# Amended Final Report on the Safety Assessment of Glyoxal[1]

**An earlier unpublished safety assessment of the preservative, Glyoxal, found insufficient data to support the safety of its use in cosmetics. Additional data needs included the types of cosmetic products in which Glyoxal is used and the typical concentration of use for each; impurities, especially with respect to selenium, chlorinated organic compounds, and the Glyoxal monomer; and dermal carcinogenesis using the methods of the National Toxicology Program's skin painting studies. Although Glyoxal is not currently reported to be used, its last reported use was in products intended to be applied to the nails. Composition data show that Glyoxal may contain formaldehyde residues. Additional data, including a dermal carcinogenicity study in which mice were treated with commercial 40% Glyoxal solutions, were subsequently provided. Glyoxal was shown to be mutagenic, but was negative in an oral and dermal carcinogenicity study. No reproductive or developmental toxicity was seen in rats or rabbits. Glyoxal powder was not irritating, and a commercial 40% Glyoxal solution produced negative to moderate irritation. In animal studies and clinical testing, Glyoxal was shown to be a sensitizer, but conflicting results made it difficult to establish the concentration of Glyoxal at which it will sensitize. Animal safety test data, however, indicate a threshold concentration of 1.25% for sensitization. Recommending that care be taken to limit the concentration of free formaldehyde, the Expert Panel concluded that Glyoxal is safe for use in products intended to be applied to the nail at concentrations $\leq$1.25%. The available data are insufficient to support the safety for other uses, if any.**

## INTRODUCTION

Glyoxal (CAS No. 107-22-2) is a bialdehyde that conforms to the formula shown in Figure 1. There are currently no reported uses of Glyoxal in cosmetic formulations (FDA 1998). Its last reported use was in nail polishes and enamels in 1993 (FDA 1993).

In 1993, the Cosmetic Ingredient Review (CIR) prepared a safety assessment of Glyoxal (Andersen 1995). That unpublished safety assessment noted that Glyoxal was genotoxic in several assays and that it could act as a tumor promoter but not as an initiator. The CIR Expert Panel issued an Insufficient Data conclusion noting the following data needs:

1. types of cosmetic products Glyoxal is currently used in and the typical concentration of use for each;
2. impurities, especially with respect to selenium and chlorinated organic compounds and the Glyoxal monomer; and
3. dermal carcinogenesis using the methods of the National Toxicology Program's skin painting studies.

In the same year the safety assessment was completed, a petition to reopen the safety evaluation was received by CIR, including a dermal carcinogenicity study and other new studies that had been newly submitted to the Environmental Protection Agency (EPA) under section 8(e) of the Toxic Substances Control Act (TSCA) and made available through the National Technical Information Service (NTIS).

This amended safety assessment of Glyoxal includes the information contained in the original safety assessment and the new material.

## CHEMISTRY

### Definition and Structure

Glyoxal (CAS No. 107-22-2) is the bialdehyde that conforms to the formula shown in Figure 1. Other names for Glyoxal include: biformal, biformyl, diformal, diformyl, ethanedial, ethanedione, glyoxalaldehyde, and oxalaldehyde (Wenninger, Canterbery, and McEwen 2000).

Glyoxal is a product of the decomposition of glucose when exposed to ionizing radiation (Chopra 1966). It occurs naturally in heated coffee (Sugimura and Sato 1983; Furihata and Matsushima 1986) and in auto-oxidized edible oils, such as sesame, safflower, and sardine oil (Hirayama et al. 1984). Drinking water will sometimes contain Glyoxal after ozonation (Ueno et al. 1991b).

### Physical Properties

Glyoxal, molecular weight 58.04 Da, has a melting point of 15°C, a boiling point of 51°C (776 mm Hg), and a flash point of 220°C; the density is 1.14 at 20°C (Lide 1993; National Research Council [NRC] 1981). It has a vapor pressure of 220.0 mm Hg at 20°C and a vapor density of 2.0 (NRC 1981). The pH of a 40% aqueous solution of Glyoxal is 2.1 to 2.7. It appears as yellow prisms or a yellow liquid; it burns with a purple flame and

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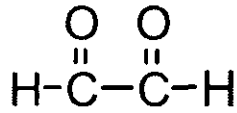

**FIGURE 1**
Chemical formula for Glyoxal.

emits green vapor. Glyoxal is soluble in water, alcohol, and ether (Lide 1993; Budavari 1989). For Glyoxal, the experimentally determined apparent Henry's Law constant is $\geq 3 \times 10^5$ M/atm; the intrinsic Henry's Law constant is $\geq 1.4$ M/atm (the intrinsic value is the apparent constant, corrected for the extent of hydration); and the hydration constant is $2.2 \times 10^5$ (Betterton and Hoffmann 1988). Henry's Law constant is a ratio of the concentration of free, unhydrated, aldehyde dissolved in the aqueous phase to the concentration in the gas phase. In the apparent constant the total aldehyde concentration in the aqueous phase (the amount present in the *gem*-diol form plus the unhydrated dissolved) is represented in the numerator of the ratio. The hydration constant is the ratio of the concentration of aldehyde present in the *gem*-diol form to the concentration of unhydrated, dissolved aldehyde.

### Chemical Reactivity

Glyoxal is highly reactive, polymerizing explosively with water (Budavari 1989).

