas of their backs. After 24 h of exposure, the patches were removed, and one set of sites was irradiated using a xenon arc solar simulator (150 W) with a continuous emission spectrum in the UVA and UVB range (290-400 nm). The sites were irradiated with twice the subject’s minimal erythema dose, the time of light exposure necessary to produce minimal perceptible erythema on the skin (which was determined prior to testing using areas other than the testing sites), using the full xenon lamp spectrum. The sites were evaluated 24 h after application, and 48 or 72 h after irradiation. A total of six applications and irradiations was made, followed by a 10- to 13-day nontreatment period. The subjects were challenged at duplicate sets of sites previously unexposed to Glutaral. After 24 h, one of the sets was irradiated with 6 J/cm² of UVA (320-400 nm) using a UVB-filtered (below 320 nm) light source. A previously untreated site was also irradiated to serve as an irradiated control. The other set of sites was not irradiated. Sites were evaluated 24 h after Glutaral application, and 24, 48, and 72 h following irradiation. At concentrations of 0.01 and 0.005%, one individual had signs of erythema at the 24- and/or 48 h challenge reading; however, at the higher concentrations there were no reactions. One other subject had irritation 24 and 48 h after the challenge irradiation, but also had irritation at the nonirradiated sites.

GLUTARAL

There was no evidence that Glutaral was a photoallergen (TKL Research, Inc., 1990b).

Occupational Studies and Observations

A number of reports have been published on contact dermatitis and respiratory effects due to occupational exposure to Glutaral in health care and funeral service workers. These studies are detailed in Table 7.

SUMMARY

Glutaral is a saturated five-carbon dialdehyde. It is a highly effective broad-spectrum antimicrobial used as a preservative in cosmetics. In 1994, the cosmetic industry reported to the FDA that Glutaral was used in 60 cosmetic formulations.

In vitro studies indicate that very little Glutaral penetrates the skin of human beings. The skin of laboratory animals had a greater degree of absorption, although the total amount of Glutaral that penetrates the skin is still low. When radioactive Glutaral was applied to the skin of rats and rabbits, it was distributed throughout the body and excreted in the urine and feces, and in expired CO₂. Although the metabolism of Glutaral has not been studied in detail, it has been suggested that Glutaral might be oxidized first to a mono- or di-carboxylic acid by aldehyde dehydrogenase and then further oxidized through an acidic intermediate to CO₂.

The acute oral LD₅₀s for rats for 1–50% solutions of Glutaral ranged from 66–733 mg/kg, and were generally greater at higher concentrations. This may have been a result of different rates of absorption due to injuries in the alimentary tract with greater concentrations or a result of differences in the mechanism of acute toxicity. In addition, acute oral LD₅₀s may vary due to different degrees of polymerization of Glutaral solutions. Larger Glutaral doses caused lesions in the alimentary tract, adrenal glands, kidneys, liver, and lungs.

Minimal toxic effects were observed when rats were given up to 1,000 ppm Glutaral in their feed or water for up to 13 weeks. At drinking water concentrations >250 ppm Glutaral, physiological compensatory changes in response to aversion-induced decreased water consumption were observed. In studies with mice, marginal to minimal toxic effects were observed with 250 and 1,000 ppm Glutaral in the drinking water. However, all of the parameters that were altered during the study returned to control values during a 6-week recovery period.

In studies with dogs, 150 and 250 ppm Glutaral in the drinking water was irritating to the gastric mucosa and induced vomiting, but no gross or microscopic evidence of gastrointestinal injury was observed.

The acute dermal LD₅₀s of 25–50% Glutaral for rabbits ranged from 897–3,045 mg/kg. Lesions were present in liver, lungs, kidneys, and spleen of animals that died. A 16-ml/kg dose of a 15% Glutaral solution caused 1 of 10 rabbits to die, and 16 ml/kg of 5 and 10% Glutaral solutions caused no deaths.

No signs of toxicity were observed when 50 µl of up to 50% Glutaral was applied to the skin of rabbits 10 times, or when 0.5 ml of 2% Glutaral was applied...
TABLE 7. Occupational exposure to Glutaral

<table>
<thead>
<tr>
<th>Study description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A female dental assistant with contact dermatitis from a 2% buffered Glutaral disinfectant. Positive patch-test to the disinfectant, but not to 2% aq. formalin.</td>
<td>Lyon, 1971</td>
</tr>
<tr>
<td>Three female dental assistants with eczematous dermatitis of the hands were patch-tested with 0.25 and 1% aq. Glutaral, several samples of moistened Glutaral-tanned glove leather, and 2.5% aq. formaldehyde. All had positive reactions to Glutaral and most of the leather samples. None reacted to formaldehyde.</td>
<td>Jordan et al., 1972</td>
</tr>
<tr>
<td>A questionnaire survey of 59 endoscopy units in Britain indicated that of 43 centers that used Glutaral to disinfect endoscopes, 16 reported staff becoming sensitive to the disinfectant; 36 individuals complained of effects, including dermatitis, conjunctivitis, and nasal irritation.</td>
<td>Axon et al., 1981</td>
</tr>
<tr>
<td>Occupational dermatoses were found in 83 of 541 hospital cleaning women. Allergic contact eczema was seen in 21 of the women, but only one case was caused by Glutaral. One female assistant nurse in the same ward had allergic contact eczema caused by Glutaral. Both women were exposed to a Glutaral disinfectant and were positive in patch tests with 1% aq. Glutaral.</td>
<td>Hansen, 1983</td>
</tr>
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<td>A woman in charge of an endoscopy unit complained of irritant conjunctivitis and breathlessness thought to be due to exposure to 2% Glutaral. Lowered air flow to her lungs was observed during the work week, but showed improvement on weekends. When a different disinfectant was used, there was only one significant fall in peak flow, which coincided with the time at which a different staff person was using Glutaral in an adjoining room.</td>
<td>Benson, 1984</td>
</tr>
<tr>
<td>Four nurses who worked in hospital endoscopy units reported respiratory symptoms after exposure to a disinfectant containing 2% aq. alkaline Glutaral. All of the nurses were atopic, and 3 had either asthma or rhinitis or both. Following 8 h in a challenge chamber that simulated work exposure, 1 nurse had a late asthmatic response, 1 had a dual nasal response, but symptoms or objective changes were observed in the other 2 nurses.</td>
<td>Osman et al., 1985</td>
</tr>
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<td>Two nurses who developed contact dermatitis after handling instruments disinfected with Glutaral were patch-tested with 1% aq. Glutaral and 2% formalin. The subjects were positive to Glutaral, and negative reactions to formalin.</td>
<td>Sanderson and Cronin, 1968</td>
</tr>
<tr>
<td>A surgical instruments nurse suffered from chronic dermatitis of the hands with marked dryness, redness, infiltration, and fissures. She had a strong positive reaction to a patch test with a disinfectant containing Glutaral.</td>
<td>Bardazzi et al., 1986</td>
</tr>
<tr>
<td>A nurse responsible for disinfecting endoscopic instruments developed contact dermatitis, with redness, dryness, and hyperkeratosis of her hands and feet. In patch tests, she had positive reactions to Glutaral and disinfectants containing Glutaral.</td>
<td>Bardazzi et al., 1986</td>
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TABLE 7. Continued

<table>
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<tr>
<th>Study description</th>
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<tr>
<td>Four nurses working in endoscopy units complained of respiratory symptoms when exposed to a disinfectant containing 2% Glutaral. In tests designed to simulate occupational exposure, each nurse had three 20-min exposures, separated by 40-min intervals. Two had positive reactions; one had both immediate and delayed nasal responses, and one had an isolated late response.</td>
<td>Corrado et al., 1986</td>
</tr>
<tr>
<td>Technologists exposed to Glutaral (1.5-3%) during tissue fixing complained of dermal, ocular, and respiratory irritation. Air samples ranged from 0 to 0.21 mg/M³ (values are below the American Conference of Governmental Industrial Hygienists Threshold Limit Value of 0.7 mg/M³). After interviewing the workers, it was found that adverse symptoms had occurred in the past, and that improved ventilation had resolved the symptoms, and that a health hazard did not exist from Glutaral exposures in this particular work environment.</td>
<td>NIOSH, 1986</td>
</tr>
<tr>
<td>An evaluation of 44 workers who used 2% Glutaral in a disinfecting agent to clean medical equipment found ocular irritation, nasal irritation, throat irritation, skin irritation, head aches, and sore throats. The concentration of Glutaral in personal breathing areas ranged from 0 to 1.6 mg/M³, and in area air samples it ranged from 0 to 1.0 mg/M³. It was concluded that a Glutaral exposure health hazard did exist in this particular work environment.</td>
<td>NIOSH, 1987a</td>
</tr>
<tr>
<td>An investigation in hospital departments where cleaning procedures used a 2% Glutaral disinfectant found air samples in personal breathing zones ranged from 0 to 1.98 mg/M³, area samples ranged from 0 to 0.74 mg/M³, and about 50% of the samples were above the ACGIH Ceiling—TLV of 0.7 mg/M³. Nurses using the disinfectant complained of ocular, respiratory, and dermal irritation. It was concluded that a Glutaral exposure health hazard did exist in this particular work environment.</td>
<td>NIOSH, 1987b</td>
</tr>
<tr>
<td>Eighty-four funeral service workers exposed to formaldehyde were patch-tested with 1% aq. Glutaral; 7% of the workers had positive reactions. A group of 38 workers not exposed to formaldehyde had negative patch results.</td>
<td>Nethercott and Holness, 1988</td>
</tr>
<tr>
<td>A hospital cleaner developed contact dermatitis of the hands 4 months after using a disinfectant containing Glutaral. He had positive reactions to patch tests with the disinfectant, a 10% solution of the disinfectant, and 0.5 and 1.0% aq. Glutaral. Standard patch tests were negative.</td>
<td>Di Prima et al., 1988</td>
</tr>
<tr>
<td>Thirteen cases of occupational hand contact dermatitis caused by Glutaral were reported between 1981 and 1987 in a clinic. Patch tests were conducted using 1% Glutaral and read after 30-60 min, and at 96 h. Nine subjects had positive responses at both readings, and 4 had positive responses only at 96 h. Sensitivity to other chemicals were noted in 10 subjects. Follow-up on the subjects indicated that dermatitis persisted for more than 6 months.</td>
<td>Nethercott et al., 1988</td>
</tr>
<tr>
<td>A dental nurse tested for contact dermatitis to dental composite resin also had positive patch test results with a disinfectant containing Glutaral.</td>
<td>Kanerva et al., 1989</td>
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*(continued)*
The vapor exposure to 2% Glutaral during cold sterilization procedures was determined to be <0.8 mg/m³ (the Swedish occupational exposure limit). The incidence of skin and respiratory irritation was significantly greater in the group exposed to Glutaral than in the unexposed group. A dose-response relationship was demonstrated. However, subjects who were patch-tested were negative to Glutaral and the other components of the disinfectant.

Nine members of an endoscopy unit were surveyed to find symptoms associated with exposure to vapor from 2% Glutaral in a disinfectant. Air samples taken for 1 h during cold sterilization ranged from 0.05 to 0.12 ppm. Eight of the workers reported ocular and nasal irritation.