### Ultraviolet (UV) Absorption

An absorption spectral analysis was conducted on glyoxal trimeric dihydrate ($C_6H_{10}O_8$). The values show no significant absorption in the UVA and UVB range (Research Triangle Institute 1989).

### Method of Manufacture

Glyoxal is synthesized by the oxidation of acetaldehyde, either by nitric or selenious acid, or by the hydrolysis of dichlorodioxane (Budavari 1989). Commercially available Glyoxal is a 40% aqueous solution of many hydrated forms in equilibrium.

### Other Formation

Murata-Kamiya et al. (1995) reported that Glyoxal is produced by exposure of DNA to an oxygen radical–forming system (5 mM $FeSO_4$-EDTA at 37°C for 60 minutes). The rate of Glyoxal generation was 17 times more efficient than that of 8-hydroxydeoxyguanosine. Shibamoto (unknown date) reported that Glyoxal is a photodegradation products of some lipids or fatty acids such as Squalene (forming 9.6 nmol Glyoxal/mg) and cod liver oil (27 nmol/mg). Glyoxal is generated when humic substances are ozonated (Matsuda et al. 1992). Glyoxal is also generated in the Maillard reaction which is involved in meat mutagen formation (Pearson et al. 1992; Glomb and Monnier 1995).

### Impurities

In dilute aqueous solution, Glyoxal exists as a mixture of the fully hydrated monomer, dimer, and trimer, with the monomer and the dimer being predominant. At concentrations of $\leq 1$ M, the monomer predominates (Whipple 1970).

Two commercial 40% Glyoxal solutions were analyzed before being used in a dermal carcinogenicity assay. The standard for one sample (European Glyoxal 40) specified 40.4% Glyoxal, 0.7% formaldehyde, 0.2% glycolaldehyde, 0.1% acid (calculated as acetic), and a trace of ethylene glycol. The standard for the second sample (Aerotex Glyoxal 40) specified 40.0% Glyoxal, 5.9% formaldehyde, 0.3% glycolaldehyde, 0.9% ethylene glycol, and 0.7% acid (calculated as acetic). Both solutions were within their respective guidelines (Bushy Run Research Center 1982).

### Analytic Methods

The detection of total aldehydes is often obtained by solution-phase spectrophotometry (NRC 1981). Steinberg and Kaplan (1984) report a method that detected low-molecular-weight aldehydes, including Glyoxal, using reversed phase liquid chromatography detection of 2,4-dinitrophenyl-hydrazone derivatives. Another method uses a fluorescent guanosine derivative to detect Glyoxal and other adduct-forming compounds on an high performance liquid chromatography (HPLC)-fluorescence detector system (Kasai et al. 1984). Ereyman (1987) proposes a method of determining Glyoxal by reacting it with phenylhydrazine hydrochloride and measuring the complex at 380 nm.

## USE

### Cosmetic

Glyoxal is described as a cosmetics preservative (Wenninger, Canterbery, and McEwen 2000). Historically, Glyoxal was used in nail polishes or enamels (FDA 1993), but no current uses are reported (FDA 1998).

### Noncosmetic

Glyoxal is used in the textile industry in as an ingredient in permanent press fabrics, as a stabilizing agent in rayon and other fibers, and as a reducing agent in the dyeing process. It is used to insolubilize proteins (such as animal glue, gelatin, and casein) and compounds with polyhydroxyl groups. It is also used in embalming fluids, leather tanning preparations, and paper coatings (NRC 1981).

## BIOCHEMICAL REACTIVITIES

The effects of Glyoxal on collagen was investigated by Bowes and Carter (1968). Powdered collagen was soaked in water overnight. The excess water was removed and 10% and 16% aqueous Glyoxal (20 ml/g collagen) were added. The pH was

then adjusted to 7.5 to 8.0 with $NaHCO_3$. Samples were incubated at room temperature for 24 hours with intermittent shaking. Formol titration and amino acid analysis (by elution) were used to measure the amount of Glyoxal bound to collagenous protein. By formol titration, 16.3 moles of amino acid groups reacted per $10^5$ g of collagen for the 10% Glyoxal solution; 13.6 mol/$10^5$ g for the 16% Glyoxal solution. Stress-strain measurements on denatured kangaroo tail tendon were used to determine the amount of Glyoxal involved in cross-linking reactions. For both concentrations of Glyoxal, 8 moles of amino acid groups per $10^5$ g collagen were cross-linked. Glyoxal had little or no effect on skin shrinkage temperature or UV absorption.

Glyoxal also reacts with the guanidino group in arginine to yield multiple adducts in vitro (Glass and Pelzig 1978). One adduct was prepared in two ways. The first combined Cbz-arginine and aqueous Glyoxal at pH 8.1, then stabilized the solution with HBr and glacial acetic acid. The second combined arginine HCl with aqueous Glyoxal in 12 M HCl. Chromatographic analysis revealed that the adduct has a structure similar to ornithine. At pH 6 to 7, the adduct in solution was stable for at least 20 hours. At pH 8 to 11.5, the adduct decomposed over a period of several hours.

## ANIMAL TOXICOLOGY

### Oral

*Acute*

A range of oral $LD_{50}$ values has been reported. The $LD_{50}$ of aqueous Glyoxal was 2.02 g/kg in male Wistar rats and 0.76 g/kg in guinea pigs (Smyth, Seaton, and Fischer 1941). In more recent studies, Glyoxal (30%) had an $LD_{50}$ of 0.2 to 0.4 g/kg in rats and 0.8 to 1.6 g/kg in guinea pigs (Eastman Kodak Company 1971). Another rat study, using 10% Glyoxal, reported a value of >5 g/kg (Younger Labs 1969b). Glyoxal (5%) had an $LD_{50}$ value of 0.4 to 0.8 g/kg in mice (Eastman Kodak Company 1971).

*Short-Term*

Groups of 12 rats (6 of each sex) were given drinking water containing 100, 300, or 1000 mg of a (40%) Glyoxal solution per kg body weight/day for 28 days. A control group of 12 rats was given untreated water. Clinical examinations and analysis of blood and urine were performed on all animals. The rats were killed at the end of the study and necropsied. No clinical signs and no mortality were noted during the study. Body weight gain and feed consumption of animals of the 100 mg/kg/day group were comparable to values for the control. A reduction in these parameters was noted in rats of the 300 mg/kg/day group and was significant in rats of the 1000 mg/kg/day group. A pronounced dose-related decrease in water consumption was noted; males of the 1000 mg/kg/day group had a significantly increased red blood cell value, most likely related to the decreased water consumption and accompanying dehydration. Male rats of the 300 mg and all rats of the 1000 mg/kg/day group had higher inorganic blood phosphorus concentrations. Rats of the high-dose group also had increased urea concentrations (no morphologic changes were found in the kidneys). No treatment-related changes were noted at necropsy or microscopic examination. The no-toxic-effect dose was 100 mg/kg/day (Société Française Hoechst 1987).

Sprague-Dawley rats received drinking water that contained 2000, 4000, or 6000 mg/L Glyoxal. Water and feed were available ad libitum. Observations were recorded daily, and body weight and water and feed consumption were measured twice a week. Animals were killed for necropsy at 30, 60, and 90 days. Significant decreases in body weight gains were seen in animals in the mid- and high-dose groups. Concomitant with this was a decrease in feed and water consumption. Feed consumption remained constant per gram of body weight. Minor swelling of the renal papillary epithelial cells and interstitial edema were observed in rats of the high-dose group at the 90-day termination. A significant increase in the kidney to the total body weight ratio was also observed in rats of the high-dose group. Glyoxalase I and II concentrations were significantly increased in the liver, and erythrocytes also were increased at 30 days for mid- and high-dose animals. Glyoxalase I was increased in the kidneys only in high-dose animals at 30 days; at 60 and 90 days, glyoxalase concentrations were comparable with controls. Observed in the mid- and high-dose animals were reductions in the activities of the following serum enzymes: aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase. These changes were accompanied by concentrations of albumin and total protein, and the albumin-to-globulin ratio was increased (Ueno et al. 1991c).

*Subchronic*

Groups of 20 Harlan-Wistar albino rats (10 each sex) received feed containing 0.03125, 0.125, or 0.25 g Glyoxal/kg body weight for 3 months. One week after the start of dosing a fourth group of rats was started on a diet containing 0.0625 g Glyoxal/kg. (Glyoxal 40% was used, but reported doses are the amount of active agent Glyoxal received.) Rats were killed at the end of dosing and various organs were examined and weighed. Liver weight, as a percentage of body weight, was increased in male rats of the highest dose group. These rats also had decreased body weight gain as compared to untreated controls. The dose that "was without significant ill-effect" was 0.12 g/kg/day (Mellon Institute 1966).

Similar to the above study, groups of Beagle dogs were fed Glyoxal at doses of 0.031, 0.065, and 0.115 g/kg body weight/day for 3 months. No changes in mortality, appetite, liver and kidney weights, gross and microscopic lesions, mean body weight changes, or various hematological and biochemistry parameters were noted in dosed dogs versus controls. The investigators rounded up the value of the highest dose and considered 0.12 g Glyoxal/kg/day to produce no "significant ill-effect" (Mellon Institute 1966).

Glyoxal, 6000 mg/L, was administered to Sprague-Dawley rats ad libidum via drinking water for 180 days (Ueno et al. 1991c). Two controls were used; one group was given feed ad libitum just as the dosed group was, and the other group was given a diet in the same amount consumed by the dosed group. Observations were recorded daily, and body weight and water and feed consumption were measured twice a week. Animals were killed for necropsy at 90 and 180 days. Two animals of the 6000 mg/L Glyoxal dose group died before 30 days. The deaths were attributed to hemorrhages in the glandular stomach. Significant decreases in body weight gains were seen in animals receiving Glyoxal and the restricted diet, but the reduction was greater in the dosed group. Animals had significantly elevated organ-to-body weight ratios for the heart, liver, and kidneys at both 90 and 180 days. A significant reduction in total protein and a significant increase in the albumin-to-globulin ratio were seen at 180 days.

National Toxicology Program (NTP) (1992) reported the findings of a drinking water study of Glyoxal Dihydrate on male and female Fischer 344 rats and B6C3F1 mice. Animals received doses of 1, 2, 4, 8, or 16 mg/ml Glyoxal Dihydrate for 90 days. In rats, all of the animals of the highest dose group were killed at day 12 due to decreased weight and feed consumption and moribundity. In male rats at the 4 mg/ml and 8 mg/ml dose concentrations, body weight gains were 90% and 75%, respectively, of that in controls. Body weight gains in female rats were reduced 9% at the 8 mg/ml dose concentration. Minor hemorrhages of the mesenteric lymph nodes, lymphoid hyperplasia of the mandibular lymph node, moderate atrophy of the salivary glands, mild renal changes, and hypospermia and atypical cells of the testes were observed in male rats in the 8 mg/ml and the 16 mg/ml dose group. Females of the 16 mg/ml dose group had thymic atrophy. All groups of male rats had some minimal lymphoid hyperplasia of the mandibular lymph node. All groups had some hemorrhages of the mesenteric lymph nodes.