A maintenance worker in a respiratory therapy department developed airborne contact dermatitis on her hands, arms, face, and neck after using a sterilizing solution containing 2% Glutaral. She was positive in patch tests with 1% aq. Glutaral.

A surveillance of occupational asthma in which physicians report occupational asthma cases to public health officials, who in turn use these case reports to identify workplaces with potential health hazards, found that 3 of 8 workers exposed to Glutaral were symptomatic (June 1, 1988–June 1, 1989).

Two hospital workers developed allergic contact dermatitis of the hands after using a Glutaral (0.13%)-phenol formulation to disinfect endoscopes. Both subjects had strong positive reactions to 1% aq. Glutaral and a 1:16 dilution of the disinfectant. Neither subject reacted to patch tests with formaldehyde.

Daily for 6 weeks. However, Glutaral produced skin irritation and slight effects on body weight, food consumption, clinical chemistry, and hematology in a 28-day dermal study with concentrations as low as 50 mg/kg/day.

Glutaral cause dose-dependent, moderate to severe skin irritation in animal studies. The no-effect irritation concentration was 1%.

Glutaral was toxic to mice and rats in inhalation studies. These studies were difficult to evaluate due to the lack of technical details concerning the production of Glutaral aerosols. The vapor phase of Glutaral at room temperature is expected to be nontoxic as a result of the small vapor pressure of Glutaral.

Glutaral was not embryotoxic, fetotoxic, or teratogenic at doses that were not highly toxic to pregnant mice, rats, and rabbits. The no observable effects level for reproductive effects was >1,000 ppm.

Positive and negative results were obtained when Glutaral was tested in the standard Ames test and the Ames test with preincubation. Glutaral gave positive and negative results, depending on bacterial strain, in forward mutation assays. In a Chinese hamster ovary cell HGPRT gene mutation assay, Glutaral was negative. It was positive and negative, depending on the laboratory, for sister chromatid.

exchanges and chromosome aberrations in Chinese hamster ovary cells. Glutaral was negative following in vivo and in vitro administration of Glutaral in unscheduled DNA synthesis in rat hepatocytes, in the *Drosophila melanogaster* sex-linked recessive lethal assay, and in the dominant lethal index test in mice.

In a 2-year drinking water study, there was a statistically significant increase in the incidence of LGLL in female rats administered 50, 150, and 1,000 ppm Glutaral. Because this increase was observed only in female rats and was not dose-response related and because the data from the untreated control group were thought to be unusually low, the authors concluded that the increased incidence of LGLL was due to a modifying influence on determinants controlling the expression of this spontaneously occurring neoplasm rather than a direct chemical carcinogenic effect.

In clinical studies, one (0.1%) positive reaction was observed when 657 dermatological patients were patch-tested with 2% Glutaral. No positive reactions were observed when 160 individuals were patch-tested with 1% Glutaral. All of 30 subjects who had contact dermatitis due to exposure to Glutaral or who were suspected to be contact-sensitive to Glutaral had positive reactions when patch-tested with 1 or 2% Glutaral. Six of nine subjects sensitized 1 year previously to Glutaral had positive patch test reactions to Glutaral. In a usage test, these subjects had negative responses on the soles of their feet, but developed severe dermatitis on all antecubital fossae. Twelve volunteers applied Glutaral to their ankles and heels daily for 4 weeks; minimal irritation was observed.

No sensitization reactions were observed in 211 subjects induced and challenged to 0.5% Glutaral. Seven (23.3%) of 30 subjects were positive for sensitization when induced with 5.0% Glutaral and challenged with 0.5% Glutaral. No cross sensitization between Glutaral and formaldehyde has been reported. Glutaral was not a phototoxin or photoallergen in clinical studies.

The threshold limit value (TLV) ceiling for Glutaral set by the American Conference of Governmental Industrial Hygienists (ACGIH) is 0.2 ppm (ACGIH, 1980). This concentration should not be exceeded even momentarily.

**DISCUSSION**

On November 20, 1987, the CIR Expert Panel released a “Notice of Insufficient Data Announcement” (IDA) in accordance with Section 30(j)(2)(A) of the CIR Procedures. In this announcement, the public was informed of the Expert Panel’s decision that the data on Glutaral were insufficient to determine whether Glutaral, under each relevant condition of use, was either safe or unsafe. The types of data required included:

1. Photosensitization studies in animals or humans; and
2. An 18-month dermal carcinogenicity study in mice (three skin-painting doses including the highest tolerable dose and one in the range of Glutaral use in cosmetic products, fresh solutions to be used each day).

In response to this notice, the Expert Panel was notified by an interested party that photosensitization data would be submitted and that a 2-year drinking water
study was in progress. The Expert Panel agreed to review carcinogenicity data from the oral study in lieu of the requested dermal data because the study was already underway and because it investigated exposure greater than expected from cosmetic use.

After reviewing the clinical photosensitization data that were submitted, the Expert Panel noted that the study was not applicable to an evaluation of the photosensitizing potential of Glutaral. Because the Expert Panel does not expect Glutaral to be a photosensitizer, additional studies are not required.

After reviewing the data from the 2-year drinking water study, the Expert Panel expressed concern about the increased incidence of LGLL observed in female rats. No dose response was observed and the investigators stated in their evaluation that the increased incidence of LGLL could not be directly attributed to Glutaral when compared with data on historical controls. The Expert Panel examined the historical control data and concluded that LGLL did appear to be a spontaneously occurring neoplasm with a varied incidence and that the incidence of LGLL in female rats was within the range of the historical control data.

Leave-On Products

The Expert Panel continues to be concerned about the carcinogenic potential of Glutaral when used in leave-on products. They concluded that the data from the 2-year drinking water study were insufficient to make a conclusion regarding the safety of this ingredient in products intended for prolonged use. The Expert Panel agreed that a 2-year dermal carcinogenicity study following National Toxicology Program procedures (rather than an 18-month skin-painting study, as was requested in the IDA) was necessary to make a conclusion.

Section 1, paragraph (p), of the CIR Procedures states that "a lack of information about an ingredient shall not be enough to justify a determination of safety." In accordance with Section 45 of the CIR Procedures, the Expert Panel issues a Final Report—Insufficient Data for leave-on products. When the requested new data are available, the Expert Panel will reconsider the Final Report conclusion for leave-on products in accordance with Section 46 of the CIR Procedures, Amendment of a Final Report.

Rinse-Off Products

The Expert Panel also addressed the issue that Glutaral is both an ocular and dermal irritant. At concentrations of 0.2% and greater, Glutaral was an ocular irritant in rabbits. The no-effects level was 0.1% Glutaral. In dermal studies with rabbits, concentrations >2% caused irritation. Sensitization data reported by Gad et al. (1986) indicated that Glutaral was a strong sensitizer in mice. However, the Expert Panel believes that the reactions observed were actually irritant in nature rather than sensitizing.

A clinical study by Marzulli and Maibach (1974) indicated the 0.5% Glutaral was not a sensitizer in test subjects induced with 0.1% Glutaral, but did produce sensitization when a sample of that group was then induced with 5% Glutaral. The Expert Panel also noted that another clinical study by Ballantyne and Berman

GLUTARAL (1984) indicated that 0.5% Glutaral was irritating to the skin, but was not sensitizing. By limiting the concentration of use to 0.5% and recognizing that contact with the skin would be brief and discontinuous when used in rinse-off products, the Expert Panel agreed that the skin irritation and sensitization potential would be minimized.

The Expert Panel noted that because Glutaral has mixed mutagenic responses in the same bacterial strains and between tests from different laboratories, the mutagenic potential of Glutaral is weak. Even though there is concern about the adequacy of the existing carcinogenicity data for leave-on products, the limited dermal absorption and weak mutagenic potential of Glutaral alleviated the Expert Panel’s concern about the use of this ingredient in rinse-off products.

The Expert Panel also reviewed the evidence of respiratory irritation and hypersensitization in both animal and occupational studies. Based on inhalation data revealing respiratory symptoms, the Expert Panel agreed that Glutaral should not be used in aerosolized cosmetic products.

CONCLUSION

Based on the animal and clinical data presented in this report, the CIR Expert Panel concludes that Glutaral is safe for use at concentrations up to 0.5% in rinse-off products. There is insufficient data to determine the safety of Glutaral in leave-on products. Glutaral should not be used in aerosolized products.

Acknowledgment: Karen Brandt, Scientific Analyst and Writer, prepared the literature review and technical analysis of this report, and Susan Pang, Scientific Analyst and Writer, updated the analysis used by the Expert Panel to evaluate this ingredient.

REFERENCES


Bushy Run Research Center. (1990) Glutaraldehyde: 13-week toxicity study in dogs with administra-

* Available for review: Director, Cosmetic Ingredient Review. 1101 17th Street, NW, Suite 310, Washington, DC 20036, U.S.A.
COSMETIC INGREDIENT REVIEW

- Bushy Run Research Center. (1994a) Glutaraldehyde: twenty-eight day repeated cutaneous dose toxicity study in Fischer 344 rats. Unpublished data submitted by Union Carbide, Bound Brook, NJ. (31 pages).*


GLUTARAL


TKL Research Inc. (1990a) Phototoxicity test (TKL Study No. 906001). Unpublished data submitted by Union Carbide Corporation, Maywood, NJ. (32 pages).*
TKL Research Inc. (1990b) Photoallergy test (TKL Study No. 907001) Unpublished data submitted by Union Carbide Corporation, Maywood, NJ. (33 pages).*

To: CIR Expert Panel Members and Liaisons

From: Christina L. Burnett

Scientific Writer/Analyst

Date: May 17, 2011

Subject: Re-review of HC Red No. 1

In 1996, the CIR Final Report on the safety of HC Red NO. 1 was published with the conclusion that this ingredient was “safe as used in hair dye formulations at concentrations of ≤ 0.5%”. A copy of the Final Report is included with this re-review.

Current uses of HC Red No. 1 can be found in Table 1. The number of uses for HC Red No. 1 has decreased from 47 to 9. All uses reported in 1996 and at present are in hair coloring products.

The European Commission’s Scientific Committee on Consumer Products (SCCP) issued an opinion on the safety of HC Red No. 1 in 2006. The opinion as well as some data that was provided to the SCCP is incorporated in this re-review. The SCCP report is available online. No other pertinent new data was discovered in a literature search for information published since 1996.

The task for the Panel at this meeting is to determine whether the conclusion on HC Red No. 1 is still valid. If it is not, an amendment should be initiated. If the conclusion is still valid, the Panel may reaffirm the original conclusion.
**HC Red No. 1 History**

**Original Report:** In 1996, the Expert Panel determined that HC Red No. 1 was safe as used in hair dye formulations at concentrations \( \leq 0.5\% \).

**June 2011:** the RR of HC Red No. 1 was presented to the Panel.
SEARCH STRATEGY FOR HC RED No. 1

March 31, 2011: SCIFINDER search for CAS No. 2784-89-6

- Limited search to references published since 1995; 103 references came back.
- Limited search to books, journals, preprints, reports, and reviews; 6 references came back.