Body weight gains in male mice were 93%, 88%, 80%, and 70% of those in controls at the 2, 4, 8, and 16 mg/ml dose concentrations, respectively. Body weight gains in female rats were 93%, 90%, and 79% of weight gain in controls at the 4, 8, and 16 mg/ml dose concentrations. Decreases in feed and water concentrations were observed in all dosed male mice and the two highest dose groups in female mice. The only histopathological findings were changes of the salivary glands in dosed male mice (NTP 1992).

### Acute Parenteral

Glyoxal (30%) had an intraperitoneal (IP) $LD_{50}$ value of <100 mg/kg in rats, and 100 to 200 mg/kg in guinea pigs. Glyoxal (5%) had an IP $LD_{50}$ of 200 to 400 mg/kg in mice (Eastman Kodak Company 1971).

### Acute Dermal

In one study, Glyoxal (10%) had an $LD_{50}$ of >5000 mg/kg in rabbits (Younger Labs 1969b). In another study Glyoxal (30%) had a cutaneous $LD_{50}$ of >20 ml/kg in guinea pigs and was classified a moderately strong skin irritant (Eastman Kodak Company 1971).

A mixture containing 1.3% Glyoxal was applied (2 g/kg body weight) to shaved and abraded skin sites on 10 albino New Zealand white rabbits (5 of each sex). The test material remained in contact with the skin for 24 hours and then the skin was washed. Twice daily observations were made for a 2-week period; reactions were scored according to the Draize standard. No animal died during the study. Slight erythema and edema were visible at the 2- and 4-hour observations. No treatment-related lesions were found at necropsy. The $LD_{50}$ for the mixture was >2 g/kg (Pharmakon Research Intl. Inc. 1984c).

In a second acute dermal study, a 40% Glyoxal solution (2000 mg/kg) was applied in a single 24-hour semiocclusive patch to clipped sites on 10 Wistar rats (5 of each sex). Sites were rinsed after patch removal. No animals died during the 14-day observation period that followed treatment; erythema was noted at the application sites. No treatment-related lesions were noted at necropsy. The $LD_{50}$ was >2 g/kg for the solution (BASF AG 1985a).

A modified Draize dermal study was conducted using six female New Zealand white rabbits (CTFA 1992). Three applications of 0.5 ml of a 40% aqueous solution of a nail enamel containing 0.5% Glyoxal were made under a topical dry patch to the clipped back or side of the animal. After 24 hours, the first application sites were scored. Then new patches were applied as before and sites were scored at 24 and 48 hours. No irritation was observed.

### Acute Inhalation

In a time saturation test, 10 SPF-Wistar rats (5 of each sex) received a single 7-hour inhalation exposure to a 40% Glyoxal solution. The calculated Glyoxal consumption reported in the study was 44.13 mg. A change in respiratory frequency was noted in all animals during the exposure period (no further details provided). No reduction in body weight was observed. No deaths occurred during the exposure period or in the 14-day observation period that followed. No macroscopic changes were found at necropsy (Société Française Hoechst 1984a).

A group of 10 SPF-Wistar rats (5 of each sex) received a single 4-hour inhalation exposure to Glyoxal powder (80% pure) at a concentration of 1.30 mg/L air. Almost 80% of the particles were between 3.0 and 10.3 $\mu$m in size. Irregular respiration, bloody tears, and bloody and crusted snouts were noted in all animals after 1 hour of exposure. Sneezing was observed in all animals 1 to 5 days following exposure. No deaths occurred during the exposure period or in the 14-day observation period that followed. No reduction in body weight was observed and no lesions were noted at necropsy. The $LC_{50}$ for male and female Wistar rats was >1.3 mg Glyoxal/L air (Société Française Hoechst 1984b).

### Ocular

A solution of 32.8% aqueous Glyoxal caused grade 5 injury to the eyes of rabbits. Grade 5 is defined as "0.02 ml yields

a score over 5.0 and 0.005 ml yields not over 5.0." Points are assigned according to corneal opacity, keratoconus, iritis, and necrosis (measured by fluorescein staining) with a maximum of 20 points (Carpenter and Smyth 1946).

Glyoxal (30%) caused moderate irritation in the rabbit eye that cleared within 48 hours without permanent injury (Eastman Kodak Company 1971).

Glyoxal (40%) was instilled into one conjunctival sac of each of three female white Vienna rabbits. The untreated eye of each rabbit served as the control. Eyes were not rinsed. No changes in the iris or corneal opacity were noted at observations made 1, 24, 48, 72 hours and 8 days after instillation. Well-defined redness and chemosis of the conjunctiva was noted in all three treated eyes at the 1-hour observation. The reactions decreased in severity with time and were scored as slight at the 72-hour reading and as normal at the day 8 observation (BASF AG 1985b).

In one study, Glyoxal powder was a severe ocular irritant when instilled into the conjunctival sac of one eye of each of three rabbits. Reactions produced had an average 1-hour maximum score of 65.6 out of a maximum possible 110 (Younger Labs 1969a). However, in another study by the same researchers, Glyoxal powder was classified as a slight ocular irritant with an average 1-hour maximum score of 6.6 (Younger Labs 1969b).

### Dermal Irritation

In two studies, Glyoxal powder or solution was applied to clipped intact skin sites on three albino rabbits and the sites were then covered with plastic strips to avoid contamination. The test material was removed after 24 hours and observations were made 1 to 168 hours postapplication. In both studies Glyoxal powder was nonirritating (Younger Labs 1969a, 1969b). In one study Glyoxal (50% solution) was nonirritating (Younger Labs 1969a). In the other study, Glyoxal (40% aqueous) was a slight irritant, producing reactions with an average 24-hour score of 1.3 (maximum possible score = 8) (Younger Labs 1969b).

Glyoxal (40%) was applied by a semiocclusive patch to the clipped upper back or flank of three female white Vienna rabbits. The test material was wiped off after 4 hours of contact. An untreated site on each rabbit served as the control. No reactions were noted at 1, 24, 48, and 72 hours after patch removal (BASF AG 1985b).

Glyoxal (40%) was applied to two intact clipped sites on each of six New Zealand white rabbits (three of each sex). (The solution also contained 0.8% ethylene glycol.) The sites were covered with dressing for a 4-hour contact period after which excess sample was removed with cleansing tissue. Readings were made at 1, 24, 48, and 72 hours and 6, 8, 10, and 14 days after the end of the contact period. Greater irritation was noted on the right side. Mild to moderate erythema was noted in all six rabbits at the 1-hour observation. Five animals also had mild to moderate edema. One animal developed severe erythema and edema on the right side by 1 to 2 days. Necrosis was noted at one site at the 24-hour observation, and in at least one site in three animals by the third day of observation. A fourth animal developed necrosis at the application site by the sixth day. The necrosis was scored as moderate severity in most cases. By day 6 all animals had desquamation (no further details); scabs were noted in four. Yellow staining was noted in all animals during the observation period. Neither erythema nor edema was noted in any animal by day 14. However, irritation continued to be present in four animals. The primary irritation scores based on readings made on days 1 to 3 was 2.58 (maximum possible score = 8) (Bushy Run Research Center 1988a).

A second irritation assay on 40% Glyoxal was conducted by Bushy Run Research Center (1988b) using the above described protocol. (The sample also contained 4% formaldehyde and 1% ethylene glycol.) Similar results were obtained at both treatment sites on the animals. Mild to moderate erythema and moderate to severe edema developed in all animals. Necrosis was noted in four rabbits after 2 days; an additional animal developed necrosis at one site by day 7. One rabbit died after 9 days; necropsy findings gave no indication that the death was treatment related. Fissuring was noted in one animal by day 10; scabs were noted in three animals after 14 days. Severe irritation (including necrosis, erythema, and edema) was noted in four of five animals through day 14. The fifth animal had minor erythema and desquamation. The primary irritation score of the test material was 5.05.

The same protocol was used in a third irritation study conducted by Bushy Run Research Center (1988c). (It is inferred the sample contained 40% Glyoxal; the sample was identified as containing 0.8% ethylene glycol.) Similar results were noted at both treatment sites in the animals. Minor to moderate erythema and moderate to severe edema was noted in all six rabbits. Necrosis was noted in two rabbits after 3 days and developed on at least one dose site in the other four rabbits by day 10. Desquamation at the dose sites was noted after day 7. Scabs were noted in all animals after 14 days; yellow staining was noted at the dose site throughout the 2-week observation period. The primary irritation score based on erythema and edema readings made during the first 72 hours was 4.55.

### Dermal Sensitization

Using the Magnusson-Kligman technique for delayed hypersensitivity, 20 guinea pigs each received three (0.1 ml) intradermal injections of Freund's Complete Adjuvant (FCA), a trade mixture containing Glyoxal, and the trade mixture emulsified in FCA. The effective Glyoxal concentration was 0.00065% (a 0.05% dilution of trade mixture containing 1.3% Glyoxal; the remaining components were not identified). One week later, the trade mixture was applied undiluted (effective Glyoxal concentration 1.3%) with a 48-hour occlusive patch to the intradermal site. Fourteen days later, animals were challenged at a previously untreated site with a 24-hour patch containing 95% of the trade mixture. Following the same protocol, a positive control (six animals) and vehicle control group (four animals) were treated with 1-chloro-2,4-dinitrobenzene and saline, respectively. Slight patchy mild redness (score of 1 out of a maximum possible 4) was noted in eight animals of the Glyoxal group at the 24-hour

observation and continued to be noted in six of the eight at the 48-hour observation. Using the Kligman scale that assigns a classification based on the percentage of animals sensitized (irrespective of reaction intensity), the trade mixture was a "moderate" sensitizer (Pharmakon Research Intl. Inc. 1984a).

In another assay using the Magnusson-Kligman technique, undiluted Glyoxal 40% was a sensitizer (Pharmakon Research Intl. Inc. 1984a).