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<td>HC Red No. 1</td>
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Total references ordered: 3
a written request for comments on CIR's prioritization scheme to FDA. He noted that FDA would be happy to review the proposed prioritization scheme and make comments.

INGREDIENTS UNDER TEST

Nonoxynols and HC Orange No. 1

Dr. Andersen noted that the tests that are being conducted on Nonoxynols and HC Orange No. 1 are well underway. It is anticipated that the results will be available for review prior to the December 12-13, 1994 Panel meeting. Dr. Andersen indicated that he definitely wants to schedule the report on Nonoxynols for review at the December meeting. A timeline has not been established for the review of HC Orange No.1; however, this report will also be ready for review in December.

REPORTS ADVANCING TO THE NEXT LEVEL

HC Red No. 1

Dr. Belsito noted that his Team concluded that HC Red No. 1 is safe for use in hair dyes at concentrations up to 0.15% after taking into consideration that this ingredient is used in semipermanent hair dyes at concentrations up to 1%, the results of subchronic dermal toxicity tests on a hair dye containing 0.15% HC Red No. 1, and that the absorption studies indicated only 1.36% dermal absorption over a 24 h period.

Dr. Belsito also indicated that if there is any interest on the part of industry to use
HC Red No. 1 at concentrations greater than 0.15%, then a 28-day dermal toxicity test at higher concentrations should be conducted.

Dr. Schroeter’s Team agreed with the proposed 0.15% concentration limit.

Dr. John Corbett noted that, in practice, the concentration limit for HC Red No. 1 in semipermanent hair dyes is 0.5%; this ingredient is not used in permanent hair dyes. Dr. Corbett questioned the Panel’s proposed 0.15% concentration limit, having noted that the no-effect-level in the subchronic oral toxicity study corresponded to 8 mg/kg/day, which is considerably in excess of what the exposure would be by dermal percutaneous absorption of either 1% or 2% of the applied dose.

Dr. Corbett also asked the Panel to re-evaluate its need for a 28-day dermal toxicity study on HC Red No. 1 at concentrations greater than 0.15% if there is interest on the part of industry to use this ingredient at these concentrations. He noted that HC Red No. 1 was non-irritating when applied to rabbits at a concentration that was much higher than 0.15%.

Dr. Shank noted that the test concentration of HC Red No. 1 in reproductive toxicity tests, teratogenicity tests, and dermal carcinogenicity tests was 0.15%, and that all of the studies involved topical application. The 0.15% concentration limit was based on the results of these studies.

Dr. McEwen stated that based on the data in the current report, HC Red No. 1 appears to be safe as used, considering that the use concentration (0.5%) is less than a multiple of 5 over the test concentration. Furthermore, he said that the exposure for animals in the carcinogenicity study is greater than what would be considered normal.

Dr. Slaga said that Dr. Shank had emphasized that the only carcinogenicity
studies that were available involved a test concentration of 0.15% HC Red No. 1, and that the concentration limit was established based on negative data (particularly from the longer duration, 23 months, study). Toxicity was not the basis for this limitation.

Dr. Klaassen acknowledged that, after all, whether or not HC Red No. 1 is a dermal carcinogen at the concentration that is being used in cosmetics is not known.

Dr. Corbett stated that he was troubled by the Panel’s discussion because, throughout the history of hair dye toxicology, the question of skin carcinogenicity has hardly ever arisen.

Considering that HC Red No. 1 is used in semipermanent hair dyes at a concentration of 0.5% (based on Dr. Corbett’s statement), Dr. Schroeter wanted to know if this is the concentration that comes in contact with the scalp during product use.

Dr. Corbett said that approximately 2% of the product is actually in contact with the scalp, and that the remainder is in contact with hair. However, the ingredient concentration that is applied (0.5%) is what actually reaches the scalp.

The Panel concluded that HC Red No. 1 is safe as used in hair dyes at concentrations up to 0.5%, and voted unanimously in favor of issuing a Tentative Report on this ingredient.

**Formic Acid**

The Panel voted unanimously in favor of issuing an Insufficient Data Announcement on Formic Acid with the following data requests:

(1) Concentration of use data
toxicity test is performed, one gets an impression of what many of those effects are, including liver injury, kidney injury, reproductive effects, and immune system effects. With respect to immune system effects, one gets some idea as to what happens to the thymus, lymph nodes, and the blood cell count. However, the results of the 28-day test do not yield any information regarding teratogenicity or carcinogenicity. Dr. Klaassen then said that if a chemical is absorbed well, one needs to consider its teratogenicity (or carcinogenicity). If the teratogenicity (or carcinogenicity) study is not performed, then there should be a legitimate reason for not doing the study. One of the reasons might be that the class of compounds to which the chemical in question belongs has never shown teratogenicity (or carcinogenicity).

4-Chlororesorcinol

The Panel voted unanimously in favor of issuing a Final Report on 4-Chlororesorcinol with the following conclusion: On the basis of the data included in this report, the CIR Expert Panel concludes that 4-Chlororesorcinol is safe as currently used in hair dye formulations.

HC Red No. 1

Dr. Bergfeld noted that the Panel had asked for confirmation of Dr. John Corbett's verbal statement at the September 12-13, 1994 Panel meeting, in which he noted that HC Red No. 1 is used at concentrations of ≤ 0.5%. She confirmed that Dr. Corbett's written statement had been received.

Dr. McEwen stated that he had not received any information on cosmetic use
concentrations of HC Red No. 1 that is contrary to that received from Dr. Corbett.

Dr. Belsito noted that the rationale (stated in report discussion) as to how the Panel arrived at a concentration limit of \( \leq 0.5\% \) for HC Red No. 1 is somewhat lacking, and should be revised. He recalled that the Panel had reviewed data indicating dermal absorption of HC Red No. 1 at a concentration of 1%; therefore, it was decided that the subchronic dermal toxicity data on 0.15% HC Red No. 1 were not as crucial.

The revised report discussion reads as follows: The CIR Expert Panel considered oral toxicity data in which animals were exposed to HC Red No. 1 in their diet at concentrations of less than or equal to 0.1%, as well as cutaneous absorption data showing that approximately 1.6% of applied HC Red No. 1 was absorbed through the skin. Based on this information and additional data summarized in the report, the Expert Panel was able to extrapolate the oral exposure to determine that HC Red No. 1 is safe as used at concentrations of \( \leq 0.5\% \).

The Panel unanimously approved the revised report discussion and voted in favor of issuing a Final Report with the following conclusion: On the basis of the animal and clinical data included in this report, the CIR Expert Panel concludes that HC Red No. 1 is safe as used in hair dye formulations at concentrations of \( \leq 0.5\% \).

**Pyrocatechol**

Dr. Belsito indicated that certain problems had arisen during the review of Pyrocatechol by his Team. He recalled that this ingredient has been banned in the European Union and that it is used in one product (leave-on product) that is not a hair
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INTRODUCTION

HC Red No. 1 has previously been reviewed by the Cosmetic Ingredient Review (CIR) Expert Panel. In 1996, the safety assessment was published with the conclusion that this ingredient is “safe as used in hair dye formulations at concentrations of \( \leq 0.5\% \)^1.

Since the original review, a few additional published studies on dermal irritation and sensitization have become available. These are included in this re-review document.

CHEMISTRY

DEFINITION AND STRUCTURE

HC Red No. 1 (CAS No. 2784-89-6) is a 2-nitro substituted phenyl-p-phenylenediamine. The molecular formula is \( \text{C}_{12}\text{H}_{11}\text{N}_{3}\text{O}_{2} \). The structure is shown in Figure 1.

Synonyms are: 4-amino-2-nitrodiphenylamine; 2-nitro-N'-phenyl-1,4-benzenediamine; and p-phenylenediamine, 2-nitro-N1-phenyl-.2

PHYSICAL AND CHEMICAL PROPERTIES

Physical and chemical properties of HC Red No. 1 can be found in the original safety assessment of HC Red No. 1.1

USE

Cosmetic

Table 1 presents the historical and current product formulation data for HC Red No. 1. According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP), HC Red No. 1 was used in a total of 47 hair coloring formulations at the time of the first safety assessment.1 An industry survey reported use concentrations of \( \leq 0.5\% \). Currently, VCRP data indicate that HC Red No. 1 is used in 9 hair coloring formulations.3 In a survey of current use concentrations conducted by the Personal Care Products Council, HC Red No. 1 is currently used at a much lower concentration of 0.07% in hair coloring products.4

The European Commission’s Scientific Committee on Consumer Products (SCCP) determined that HC Red No. 1 at a maximum final concentration of 1% does not pose a risk to the health of the consumer, apart from sensitization.5

TOXICOKINETICS

Absorption, Distribution, Metabolism, and Excretion

Dermal/Percutaneous

In an in vitro percutaneous absorption study provided to the SCCP, human dermatomed skin mounted in glass diffusion cells were treated with 200 \( \mu \text{g/cm}^2 \) \([^{14}\text{C}]\)-HC Red No. 1 for 30 minutes and then washed with natural sponges soaked in 3% Teepol®.5 Receptor fluid samples were taken at 0.5, 1, 2, 4, 6, 24, and 48 h after application. At the end of the experiment, the skin was washed again and then stripped with tape. The receptor fluid samples, sponges, tape strips, residual skin, and donor chamber were analyzed for radioactivity to determine penetration rates and distribution. The amount of \([^{14}\text{C}]\)-HC Red No. 1 recovered was 0.089 ± 0.075 \( \mu \text{g/cm}^2 \) (0.044%) in the flange, 0.164 ± 0.120 \( \mu \text{g/cm}^2 \) (0.082%) in the donor chamber, 1.14 ± 0.560 \( \mu \text{g/cm}^2 \) (0.566%) in the receptor fluid, 195 ± 13.6 \( \mu \text{g/cm}^2 \) (97.5%) in the 0.5 h skin wash, 1.12 ± 0.794 \( \mu \text{g/cm}^2 \) (0.560%) in the 48 h skin wash, 0.199 ± 0.111 \( \mu \text{g/cm}^2 \) (0.099%) in the stratum corneum, and 0.208 ± 0.159 \( \mu \text{g/cm}^2 \) (0.104%) in the remaining epidermis/dermis. It was calculated that 1.34 ± 0.720 \( \mu \text{g/cm}^2 \) (~0.67%) HC Red No. 1 was available systemically. Total recovery was 99%.
TOXICOLOGICAL STUDIES
Repeated Dose Toxicity

Oral – Non-Human
In data provided to the SCCP, groups of 10 Sprague Dawley rats (5 males and 5 females per dose group) received 0, 50, 150, 300, or 600 mg/kg bodyweight/day HC Red No. 1 in PEG 400 by oral gavage.\textsuperscript{5} The rats received the test material once a day for 14 consecutive days. Organization for Economic Cooperation and Development (OECD 408) guidelines were followed. No treatment-related mortality was observed in any of the rats. Deaths that occurred in 150 and 600 mg/kg/day females were due to injuries sustained during dosing. Staining of the body and urine occurred but was expected due to the test material being a dye. Clinical observations that may indicate test-material induced toxicity included dark material around the nose, decreased defecation, soft stools, dehydration, reduced weight gain and reduced food consumption. These observations were mainly noted in the 600 mg/kg/day males and females. Decreased erythrocytes, increased mean corpuscular volume, increased mean corpuscular hemoglobin, polychromatic red blood cells, increased alanine aminotransferase and increased total bilirubin were observed in the 300 and 600 mg/kg/day males and females and in the 150 mg/kg/day females. Changes were also noted in total protein, albumin, A/G ratio, and potassium (details not provided). At gross necropsy, blackish-purple and/or enlarged spleen was observed in the 300 and 600 mg/kg/day males and females. Spleen weights were increased in the 300 and 600 mg/kg/day males and females and in the 150 mg/kg/day females. Liver weights were increased in the 300 mg/kg/day males and females and also in the 150 and 600 mg/kg/day females. The study concluded that the no-observed-adverse-effect-level (NOAEL) was 50 mg/kg bodyweight/day HC Red No. 1.