In a test using the Ritz and Buehler technique, Bio/Dynamics Inc. (1988) applied occlusive dermal patches containing 1.25%, 5%, or 20% Glyoxal to groups of 15 albino guinea pigs for 6 hours. A total of nine induction exposures were applied within a 3-week period. Following a 2-week, nontreatment period, animals were challenged (at a previously unexposed site) with 0.01%, 0.03%, 0.1%, 0.3%, and 1.0% Glyoxal. A second challenge was performed 1 week later using 0.3%, 1.0%, and 3.0% Glyoxal. In addition to the positive control that was treated with dinitrochlorobenzene (DNCB), two groups of animals that had not been induced were also maintained to provide irritation data. One group was treated with the challenge 0.01% to 1.0% Glyoxal solutions, and the other group was treated with the 0.3% to 3.0% rechallenge solutions.

Three animals (one from each Glyoxal induction group) died during the study; it was unclear to the researchers if the deaths were treatment related. Orange staining of the skin was noted in all Glyoxal treated animals but it did not interfere with visual evaluation. During induction, slight dermal reactions were noted in a few animals of the 1.25% Glyoxal group following the sixth exposure. The reactions increased in severity with subsequent induction exposures but did not become severe. Animals treated with 5% and 20% Glyoxal had increased dermal response beginning after the fourth induction exposure. It was suggested that Glyoxal was a concentration-dependent primary irritant and cumulative irritant.

Animals of the 1.25% induction group did not react to challenge concentrations of 0.01%, 0.03%, or 0.1% Glyoxal. One animal reacted to the 0.3% challenge dose; this animal was also the only responder to the 1.0% challenge dose. Upon rechallenge, one animal (not the one that responded to the primary challenge) reacted to 0.3% Glyoxal (noted at the 48-hour observation) and continued to respond to higher rechallenge concentrations. Another guinea pig responded to the 1.0% rechallenge dose (noted at the 48-hour observation) and also responded to the subsequent 3.0% rechallenge dose. Three others responded to the 3.0% rechallenge dose; one of these three was the animal that had responded to the $\geq$0.3% primary challenge doses.

No reactions were noted in animals of the 5% induction group to challenge concentrations of 0.01% and 0.03% Glyoxal. One animal reacted to the 0.1% challenge doses; reactions of increased severity were noted in this animal to the 0.3% and 1.0% challenges. Two other animals also reacted to the 1.0% challenge dose. Upon rechallenge, nine animals responded to 0.3% Glyoxal and also reacted to the higher rechallenge concentrations. Another three animals reacted to the 1.0% rechallenge dose (and, subsequently, to the higher concentration), and another two reacted to the 3.0% rechallenge dose.

No reactions were noted in the 14 animals of the 20% induction group to challenge concentrations of 0.01%, 0.03%, 0.1%, and 0.3% Glyoxal. Three animals reacted to the 1.0% challenge dose. None reacted to the 0.3% rechallenge dose. Eight responded to the 1.0% rechallenge dose (and subsequently, to 3.0%), and another four reacted to the 3.0% rechallenge dose. Edema was noted in 3 of the 12 animals that responded to 3.0% Glyoxal.

No reactions were noted in animals that had not received the induction patches, but were exposed to the 0.01% to 1.0% Glyoxal challenge solutions or the 0.3% to 3.0% rechallenge solutions. Glyoxal induced dermal sensitization in guinea pigs exposed to concentrations of 1.25%, 5%, and 20% and that responded to challenge concentrations of $\geq$0.3%. The researchers noted but could not explain the greater sensitization observed in animals of the 5% induction group as compared to animals of the 20% group (Bio/Dynamics Inc. 1988).

## REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Glyoxal was selected by the NTP for assessment of oral developmental toxicity in rats and rabbits. Due to the instability of Glyoxal, the studies were done on Glyoxal Trimeric Dihydrate (NTP 1993, 1994). It has the structure shown in Figure 2.

The oral route was selected because it was the most likely route of human exposure. In pilot studies using Sprague-Dawley rats, Glyoxal Trimeric Dihydrate was administered by gavage at doses of 200, 800, 1200, 1600, and 2000 mg/kg on gestation days (GD) 6 to 15. All dams of the 2000 and 1600 mg/kg groups and five of eight dams of the 1200 mg/kg group died or had to be killed before GD 17. Rabbits that received $\geq$800 mg/kg had rough coat, vaginal discharge, lethargy, respiratory distress and diarrhea. No abnormal clinical signs were noted in dams of the 200 mg/kg group during the study. Decreased maternal weight gain was noted in all treated animals. Decreased litter size, increased resorption incidence per litter, increased incidence of nonlive implants per litter, and decreased fetal body weight, were observed at the 1200 mg/kg dose. Six of eight litters of the 800 mg/kg group, and two of three litters of the 1200 mg/kg group were completely resorbed (NTP 1993).

Subsequently, a definitive developmental toxicity study was conducted. Groups of 26 Sprague-Dawley rats were dosed by

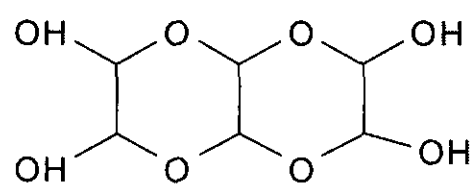


**FIGURE 2**
Chemical structure of Glyoxal Trimeric Dihydrate (NTP [lab supplement] 1993, 1994).

gavage with 50, 150, or 300 mg/kg/day Glyoxal Trimeric Dihydrate on GD 6 to 15. All rats were killed on GD 20 and the dams and fetuses were examined. No maternal lethality was observed. Pregnancy rates were comparable to the rate for untreated controls. Increased water consumption was noted in all treated rats, whereas decreased feed consumption was noted during dosing days in rats of the high-dose group. Maternal body weight gain was decreased in dams of the 300 mg/kg/day group compared to control dams. Rooting behavior was noted in animals of the 150 and 300 mg/kg/day groups but decreased after the dosing period was completed. At necropsy, no dose-related effects were noted on maternal liver weight. No differences were noted between treated and control animals in the frequency of postimplantation loss, mean fetal body weight per litter, or external, visceral, or skeletal malformations. A no-observable-adverse-effect level (NOAEL) of $\geq$300 mg/kg/day was established. At that dose mild maternal toxicity was indicated by reduced maternal body weight and feed consumption (NTP 1994).