In a 91 day study provided to the SCCP, groups of 15 male and 15 female Sprague Dawley rats received 0, 2, 5, or 20 mg/kg bodyweight/day HC Red No. 1 in PEG 400 by oral gavage.\textsuperscript{5} Additional groups of 5 animals of both sexes of the 0 and 20 mg/kg/day dose groups were used to assess recovery for 28 days post-treatment. OECD 408 guidelines were followed. No treatment-related mortality or clinical abnormalities aside from those associated with the test material being a dye were observed. Observations in clinical chemistry included, increased calcium (middle and high dose groups in males, high dose group in females), total protein (particularly the male high dose group), albumin (in female groups), globulin (high dose groups in males, middle and high dose groups in females), and sodium (in high dose group females). It was speculated that these clinical chemistry observations were from dehydration or renal dysfunction, but the findings were within historical control ranges and there was no notable changes in the renal histopathology or urinalysis parameters. Statistically significant increased MCH was observed in 4 males of the 20 mg/kg/day group at the end of the treatment while increased leukocytes and lymphocytes (on day 91/92) were observed in the females of the 5 and 20 mg/kg/day dose groups. Decreased erythrocytes were observed in the females of the 20 mg/kg/day dose group on day 119. There were no significant changes in the ophthalmology, gross necropsy, or organ weight data with the exception of decreased absolute and relative pituitary weight for females of the 5 mg/kg/day dose group and a decreased relative thymic weight for males in the 20 mg/kg/day dose group. The study concluded that the NOAEL was 20 mg/kg bodyweight/day HC Red No. 1; however, due to the decreased relative thymic weight observed in the males, the SCCP set the NOAEL at 5 mg/kg bodyweight/day HC Red No. 1.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY
In the 91 day study described above, the estrous cycle length and normality were evaluated in the female rats during treatment with HC Red No. 1, while sperm was collected from the male rats after they were killed and evaluated for sperm enumeration, motility, and morphology.\textsuperscript{5} A statistically significant decrease in the percent sperm motility was observed in the 2 mg/kg/day dose group, but the change was not considered treatment-related since decreases were not observed in the 5 or 20 mg/kg/day dose groups.
In data supplied to the SCCP, the potential of HC Red No. 1 to cause toxic effects on embryonic and fetal development was studied in mated female Crl:CD(SD)IGS BR/VAF/Plus rats.\(^5\) A range finding study was performed separately to determine the doses to be used in the study. In the main study, groups of 25 mate females received 0, 25, 75, or 125 mg/kg bodyweight/day HC Red No. 1 in PEG 400 via oral gavage once daily on days 6-20 of gestation. The control group received PEG 400. Mortalities, clinical observations, body weights and feed consumption values were recorded. The rats were killed on day 21, and the gravid uterus was excised, weighed and examined for number and distribution of corpora lutea, implantation sites and uterine contents. A gross necropsy was performed. Fetuses were weighed, sexed, and submitted for external examination.

No mortalities occurred in the dams during treatment. One dam of the 125 mg/kg/day dose group began delivering on day 20 and was subsequently killed. Orange, red, or purple urine was observed in all dose groups. Additionally, purple fur and urine-stained abdominal fur was observed in the 75 and 125 mg/kg/day dose groups. Black spleens, due to the test substance being a dye, were observed in 2 rats in the 75 mg/kg/day dose group and at significant numbers in the 125 mg/kg/day dose group. One rat in the 125 mg/kg/day dose group had a rough spleen. At the end of the treatment, mean body weight in the 125 mg/kg/day dose group and corrected maternal body weight in the 75 and 125 mg/kg day dose groups were significantly reduced. Body weight losses were observed in all dose groups on days 6-9 and for the entire dosage period in the 125 mg/kg/day group. Body weight gains were significantly reduced in the high dose group for the entire dosage and gestation periods, and the net body weight gains for the gestation period were significantly reduced in the 75 and 125 mg/kg/day dose groups. Absolute and relative feed consumption values were significantly reduced in the 75 and 125 mg/kg/day dose groups during the entire dosage and gestation periods while absolute feed consumption values were significantly reduced in the 25 mg/kg/day dose group on days 6-9. There were no treatment-related effects on litter parameters nor external malformations or anomalies in fetuses in any dose group. The maternal and fetal NOAELs for HC Red No. 1 in this study were 25 mg/kg/day and 125 mg/kg/day, respectively.\(^5\)

**GENOTOXICITY**

**In Vitro**

The genotoxicity of HC Red No. 1 was investigated in an Ames study supplied to the SCCP.\(^5\) *Salmonella typhimurium* strains TA98, TA100, TA 1535, and TA 1537 and *Escherichia coli* strain WP2 uvrA (pKM101) were utilized for the study. The study was conducted in 3 experiments in accordance with OECD 471 guidelines. Experiment 1 used a concentration range of 99.8% HC Red No. 1 of 2.5-5000 µg/plate with and without S9 metabolic activation; experiment 2 used a concentration range of HC Red No. 1 of 7.5-5000 µg/plate with and without S9; and experiment 3 used a concentration range of 25-5000 µg/plate with TA 1535 only and without S9. In experiments 1 and 2, HC Red No. 1 did not induce an increase in the number of revertants when compared to the controls. In experiment 3, a non-dose responsive increase in the number of revertant was observed, but this effect could not be duplicated in an additional test. The study concluded that HC Red No. 1 is not genotoxic.

HC Red No. 1 was studied for gene mutations in mouse lymphoma L5178Y TK\(^{-}\) cells according to data provided to the SCCP.\(^5\) After a range-finding test to measure cytotoxicity, 2 independent experiments were performed. The concentrations for experiment 1 ranged from 1.5-140 µg/ml, without S9 metabolic activation and from 5-30 µg/ml with S9 activation. In experiment 2, the concentrations were 0.5-100 µg/ml without S9 metabolic activation. The study was performed in accordance with OECD 476 guidelines. HC Red No. 1 did not affect osmolarity or pH when compared to control values. The appropriate level of toxicity was reached with and without S9 activation and visual precipitation was not
observed at any concentration. No concentration-related increases in mutant frequency with or without S9 activation was observed in either experiment with HC Red No.1. This study concluded that HC Red No. 1 is not genotoxic at the \( tk \) locus of mouse lymphoma cells.

The potential for HC Red No.1 to induce chromosomal aberrations in Chinese hamster ovary (CHO) cells was reported in data submitted to the SCCP. Two independent experiments were performed. In experiment 1, cells were incubated for 4 h with HC Red No. 1 without S9 metabolic activation at concentrations ranging from 25-100 \( \mu \)g/ml. In experiment 2, cells were incubated for 4 h with HC Red No. 1 with S9 at concentrations ranging from 50-120 \( \mu \)g/ml or for 20 h without S9 at concentrations ranging from 20-80 \( \mu \)g/ml. The study was performed in accordance with OECD 473 guidelines. HC Red No. 1 did not affect osmolarity or pH when compared to control values. Precipitation was observed at concentrations \( \geq 100 \mu \)g/ml. The mitotic index did not show a 50% reduction in the highest concentrations tested; however, HC Red No.1 induced sufficient toxicity with a 60% decrease in relative growth inhibition at the highest concentration tested when compared to the controls. HC Red No. 1 without S9 activation during 4 and 20 h incubations caused a concentration-dependent statistically significant increase in cells with structural chromosomal aberrations. In cells incubated with S9 activation, a concentration-dependent statistically significant increase in the frequency of cells with chromosomal aberrations was observed. No increase in the frequency of polyploidy and/or endoreduplicated cells were observed. It was concluded that HC Red No. 1 was clastogenic in this study.

**In Vivo**

In data supplied to the SCCP, the genotoxic potential HC Red No. 1 was studied in a micronucleus test using male ICR mice. A dose range finding experiment preceded the main study. In the main study, groups of 5 mice received single doses of 500, 1000, or 2000 mg/kg bodyweight HC Red No. 1 via oral gavage. Bone marrow cells were collected at 24 or 48 h (for the 2000 mg/kg dose group and the vehicle control). The samples were prepared in accordance with OECD 474 guidelines. The ratio between polychromatic and 1000 erythrocytes (PCE/EC) was determined. In the dose range finding study, toxic effects observed included piloerrection, lethargy, and red urine. In the main study, no treatment-related mortalities were observed. Lethargy, signs of red skin tone and red urine were observed in the mice of the 2000 mg/kg dose group. A reduction in the PCE/EC ratio of up to 15% was observed in the treatment groups when compared to the control groups. There was no statistically significant increase in micronuclei in the treatment groups when compared to the controls. The study concluded that HC Red No. 1 was not genotoxic in this micronucleus assay.

**SENSITIZATION**

**Dermal – Non-Human**

In data provided to the SCCP, an LLNA study of 0.25%, 0.5%, 1.0%, and 2.0% HC Red No. 1 had stimulation indices (SI) of, 0.35, 1.06, 1.04, and 1.52, respectively. The positive control, 2% p-phenylenediamine (PPD) yielded an SI of 7.10. The authors of the study concluded that HC Red No. 1 did not induce skin sensitization. Another LLNA study reported to the SCCP tested HC Red No. 1 at the same concentrations. The SI were 3.49 for 0.25%, 1.73 for 0.5%, 1.51 for 1.0%, and 4.27 for 2.0% HC Red No. 1. PPD at concentrations of 0.25%, 0.5%, 1.0%, and 2.0% yielded SI of 3.66, 9.13, 11.66, and 22.59, respectively. The authors of this second study concluded that HC Red No. 1 can induce skin sensitization in mice. However, the SCCP felt that these studies were not conducted in compliance with proper methods for LLNA studies.

**HAIR DYE EPIDEMIOLOGY**

Hair dyes may be broadly grouped into oxidative (permanent) and direct (semipermanent) hair dyes. The oxidative dyes consist of precursors mixed with developers to produce color, while direct hair dyes are a preformed color. HC Red No.
1 is a direct hair dye ingredient. While the safety of individual hair dye ingredients are not addressed in epidemiology studies that seek to determine links, if any, between hair dye use and disease, such studies do provide broad information. The CIR Expert Panel noted the conclusions of these reviews, including that personal use of hair colorants cannot be evaluated as to its carcinogenicity and that occupation as a hairdresser or barber entails exposures that are probably carcinogenic. Insufficient evidence exists to support a causal association between personal hair dye use and a variety of tumors and cancers such as acute leukemia, bladder cancer, multiple myeloma, and non-Hodgkin’s lymphoma, and the epidemiological evidence for personal use of hair colorants is inadequate and is not classifiable as to its carcinogenicity to humans. A detailed summary of the available hair dye epidemiology data is available at http://www.cir-safety.org/findings.shtml.
Table 1. Historic and current uses and concentrations of HC Red No.1.

<table>
<thead>
<tr>
<th>Duration of Use</th>
<th>1996</th>
<th>2011</th>
<th>Conc. of Use (%) 1996</th>
<th>Conc. of Use (%) 2011</th>
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<td><strong>Totals</strong></td>
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<td><strong>&lt; 0.5</strong></td>
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<td>Dermal Contact</td>
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<td>NA</td>
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<td>NA</td>
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<td>Hair-Coloring</td>
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<td>Baby Products</td>
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</table>
References


Memorandum

TO: F. Alan Andersen, Ph.D.
   Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: John Bailey, Ph.D.
   Industry Liaison to the CIR Expert Panel

DATE: January 7, 2011

SUBJECT: Concentration of Use by FDA Product Category: HC Red No. 1
## Concentration of Use by FDA Product Category

**HC Red No. 1**

<table>
<thead>
<tr>
<th>Product Category</th>
<th>Concentration of Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hair dyes and colors (all types requiring caution statement and patch test)</td>
<td>0.07%</td>
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</table>

Information collected in 2010  
Table prepared January 6, 2011
**FDA 2011 VCRP Data**

| HC RED #1 | 8 | 06A - Hair Dyes and Colors |
| HC RED #1 | 1 | 06B - Hair Tints |
Final Report on the Safety Assessment of H.C. Red No. 1

Abstract: H.C. Red No. 1 is an aromatic compound used as a colorant in semipermanent hair dyes and colors. This ingredient is reportedly used in almost 50 products; one manufacturer reports current use concentrations of \( \leq 0.5\% \). These products will generally have a warning statement and patch test instructions that should be followed to determine whether each individual user is sensitive to the product before use. In a study performed using human female cadaver skin, the percutaneous absorption of H.C. Red No. 1 was linear for the first 4 h, with total absorption of 1.68\% after 48 h. The oral median lethal dose was between 2.5 and 5.0 g/kg for male rats and 0.625 and 1.25 g/kg for female rats. Short-term oral feeding of H.C. Red No. 1 to rats had effects on several organ weights and resulted in liver and splenic lesions. Dermal exposure to almost twice the oral concentration produced no evidence of toxicity. In rabbits, H.C. Red No. 1 was not a dermal irritant, but it was a mild ocular irritant. H.C. Red No. 1 was a contact sensitizer, but not a photosensitizer, in guinea pigs. No reproductive or developmental toxicity was observed when a formulation containing 0.15\% H.C. Red No. 1 was applied dermally to rats, and neither fetotoxic nor teratogenic effects were seen in rats fed \( \leq 0.1\% \) H.C. Red No. 1. No evidence of mutagenic potential was seen in most bacterial and mammalian assays. No carcinogenic effects were reported for mice dosed dermally with H.C. Red No. 1, but several possible effects were seen in a rat skin-painting study with 0.15\% H.C. Red No. 1, including liver enlargement, parathyroid and hepatocellular hyperplasia, hepatocellular hypertrophy, hyperkeratosis in several locations, and dermatitis. Whether these effects were compound-related was unclear. A repeated-insult patch test of a 3\% slurry of H.C. Red No. 1 completed on 103 individuals with normal skin reported one possible and one definite sensitization reaction. Because the ingredient is used at a low concentration and very little is actually absorbed, the oral exposure data using concentrations of \( \leq 0.1\% \) represent a much higher exposure than would occur through the skin. The general absence of toxicity in such oral studies supports the safety of use of H.C. Red No. 1 in hair dye formulations at concentrations of \( \leq 0.5\% \). Key Words: H.C. Red No. 1—Hair dye—Mild ocular irritation—Contact sensitization.

H.C. Red No. 1 is a color additive that functions as a colorant in hair dyes and colors (Wenninger and McEwen, 1992).

1Reviewed by the Cosmetic Ingredient Review Expert Panel.
Address correspondence and reprint requests to Dr. F. A. Andersen at Cosmetic Ingredient Review, 1101 17th Street NW, Suite 310, Washington, D.C. 20036, U.S.A.
CHEMISTRY

Definition and Structure

H.C. Red No. 1 (CAS No. 2784-89-6) is the hair color that conforms to the formula shown in Fig. 1 (Wenninger and McEwen, 1993). H.C. Red No. 1 is also known as 4-amino-2-nitrodiphenyiamine (Wenninger and McEwen, 1993; Clairol, 1994a); 2-nitro-N'-phenyl-1,4-benzenediamine (Wenninger and McEwen, 1993; Clairol, 1994b); 2-nitro-N'-phenyl-1,4-benzenediamine (Clairol, 1994a); 1,4-benzenediamine, 2-nitro-N(1)-phenyl-; p-phenylenediamine, 2-nitro-N(1)-phenyl-; and 2-nitro-4-aminozophenylamine (Chemline, 1994).

Physical and Chemical Properties

H.C. Red No. 1 is a dark brown crystalline material, molecular weight 229, that is soluble in ethanol and insoluble in water, with a melting point in the range of 98–101°C (Clairol, 1994b). The empirical formula for H.C. Red No. 1 is C_{12}H_{11}N_{3}O_{2}.

Manufacture and Production

The following three-step process is used in the manufacture of H.C. Red No. 1: acetylation of 4-fluoro-3-nitroaniline, condensation with aniline, and hydrolysis (Clairol, 1994a). Published data on the ultraviolet absorbance of H.C. Red No. 1 were not found.

Analytical Methods

Published analytical methods data on H.C. Red No. 1 were not found.

Impurities

It is specified that H.C. Red No. 1 must exist as a minimum of 95% H.C. Red No. 1, with a maximum of 2% each of ash and 4-acetamido-2-nitrophenylamine (Clairol, 1994a).

USE

Cosmetic

H.C. Red No. 1 is reported to function as a hair colorant in hair dyes and colors (Wenninger and McEwen, 1992). Product formulation data submitted to the Food and Drug Administration (FDA) in 1994 reported that H.C. Red No. 1 is used in 47 cosmetic formulations (see Table 1) (FDA, 1994). H.C. Red No. 1 is reported

![Chemical formula for H.C. Red No. 1 (Wenninger and McEwen, 1993).](attachment:chemical_formula.png)
to be used in semipermanent hair colors at a concentration of \( \leq 0.5\% \) (Clairol, 1994b; 1994c). Hair coloring formulations are applied to or may come in contact with hair, skin (particularly at the scalp), eyes, and nails. Individuals who dye their hair may use such formulations once every few weeks, whereas hairdressers may come in contact with products containing these ingredients several times a day. Under normal conditions of use, skin contact with hair dye is restricted to 30 min. The hair dyes containing H.C. Red No. 1, as coal tar hair dye products, are exempt from the principal adulteration provision and from the color additive provisions in sections 601 and 706 of the Federal Food, Drug, and Cosmetic Act of 1938, when the label bears a caution statement and patch-test instructions for determining whether the product causes skin irritation. The following caution statement should be displayed conspicuously on the labels of coal tar hair dyes:

**Caution**—This product contains ingredients that may cause skin irritation on certain individuals, and a preliminary test according to accompanying directions should be made. This product must not be used for dyeing eyelashes or eyebrows; to do so may cause blindness.

At its February 11, 1992, meeting, the Cosmetic Ingredient Review Expert Panel issued the following policy statement on coal tar hair dye product labeling:

The Cosmetic Ingredient Review (CIR) Expert Panel has reviewed the cosmetic industry’s current coal tar hair dye product labeling, which recommends that an open patch test be applied and evaluated by the beautician and/or consumer for sensitization 24 hours after application of the test material and prior to the use of a hair dye formulation.

Since the recommendation on the industry’s adopted labeling establishes a procedure for individual user safety testing, it is most important that the recommended procedure be consistent with current medical practice. There is a general consensus among dermatologists that screening patients for sensitization (allergic contact dermatitis) should be conducted by the procedures used by the North American Contact Dermatitis Group and the International Contact Dermatitis Group (North American Contact Dermatitis Group, 1980; Eiermann et al., 1982; Adams et al., 1985). These procedures state that the test material should be applied at an acceptable concentration to the patient, covered with an appropriate occlusive patch, and evaluated for sensitization 48 and 72 hours after application. The CIR Expert Panel has cited the results of studies conducted by both the North American Contact Dermatitis Group and the International Contact Dermatitis Group in its safety evaluation reports on cosmetic ingredients (Elder, 1985).

During the August 26–27, 1991 public meeting of the CIR Expert Panel, all members agreed that the cosmetic industry should change its recommendation for the evaluation of the open patch test from 24 hours to 48 hours after application of the test material.

The industry was advised of this recommendation and asked to provide any compelling reasons why this recommendation should not be made by the Expert Panel and adopted by the cosmetic industry. No opposition to this recommendation was received.
H.C. RED NO. 1

At the February 11, 1992 public meeting of the CIR Expert Panel, this policy statement was adopted.

Published data on the international cosmetic use of H.C. Red No. 1 were not found, nor were there any noncosmetic uses.

GENERAL BIOLOGY

Percutaneous Absorption

The rate of percutaneous absorption of \(^{14}\text{C}-\text{H.C. Red No. 1}\) from a commercial semipermanent dye base was examined using human female cadaver skin mounted in static Franz diffusion chambers (Clairol 1994d). \(^{14}\text{C}-\text{H.C. Red No. 1}\) was mixed with H.C. Red No. 1 to achieve a concentration of 1%, and the specific activity was equivalent to 0.156 \(\mu\text{Ci/mg}\). Skin integrity was assessed by determining the permeation rates of \(^3\text{H}_2\text{O}\) over a 1-h period; the acceptable range was \(\leq 1.5\) mg/cm\(^2\)/h. The dye was applied to the stratum corneum side of the skin at a dose of 10 mg/cm\(^2\) for 30 min, after which the dye was removed by rinsing with 2 ml of water. After 1, 2, 4, 6, 8, 24, 30, and 48 h, 200-\(\mu\)l samples were withdrawn from the receptor chamber and assayed for carbon 14 by scintillation counting. The rate of percutaneous absorption was linear for \(~4\) h following exposure; the rate of absorption was then reduced and essentially reached zero by 24 h. The total skin absorption at 24 and 48 h was 1.6 \(\pm\) 0.01 and 1.68 \(\pm\) 0.01%, respectively. (If data from one cell that had a much higher level of absorption was treated as an outlier, the mean total absorption was 1.36%.) The average skin permeability coefficient was \(1.11 \times 10^{-6}\) cm/h. Mass balance indicated that total radioactivity recovery was \(~100\)% with \(>95\)% being present in the rinsates used to remove the dye from the skin. The remainder of the radioactivity was distributed among the receptor fluid, the filter paper support, and the skin.