In a pilot study using pregnant New Zealand white rabbits, Glyoxal Trimeric Dihydrate, at doses of 200, 800, 1000, 1200, and 1500 mg/kg/day, was administered by gavage on GD 6 to 19. Maternal mortality was 100% at doses $\geq$800 mg/kg/day. Maternal weight gain and corrected weight gain were below values for controls, but the differences were not statistically significant. One of seven rabbits of the 200 mg/kg/day group delivered prior to GD 30. No other adverse effects were noted (NTP 1993). In a repeat pilot study, pregnant rabbits received either 400 or 600 mg/kg/day Glyoxal Trimeric Dihydrate on GD 6 to 19. Clinical signs of toxicity were observed in 6 of 10 animals of the 400 mg/kg/day group and in all 8 animals of the 600 mg/kg/day group. By GD 18 all rabbits of the 600 mg/kg/day group died or were killed. Necropsy was done on surviving animals of the 400 mg/kg/day group on GD 30. Two of 10 animals of this group aborted prior to necropsy; decreased fecal output was noted in another four animals. Maternal weight gain and corrected weight gain were significantly decreased compared to controls (NTP 1992).

In the definitive study, a group of 26 rabbits was administered 50 mg/kg/day Glyoxal Trimeric Dihydrate on GD 6 to 19. No other doses were used due to severe toxicity observed at larger doses (unpublished data). Rabbits were killed on GD 30. A reduction in feed consumption and body weight gain was noted during the dosing period. The reduction in body weight gain was significant on GD 6 to 9. No treatment-related effect on maternal liver weight was noted at necropsy. No differences were noted between treated and control animals in the frequency of postimplantation loss, mean fetal body weight per litter, or external, visceral, or skeletal malformations. The NOAEL for maternal effects was <50 mg/kg/day and the level for developmental toxicity was 50 mg/kg/day (NTP 1993).

## GENOTOXICITY

Glyoxal is reported to be a mutagen in renaturation assays, unscheduled DNA synthesis (UDS) assays, the Ames assay, the *Escherichia coli* SOS chromotest, the *Bacillus subtilis* liquid rec-assay, the rat hepatocyte primary DNA repair test (single strand breaks found, but no DNA cross-linking), sister chromatid exchange assays, Chinese hamster ovary (CHO) and Chinese hamster V79 chromosome aberration assays, the CHO/HGPRT gene mutation assay (only with metabolic activation), the mouse lymphoma L5178y/TK$^{+/-}$ system, and in vivo in the rat, where UDS and increased alkaline elution of DNA were seen in glandular stomach tissue and single strand breaks in liver tissue DNA (not seen in kidney, spleen, pancreas, and lung). It was negative in the C3H/10T1/2 cell transformation assay, and the in vivo mouse micronucleus assay. Both positive and negative genotoxicity were seen in the in vivo Drosophila sex-linked recessive test. Table 1 summarizes in vitro genotoxicity studies and Table 2 summarizes in vivo genotoxicity studies.

## CARCINOGENICITY

### Dermal

In a report of a study done at the Bushy Run Research Center (1982), groups of 40 C3H/HeJ mice were treated three times weekly throughout their lifetime with applications of one of two commercial 40% Glyoxal solutions (1:8 dilution of commercial solution in deionized water). Impurities in each solution (European Glyoxal 40 and Aerotex Glyoxal 40) are described in the Impurities section. The effective concentration of Glyoxal tested was 4.5%. The solution (25 $\mu$l) was applied to the clipped skin of the back. Deionized water was applied to control mice. The mice were observed daily for mortality and were examined once a month for lesions.

The last mouse died 2 years after the start of the study. Animals treated with either Glyoxal solution had statistically significant longer mean survival times than did the controls. (Mean survival was 580 days Aerotex Glyoxal 40–treated mice, 594 days for European Glyoxal 40–treated mice, and 488 days for the control.) Necropsy was performed on all animals.

Neither dermal nor subcutaneous neoplasms were found in mice treated with Aerotex Glyoxal 40. Dermal inflammation and necrosis were observed in 10 of the 40 mice. Epidermal hyperplasia was noted in two mice. Similarly, no skin neoplasms were found in mice treated with European Glyoxal 40. One mouse of this group had an infiltrative fibrosarcoma on the left rib cage and axilla. However, this neoplasm type occasionally occurs in control mice. No neoplasms were observed in control mice.