ANIMAL TOXICOLOGY

Acute Toxicity

Groups of five male and five female Sprague-Dawley rats were dosed orally with H.C. Red No. 1 as a 10% suspension in 3% acacia; the males were dosed with 1,250, 2,500, or 5,000 mg/kg and the females with 625, 1,250, and 5,000 mg/kg. The animals were observed for 7 days (Clairol, 1987a). Signs of toxicity were not recorded. All male and female rats of the 5,000 mg/kg dose group died within 24 h of dosing. All male rats in the other dose groups survived. Three female rats dosed with 1,250 mg/kg H.C. Red No. 1 died—one within 24 h, one within 2 days, and one within 4 days. No female rats of the 625 mg/kg dose group died within 7 days of dosing. The oral median lethal dose for male rats was between 2,500 and 5,000 mg/kg and for female rats was between 625 and 1,250 mg/kg H.C. Red No. 1.

Short-term Toxicity

Groups of Sprague-Dawley rats were fed H.C. Red No. 1 in the diet for 4 weeks in a pilot study in order to determine the dosages to be used in a longer-term
study, which will be described later in this review (Bristol-Myers Products, 1992). Twenty rats per group (10 per sex) were fed 0.05, 0.1, 0.2, 0.4, 0.6, or 0.8% w/w H.C. Red No. 1 in feed: a control group was fed untreated feed. Body weights and feed consumption were recorded weekly. The physical condition of the animals was observed daily, and pharmacologic and/or toxicologic observations were made weekly. After 4 weeks of dosing, necropsy was performed on the fasted animals. The liver, kidneys, and spleen from rats in each test group were weighed, and selected clinical chemistry evaluations were performed.

All animals survived until study termination. During the study, discolored urine and hair were observed in the test animals. When compared with control values, body weights and feed consumption were statistically significantly lower among animals of the 0.2–0.8% dose groups from weeks 1–4, with the exception of body weights and feed consumption for males of the 0.2% dose group during weeks 1 and 2 and feed consumption for all females during week 2. The only other statistically significant difference between test and control animals was a decrease in feed consumption by females of the 0.1% test group during week 1.

Statistically significant differences in many of the hematologic and clinical chemistry parameters were observed between test and control animals, especially those of the ≥0.1% test groups. Statistically significant increases were observed in the absolute and relative liver weights of males and females of the 0.4–0.8% dose groups, in the relative and absolute spleen weights of females of the 0.2–0.8% dose groups, in the relative kidney weights of females of the 0.4–0.8% groups, and in absolute kidney weights of males of the 0.6–0.8% dose groups. Compound-induced lesions were observed in the livers of female rats of the 0.8% test group and in the spleens of females of the 0.2 and 0.8% dose groups. Similar, but less prominent lesions were noted in the spleens of a few males does with 0.1% H.C. Red No. 1.

Subchronic Toxicity

Dermal

Groups of 12 New Zealand White rabbits, six males and six females per group, were used to determine the percutaneous toxicity of a semipermanent hair dye formulation containing 0.15% H.C. Red No. 1 (Burnett et al., 1976). One milliliter per kilogram of mixture was applied undiluted twice weekly for 13 weeks to clipped sites on the dorsolateral aspects of the thoracic-lumbar area (one on each side of the midline), with the sites being alternated to minimize dermal irritation. The application sites on three animals per sex per group were abraded for the first dose of each week. The animals were restrained for 1 h following dosing, and the test site was then washed and rinsed. Three groups of negative control animals were treated in the same manner as the test animals, with the exception that no dye was applied. All animals were weighed weekly. Hematological, clinical chemistry, and urinary determinations were made at study initiation and after 3, 7, and 13 weeks. All animals were killed after 13 weeks and examined grossly. Various ratios of organ to body weight were determined, and a number of tissues were

J Am Coll Toxicol, Vol. 15, No. 4, 1996
examined microscopically. No evidence of compound-induced toxicity was found, no gross abnormalities were seen at necropsy, and no test article–related microscopic lesions were reported. No discoloration of the urine due to administration of the hair dye formulation was observed.

**Oral**

Male and female Sprague-Dawley rats were fed H.C. Red No. 1 at dietary concentrations of 0.01, 0.03, and 0.1% for 13 weeks; the 0.01% dose group consisted of 40 males and 45 females, and the 0.03 and 0.1% dose groups consisted of 40 males and 55 females (Bristol-Myers Products, 1992). A control group of 40 males and 45 females was fed normal feed. (Some of the animals used in this study were also used in teratology, dominant lethal, or micronucleus studies, which are summarized later in this report.) Ten rats per sex per group were dosed until week 27.

Animals were observed daily for death or moribundity and weekly for signs of toxicity. Body weights and feed consumption were measured weekly. At 13 weeks, blood was obtained by retroorbital bleeding from five males and five females of the control and high-dose groups to determine methemoglobin formation. At both the 13- and 27-week necropsy, blood was taken from 10 rats/sex/group for hematologic and clinical chemistry evaluation. The recovery animals were necropsied at week 28; the recovery animals consisted of the 20 males per group used in the dominant lethal study and 10 females of both the mid- and high-dose groups that were fed control feed between weeks 20 and 28.

No statistically significant differences in mean body weight among males were observed during weeks 0–13 of the study, and a statistically significant difference in mean body weight among females was observed only during week 10 between animals of the mid-dose and control groups. After the interim necropsy, body weights for males and females were similar throughout the remainder of the study. Feed consumption was statistically significantly increased during week 10 for males of the high-dose group. Feed consumption was statistically significantly lower for females of the low-dose group during week 1; for females of the mid-dose group during weeks 0–5, 7, and 10; and for females of the high-dose group during weeks 0–4 and 9. Females of the mid- and high-dose group consumed less than control females throughout weeks 0–13. Feed consumption was basically similar between test and control males and females throughout the remainder of the study. No statistically significant differences were observed in body weights of the male or female recovery rats compared with controls.

The urine of animals of all dose groups was rust-colored throughout the study. Several animals of the high- and mid-dose groups had discolored (red) hair by weeks 8 and 16, respectively. One female of the mid-dose group experienced rapid, labored breathing and had significant weight loss during week 9 and was moribund in week 10; multiple dark areas on the lungs, pale and mottled kidneys, a dark liver, and very little body fat were observed at necropsy. A male of the high-dose group (which was in the dominant lethal study) was found dead during week 21; red lungs, blood around the nose and mouth, a small spleen, and dark
material in the stomach were found at necropsy. No other statistically significant, compound-induced alterations were noted.

The only hematologic parameter that was statistically significantly different was an increase in the percentage of segmented neutrophils in females of the high-dose group after 13 weeks. Statistically significant differences in clinical chemistry values included an increase in total bilirubin in high-dose females after 13 weeks and increases in serum triglycerides and blood urea nitrogen in high-dose males and in serum sodium concentrations in mid- and high-dose females after 27 weeks. H.C. Red No. 1 did not induce methemoglobin formation.

There was a statistically significant increase in absolute and relative liver weights after 13 weeks and in relative kidney and relative liver weights after 27 weeks in males of the high-dose group. No statistically significant differences in organ weights were observed in recovery males or females. The only H.C. Red No. 1-related change noted at the 13-week necropsy was an increase in pigment in the spleens of females of the mid- and high-dose groups; the pigment was primarily composed of hemosiderin owing to the breakdown of red blood cells. The investigators described the increased deposition of pigment as an exacerbation of a normal occurrence in female rats. Only limited microscopic examination was made after 27 weeks because of a loss of tissues; no compound-induced alterations were recorded for the available tissues.

Dermal Irritation

The dermal irritation potential of H.C. Red No. 1 was assessed using six New Zealand White rabbits (Clairol, 1987b). H.C. Red No. 1, 500 mg, was applied as an aqueous slurry for a 1-sq-in area of shaved intact skin without occlusive patches. After 24 h, the residual material was removed, and the test site was scored according to the method of Draize at 24 and 72 h. Neither erythema nor edema was present 24 or 72 h after application; H.C. Red No. 1 was nonirritating upon nonocclusive application.

Dermal Sensitization

A guinea pig maximization test was performed according to the methods of Kligman-Magnusson using 10 female Hartley albino guinea pigs to determine the contact sensitization potential of H.C. Red No. 1 (Clairol, 1979a). A 4 × 6-cm area was clipped free of hair for induction, which consisted of an intradermal injection of 0.1% H.C. Red No. 1 in propylene glycol with Freund's complete adjuvant (FCA) followed 1 week later by the application of an occlusive patch of 25% H.C. Red No. 1 in propylene glycol for 48 h. The application area was pretreated with 10% sodium lauryl sulfate 24 h before patch application. The animals were first challenged 2 weeks later with a 24-h occlusive patch of 5% H.C. Red No. 1; 1 week later a second challenge was performed with a 24-h occlusive patch of 2% H.C. Red No. 1. Erythema and edema were observed at the test sites following challenge. There was evidence of contact sensitization to H.C. Red No. 1 in all animals at both the 5% and 2% challenges.

Because H.C. Red No. 1 produced a contact sensitization reaction in the pre-

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vious test, the contact sensitization potential of H.C. Red No. 1 was assessed in an open epicutaneous test performed according to the method of Schultz using 10 female Hartley albino guinea pigs (Clairol, 1979b). Eighteen 0.5-ml applications of 3% H.C. Red No. 1 in aqueous vehicle containing 10% isopropanol, 2% Tween 80, and 2% hydroxyethylcellulose solution were made 5 days per week for 3 weeks and for 3 consecutive days during the fourth week to a shaved area on the left flank of each animal. The animals were challenged 2 weeks later with a 24-h application of the test material at a site on the opposite flank; observations were made after 24, 48, and 72 h. Contact sensitization was indicated by erythema, which was observed in seven animals after 24 h and in four animals after both 48 and 72 h; edema was not noted.

Photosensitization

The photosensitization potential of H.C. Red No. 1 was assessed using a group of 16 Hartley albino guinea pigs, eight per sex; a positive control group of eight guinea pigs, four per sex, was used (Clairol, 1987c). H.C. Red No. 1, 10%, was dissolved in 80% DAE (40% dimethylacetamide/30% acetone/30% ethanol) and 20% physiological saline; 5% musk ambrette was dissolved in the same vehicle and used as the positive control. The light source was a 150-W xenon lamp that emitted UVA, UVB, and visible light. The minimal erythematous doses (MED) for UVB and UVB were determined to be 14 min and 90 s, respectively. During week 1 of induction, 0.1 ml of the test material was applied to a 1.8-cm-diameter depilated site on the nuchal area for 4 consecutive days. One hour after application, the animals were irradiated with 0.5 MED of UVA; a WG-354 glass filter was used to remove UVB waves. Each animal was scored for dermal irritation according to the Draize scale 24 h after application. During weeks 2 and 3 of induction, 0.1 ml of the test material was again applied to the same depilated site for 4 days. One hour after application, the animals were irradiated with 1 MED of UVB. On days 1 and 3 of these weeks, each animal was given an intradermal injection of FCA in physiological saline (1:1) on four different sites surrounding the test area.

Two weeks after the last week of induction, the animals were challenged with three daily applications of a 5% H.C. Red No. 1 or musk ambrette solution on three different sites of the left lumbar area (1.8 cm diameter); each site was scored 24 h after each application for irritation. One hour after each application, the animals were irradiated with 0.5 MED of UVB at site 1, and with 0.5 MED of UVB at site 2; they received no UV irradiation at site 3. Two weeks after the initial challenge, the animals were rechallenged with 0.1 ml of a 1% H.C. Red No. 1 solution on a depilated 1-sq-in area on the right flank, which was not irradiated.

No erythema or edema was observed for any of the animals during induction; however, significant irritation was seen at the FCA injection site in all animals. The skin was discolored (rose colored) at the site of H.C. Red No. 1 application. Upon challenge, 13 of 16 animals responded (irritation score ≥1) at all three challenge sites, and there was no difference in the degree of irritation between these sites. On rechallenge, seven of 16 animals had irritation. H.C. Red No. 1 produced a contact allergic reaction, but not a photoallergic reaction, in guinea pigs.
Ocular Irritation

The ocular irritation potential of H.C. Red No. 1 was assessed using four New Zealand White rabbits in a modification of the Federal Hazardous Substances Act testing method using Draize scoring (Claire, 1987d). One hundred milligrams H.C. Red No. 1 was placed in the conjunctival sacs of each rabbit. The eyes of two of the rabbits were rinsed 20 s following application. Mild effects were observed (scores of 1 and 2) and were limited to the conjunctiva (redness, swelling, and discharge). There was no corneal or iridal involvement. The unrisened and rinsed eyes appeared normal by the second and third days, respectively, following application.

Reproductive and Developmental Toxicity

Dermal

Groups of 20 gravid Charles River CD rats were used to evaluate the teratogenic potential of a semipermanent hair dye formulation containing 0.15% H.C. Red No. 1 (Burnett et al., 1976). The formulation was applied topically at a dose of 2 ml/kg to a shaved dorsocapular area on days 1, 4, 7, 10, 13, 16, and 19 of gestation. Three negative control groups of rats were shaved but not dosed, and rats of a positive control group were dosed orally by gavage with 250 mg/kg acetylsalicylic acid on days 6–16 of gestation. Feed and water were available ad libitum. All animals were weighed on the days of dosing and killed on day 20 of gestation. The only reported observation was a change in color of the skin and hair at the site of application. No signs of toxicity were reported. Body weight gains and mean feed consumption were similar for animals of the treated and negative control groups. A semipermanent hair dye formulation containing 0.15% H.C. Red No. 1 did not produce embroyotoxic or teratogenic effects in Charles River CD rats.

A multigeneration reproduction study was conducted using groups of 80 Sprague-Dawley rats (40 rats per sex), which received topical applications of a semipermanent hair dye formulation containing 0.15% H.C. Red No. 1 (International Research and Development Corporation, 1977). A dose of 0.5 ml was applied twice a week to a shaved area of the back ~1 in in diameter. (The initial dose was 0.2 ml per application, which was increased by increments of 0.1 ml per application weekly until reaching 0.5 ml per application.) Successive applications were made to adjacent areas on successive application days to minimize dermal irritation. Three negative control groups of rats were shaved but not dosed. When the rats were 100 days old, they were mated to produce an F_{1a} generation, which was eventually used in a carcinogenicity study. The F_0 generation was then reduced to 20 animals per group, remated to produce an F_{1b} generation, and then killed following weaning of the F_{1b} litters. Twenty male and 20 female rats per group were chosen from the F_{1b} litters and mated after 100 days to produce F_{2a} and F_{2b} litters. Five male and five female F_{1b} parents were necropsied after weaning of the F_{2b} litters.

Again following the same procedures, 20 male and 20 female F_{2} parents per

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group were selected from the F2b litters and mated to produce F3a, F3b, and F3c litters. After weaning the F3b litters, one weaning per litter per group was necropsied; the pups of the F3a and F3c litters were killed after weaning. Parental generations were observed daily for changes in general behavior and appearance, and detailed observations were recorded weekly. Body weights and feed consumption were measured weekly. The pups were counted and weighed as a litter on days 0, 4, and 14 of lactation. On day 21 of lactation, the pups were counted, sexed, and examined for pharmacological effects.

Dermal reaction consisting of mild scabbing, fissuring, atonia, and a leathery texture occurred intermittently throughout the treatment period in each generation. No dose-related pharmacotoxicological signs were found, and body weight gains, feed consumption, and survival were comparable for treated and control rats in each generation. During week 61, siaaladenitis was noted in some test and control animals; this condition regressed at week 63 but was followed by an increased incidence of respiratory congestion in both test and control animals. The respiratory congestion persisted in the F2 parents during the production of successive litters. Litter size and pup body weights were similar for test and control groups. Fertility, gestation, survival, and live birth indexes were comparable between test and control animals for the F0, F1, and F2 parents. The F2 parents had markedly reduced fertility indexes for the three separate matings, but there were no significant differences between the control and test groups with respect to fertility. The researchers did not report that respiratory congestion was a significant factor in the reduction of fertility indexes. The results of a special study established that the decreased fertility was due to reproductive tract changes in both the treated and control rats and therefore was not related to the test article. No gross or microscopic treatment-related lesions were observed in F1b parental rats or F3b weanling rats. The topical application of a semipermanent hair dye formulation containing 0.15% H.C. Red No. 1 did not affect the reproductive performance of rats.

Oral

As reported earlier, animals from a subchronic feeding study were used in a developmental toxicity study (Bristol-Myers Products, 1992). After 15 weeks of dosing, 25 females per group (0, 0.01, 0.03, or 0.1% H.C. Red No. 1 in feed) were fed control diet throughout mating. Females were observed daily for general appearance and toxicity. Body weights were measured at study initiation and on days 6, 9, 12, 15, and 20, and feed consumption was determined for 10 gravid rats/group on days 11–12 and 19–20 of gestation. The animals were killed on day 20 of gestation. There were no statistically significant differences in body weights or feed consumption between the test and control groups during gestation; mean body weight gains were statistically significantly lower for dams of the high-dose groups during days 6–9 and days 0–9, 0–12, and 0–15. Gravid uterine weights and maternal carcass weights were not statistically significantly different between test and control groups. There were no statistically significant differences between test and control groups with regard to any cesarean-section observations or fetal

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morphological examinations. H.C. Red No. 1 did not produce a fetotoxic or a teratogenic response.

**MUTAGENICITY**

An Ames test was performed using *Salmonella typhimurium* strains TA1535, TA1537, TA1538, and TA98 in which H.C. Red No. 1 was assayed for mutagenic potential at concentrations of 25–5,000 μg in the presence of metabolic activation (Clairol, 1991). Positive and negative controls were used. H.C. Red No. 1 did not increase the number of revertants in any of the *S. typhimurium* strains in the presence of metabolic activation. An assay was performed to evaluate the potential of H.C. Red No. 1 to induce unscheduled DNA synthesis (UDS) in primary cultures of rat hepatocytes by measuring the incorporation of tritiated thymidine into DNA (Pharmakon Research International, Inc., 1993). H.C. Red No. 1 was tested in triplicate at a dose range of 0.1–2,500 μg/ml in dimethylsulfoxide (DMSO) with concurrent untreated, negative, and positive controls. Upon visual inspection, it was determined that 25 μg/ml was the highest dose to be scored owing to toxicity; cultures dosed with 1, 5, and 10 μg/ml were also evaluated. H.C. Red No. 1, 1–25 μg/ml, was negative in inducing UDS (repair) in rat primary hepatocytes.

A chromosomal aberration study was performed using Chinese hamster ovary (CHO) cells to evaluate the clastogenic potential of H.C. Red No. 1 (Integrated Laboratory Systems, 1993). H.C. Red No. 1 in DMSO was tested at a dose range of 7.5–100 μg/ml in the presence and absence of metabolic activation. Cyclophosphamide and mitomycin C were used as the positive controls in the presence and absence of metabolic activation, respectively. Without metabolic activation, chromosomal aberrations were evaluated only for the cultures treated with 7.5–25 μg/ml owing to excessive toxicity to the cultures dosed with 50–100 μg/ml. H.C. Red No. 1 did not induce a significant increase in the percentage of metaphase cells containing at least one aberration. The mitotic index (MI) was significantly decreased, but the percentage of polyploid metaphase cells was not significantly different. Based on pairwise comparisons, CHO cells dosed with 7.5–100 μg/ml in the absence of metabolic activation had a depressed MI. With metabolic activation, chromosomal aberrations were evaluated for the cultures treated with 50–100 μg/ml. Over this dose range, H.C. Red No. 1 induced a significant increase in the percentage of metaphase cells containing at least one aberration. Again, the MI was depressed, while the percentage of polyploid metaphase cells was not significantly altered. Using pairwise comparisons, the aberration response, consisting primarily of chromatid-type breaks and rearrangements, was significantly increased, and the MI was significantly depressed only for cultures dosed with 100 μg/ml in the presence of metabolic activation.

During the previously described subchronic feeding study, bone marrow smears were prepared from the femurs of five males per group (0, 0.01, 0.03, or 0.1% H.C. Red No. 1 in feed) at the time of the 13-week necropsy and processed for a micronucleus assay (Bristol-Myers Products, 1992). There were no statistically significant differences in the number of micronucleated polychromatic erythro-

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cytes between the control and test groups. H.C. Red No. 1 did not have a clastogenic effect.

Animals from the previously described subchronic feeding study were also used in a dominant lethal study (Bristol-Myers Products, 1992). After 20 weeks of dosing, 20 males per group (0, 0.01, 0.03, or 0.1% H.C. Red No. 1 in feed), which were fed control feed throughout the remainder of the study, were mated with nondosed females. The males were weighed weekly and observed daily for general condition and signs of toxicity. Females were also observed daily; weighed on days 0, 12, and 17 of gestation; and killed on day 17 of gestation. No statistically significant differences in body weights of gravid females were observed between the test and control groups. There were also no statistically significant differences reported for any litter or reproduction parameters.

CARCINOGENICITY

A 23-month skin-painting study was performed using groups of 100 Eppley Swiss Webster mice (50 males and 50 females per group) to determine the carcinogenic potential of a semipermanent hair dye formulation containing 0.15% H.C. Red No. 1 (Burnett et al., 1980). A 0.05-ml sample of the test solution was applied undiluted to a 1-cm² area of clipped skin of the interscapular region. A group of negative controls was shaved but not dosed. Observations were made daily, and body weights were measured monthly. After 9 months, 10 male and 10 female animals from each group were necropsied, and liver and kidney weights were determined. Gross and microscopic examinations were made for all animals found dead, killed due to moribund condition, or killed at study termination. Relative and absolute liver and kidney weights were not significantly different from control values. No dose-related neoplasms were observed. A semipermanent hair dye formulation containing 0.15% H.C. Red No. 1 applied dermally for 23 months did not induce a carcinogenic effect.

F₁₀ generation Sprague-Dawley rats from a previously described reproduction study (International Research and Development Corporation, 1977) were used to determine the carcinogenic potential of a semipermanent hair dye formulation containing 0.15% H.C. Red No. 1 (International Research and Development Corporation, 1979). Twice a week a dose of 0.5 ml of the hair dye formulation was applied topically to a shaved area of the back, (~1 in in diameter) of 120 rats, 60 per sex, for 12 months. (The initial dose was 0.2 ml per application, which was increased by 0.1 ml per application increments weekly until reaching 0.5 ml per application.) Successive applications were made to adjacent areas to minimize dermal irritation. Three negative control groups of 120 rats were shaved but not dosed.

The rats were observed daily for signs of toxicity and mortality; detailed observations were recorded weekly. Body weights were measured weekly for the first 14 weeks and monthly thereafter; feed consumption was determined weekly. Biochemical measures were determined from blood and urine samples that were collected from five male and five female fasted rats per group at 3, 12, 18, and 24 months. Five male and five female rats per group were killed after 12 months. No

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signs of toxicity were observed. Test rats had a slightly greater incidence of skin lesions from various locations, including ulceration, scabbing, abscess formation, and thickening. Coloration of the hair and skin at the application site was observed in several treated rats, but it was not considered to be pathologically significant. Body weight gains, survival, hematological values, and biochemical measures were similar for rats of the treated and control groups. After 3, 12, and 24 months, the animals consistently had dark straw-colored urine; three and nine rats had dark brown urine at 12 and 18 months, respectively.

The incidence of enlarged and/or firm livers was slightly greater in the test group compared with the controls; this result was considered to be "possibly compound related." Other lesions considered to be possibly compound related for males and females of the test group include parathyroid gland hyperplasia, hepatocellular hypertrophy or hyperplasia, and hyperkeratosis and dermatitis from a variety of locations. There was a considerably higher incidence of this last effect. Several male test rats had hyperkeratosis and/or acanthosis involving the gastric mucosa, which was also possibly compound related.

The incidence of hematopoiesis in the livers of test rats was somewhat greater than that of all controls; the significance of this increase was not determined. For female test animals, the incidence of pituitary adenomas was significantly higher compared with females from two of the three control groups, and the incidences of mammary adenocarcinaoma/mammary carcinoma were significantly higher compared with females in one of the three control groups; however, these differences were not considered biologically significant. Actuarial (life table) analyses did not report significant variations in indexes of tumor bearing in the test animals compared with the control groups by sex.

**CLINICAL ASSESSMENT OF SAFETY**

**Sensitization**

A repeated-insult patch test was performed to determine the sensitization potential of H.C. Red No. 1 (TKL Research, Inc., 1987). Of the 105 initial subjects, 103 completed the study. During the induction phase, H.C. Red No. 1 was applied as 0.2 ml of a 3.0% slurry in a bland base to the infrascapular area of the back under an occlusive patch for nine consecutive applications. Patches were removed 24 h after application, and the test sites were read either 48 or 72 h after application. The challenge patch was applied to a previously untested area 2 weeks after the last induction patch; the patch was removed after 24 h and scored 48 and 72 h after application. One subject had reactions indicative of presensitization, and another subject had reactions indicative of sensitization to H.C. Red No. 1.

**Epidemiology**

Between 35% and 45% of American women dye their hair, often at monthly intervals, over a period of years (Cosmetic, Toiletry, and Fragrance Association, 1993). This estimate is drawn from market research data on hair dye product use.

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generally from females aged 15–60. A number of epidemiologic studies have investigated the association between cancer and occupation as a hairdresser or barber and between cancer and personal use of hair dyes. The World Health Organization’s International Agency for Research on Cancer (IARC) empaneled a Working Group on the Evaluation of Carcinogenic Risks to Humans to review all available data on these issues. The Working Group met October 6–13, 1992, in Lyon, France (IARC, 1993).

The charge to the IARC Working Group was to ascertain that all appropriate data had been collected and were being reviewed; to evaluate the results of the epidemiological and experimental studies and prepare accurate summaries of the data; and to make an overall evaluation of the carcinogenicity of exposure to humans. The IARC Working Group concluded that: “there is inadequate evidence that personal use of hair colourants entails exposures that are carcinogenic.” Hence: “Personal use of hair colourants cannot be evaluated as to its carcinogenicity (Group 3).” The IARC Working Group also concluded that: “there is limited evidence that occupation as a hairdresser or barber entails exposures that are carcinogenic.” Hence: “Occupation as a hairdresser or barber entails exposures that are probably carcinogenic (Group 2A)” (IARC, 1993). The CIR Expert Panel concludes that the relevance of the occupational data and conclusion to individuals using hair dyes is unclear.

SUMMARY

H.C. Red No. 1, a color additive that functions as a colorant in hair dyes and colors, is a dark brown crystalline material. It is soluble in ethanol and insoluble in water. It is manufactured by a three-step process and must exist as a minimum of 95% H.C. Red No. 1. In 1994, data submitted to the FDA reported that H.C. Red No. 1 was used in 47 hair dye and color formulations; data received from industry reported that it is used at concentrations of ≤0.5% in oxidative and semipermanent hair dye formulations. Hair dyes containing H.C. Red No. 1, as coal tar hair dyes, are exempt from the principal adulteration provision and from the color additive provisions in sections 601 and 706 of the Federal Food, Drug, and Cosmetic Act of 1938 when the label bears a caution statement and patch-test instructions for determining whether the product causes irritation.

The rate of percutaneous absorption of 1% H.C. Red No. 1 was linear for ~4 h following exposure. The total skin absorption at 24 and 48 h was 1.6 ± 0.01 and 1.68 ± 0.01%, respectively. The oral median lethal dose of H.C. Red No. 1 for male and female rats was between 2,500 and 5,000 mg/kg and between 625 and 1,2500 mg/kg, respectively. In a short-term oral study in which rats were fed ≤0.8% H.C. Red No. 1, some statistically significant differences in liver, spleen, and kidney weights were observed between some of the test and control groups. Dose-related lesions were observed in the livers and spleens of some female test rats; similar but less prominent splenic lesions were observed for some male rats. In a dermal subchronic toxicity study of a semipermanent hair dye formulation containing 0.15% H.C. Red No. 1, the urine of rats was not discolored, and no evidence of compound-induced toxicity was found. In an oral subchronic study in
which rats were fed \( \leq 0.1\% \) H.C. Red No. 1, animals of all dose groups had rust-colored urine throughout the study, the hair of some animals was discolored red, the liver and spleen weights of some males were increased, and there was an increase in splenic pigmentation in some dosed females after 13 weeks; H.C. Red No. 1 did not induce methemoglobin formation. An aqueous slurry of 500 mg H.C. Red No. 1 was nonirritating to rabbits when applied under nonocclusive conditions. H.C. Red No. 1 did produce contact sensitization in guinea pigs under both open and occlusive conditions, but it did not produce a photoallergic reaction. The application of 100 mg H.C. Red No. 1 in the conjunctival sacs of rabbit eyes resulted in mild effects.

The ability of a semipermanent hair dye formulation containing 0.15% H.C. Red No. 1 to induce developmental toxicity (including teratogenicity) or reproductive effects upon dermal application was examined. In a developmental toxicity study, the formulation caused a change in the application site skin and hair color, but it did not produce embryotoxic or teratogenic effects. The formulation did not affect reproductive performance in a multigeneration study. In an oral study in which rats were fed \( \leq 0.1\% \) H.C. Red No. 1, neither a teratogenic nor a fetotoxic response was observed. H.C. Red No. 1 produced no evidence of mutagenic potential in an Ames test, was negative in inducing UDS in rat primary hepatocytes, did not have a clastogenic effect in a micronucleus assay, and produced no dominant lethal effects. Based on pairwise comparisons, CHO cells dosed with 100 \( \mu \text{g/ml} \) and 7.5–100 \( \mu \text{g/ml} \) H.C. Red No. 1 had a depressed MI in the presence and absence of metabolic activation, respectively; in the presence of metabolic activation, the aberration response was also significantly increased.

A semipermanent hair dye formulation containing 0.15% H.C. Red No. 1 did not produce carcinogenic effects in a 23-month mouse skin-painting study. However, in a second dermal carcinogenicity study with a semipermanent hair dye formulation containing 0.15% H.C. Red No. 1 using rats, discolored urine was reported, and the following, possibly compound-related alterations were observed: enlarged and/or firm livers, parathyroid gland hyperplasia, increased frequency of hepatocellular hypertrophy or hyperplasia, and increased incidence of hyperkeratosis and dermatitis from a variety of locations for male and female test rats, and hyperkeratosis and/or acanthosis involving the stomach mucosa in some male test rats. In a human repeated-insult patch test of a 3.0% slurry of H.C. Red No. 1, one subject had reactions indicative of presensitization, and another subject had reactions indicative of sensitization.

DISCUSSION

The CIR Expert Panel considered oral toxicity data in which animals were exposed to H.C. Red No. 1 in their diet at concentrations of \( \leq 0.1\% \), as well as cutaneous absorption data showing that \( \sim 1.6\% \) of applied H.C. Red No. 1 was absorbed through the skin. Based on this information and additional data summarized in the report, the Expert Panel was able to extrapolate the oral exposure to determine that H.C. Red No. 1 is safe as used at concentrations of \( \leq 0.5\% \).

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CONCLUSION

On the basis of the animal and clinical data included in this report, the CIR Expert Panel concludes that H.C. Red No. 1 is safe as used in hair dye formulations at concentrations of \( \leq 0.5\% \).


REFERENCES


Bristol-Myers Products. (1992) Final report study no. GLP-90001. The toxicity of H.C. Red No. 1 (4-amino-2-nitrodiphenylamine) in the Sprague Dawley rat: effects on growth, reproduction, and fetal development (three/six month combined subchronic toxicity, teratology, dominant lethal and micronucleus study in rats.) Unpublished data submitted by Clairol, Inc. (681 pages).*


Clairol. (1987b) Primary skin irritation study using rabbits (report dated January 8). Unpublished data submitted by Clairol, Inc. (3 pages).*

Clairol. (1987c) Photosensitization potential of H.C. Red No. 1 (and Acid Orange no. 3) in guinea pigs. Study no. 87007 (reported dated July 7). Unpublished data submitted by Clairol, Inc. (14 pages).*


Clairol. (1994a) Chemistry, nomenclature, and specifications. Unpublished data submitted by Clairol, Inc. (1 page).*

Clairol. (1994b) General data. Unpublished data submitted by Clairol, Inc. (1 page).*

Clairol. (1994c) Personal correspondence from P. Nicholas to F. A. Andersen regarding Clairol’s use concentration of H.C. Red No. 1 (1 page).*


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