Miyakawa et al. (1991) reported a study in which female CD-1 mice, 20 per group, were shaved and painted with 500 $\mu$mol of 40% aqueous Glyoxal for 5 weeks. The Glyoxal was dissolved in 0.1 ml DMSO per 50 $\mu$mol Glyoxal. In addition, half of these mice were painted with a known tumor promoter, 12-*O*-tetradeconaylphorbol-13-acetate (TPA). The positive control used was 7,12-dimethylbenz[*a*]anthracene (DMBA) (with and without TPA); the negative controls used were DMSO and TPA, only Glyoxal, or only TPA. There was no significant

**TABLE 1**
Glyoxal genotoxicity in vitro

| Strain/assay | Concentration[a] | Results | References |
|---|---|---|---|
| | | **DNA assays** | |
| Enzyme degradation/ renaturation | 0.5% | Glyoxalation increases resistance to DNase, reduces ability to renature | Brooks and Klamerth 1968 |
| Renaturation | 0.33% (trimer dihydrate) | Inhibition of C:G bonding, reduction in renaturation of DNA | Birnboim and Mitchel 1978 |
| Renaturation | $1.1 \times 10^{-3}$–$1.7 \times 10^{-2}$ M at 80°C for 5 minutes followed by gradual removal by dialysis | Inhibition of renaturation is a linear function moles of bound Glyoxal. Fully Glyoxalated DNA has a melting temperature depression of 12°C | Hutton and Wetmur 1973 |
| | | **Bacterial assays** | |
| TA100 | 40 $\mu$g/plate | Mutagenic; with S-9 or catalase, mutagenicity reduced | Yamaguchi and Nakagawa 1983 |
| TA98, TA100 | 10 $\mu$g/plate–10 mg/plate | Mutagenic in TA100, not mutagenic in TA98 | Bjeldanes and Chew 1979 |
| TA98, TA100 | NR | Mutagenic in TA100, not mutagenic in TA98 | Sasaki and Endo 1978 |
| TA100, TA104 | 50, 100 $\mu$g/plate | Mutagenic; with glyoxalase I and II, glutathione, 2,5-diphenylfuran, 2,5-dimethylfuran, and singlet $O_2$ scavengers, mutagenicity reduced | Ueno et al. 1991b |
| TA102, TA2638 | 1000 $\mu$g/plate | Mutagenic | Levin et al. 1982 |
| TA104 | NR (2250 revertants/$\mu$mol) | Mutagenic | Marnett et al. 1982 |
| TA100, TA102, TA104 | 5, 10, 50, 100, 500 $\mu$g/plate | Mutagenic with S-9 | Shane et al. 1988 |
| TA97, TA98, TA100, TA102, TA104 | 30, 60, 120 $\mu$g/plate | Mutagenic without S-9 in TA100, TA102, TA104; mutagenic with S-9 in TA100; not mutagenic in TA97, TA98 | Sayato, Nakamuro, and Ueno 1987 |
| TA 98, TA1535, TA100, TA1537 | 3.15–100,000 nl/plate ± S9 (10 doses) | At border of +/− result equivocal; marginal increase of revertants in TA1537 with S9; stronger response in TA100 at very high doses both with/without S9 | BASF 1979 |
| TA100, TA1535, TA1537, TA1538, TA98, *E. coli* WP2uvrA | 10–1000 $\mu$g/plate ± S9 (three doses) | Without S9: toxic at 100 and 1000 $\mu$g/plate<br>With S9: dose-dependent positive in TA100; upon retest with lower doses, positive results were noted in TA100 at $\geq$10$\mu$g/plate | American Cyanamid 1977 |
| TA100, TA1535, TA1537, TA1538, TA98, *E. coli* WP2uvrA | 4–5000 $\mu$g/plate ± S9 (six doses) | Without S9: dose-dependent increase in TA100<br>With S9: increased revertants in TA100 and TA1535 | Hoechst AG 1984 |
| TA102 | 4–5000 $\mu$g/plate ± S9 (six doses) | Without S9: dose-dependent increase in revertants<br>With S9: "relevant" increase in revertants | Hoechst AG 1986 |
| TA1535, TA1537, TA1538, TA98, TA100 | 100–10,000 $\mu$g/plate (test material was a mixture containing 1.3% Glyoxal) | Dose-related increase in mutation (base-pair substitution) frequency in TA100 with metabolic activation; similar results observed in a retest; results considered equivocal as S9 may have played a role | Pharmakon Research Intl. Inc. 1984b |
| *E. coli* SOS chromotest (PQ37) | 0.1, 0.3, 0.6 mM in DMSO | Mutagenic | Von der Hude et al. 1988 |
| *B. subtilis* liquid rec-assay | varied | Strongly DNA damaging,with or without S-9 | Matsui, Yamamoto, and Yamada 1989 |

**TABLE 1**
Glyoxal genotoxicity in vitro *(Continued)*

| Strain/assay | Concentration[a] | Results | References |
|---|---|---|---|
| | | **Mammalian cell assays** | |
| CHO AUXB1 revertants | NR | Dose-dependent increase in the number of revertants | Taylor and Wu 1980 |
| CHO/HGPRT forward mutation | 37.5–600 $\mu$g/ml ± S9 activation (cytotoxic at 1000 $\mu$g/ml) | Increased mutation at 600 $\mu$g/ml with S9; not significant compared to (+) *controls, DMN, and EMS, but was* considered a suspect mutagen | Pharmakon Research Intl. Inc. 1982d |
| CHO/HGPRT forward mutation | 0.1–1.5 mg/plate ± S9 activation (five doses) | Negative without S9; with S9, dose-dependent response between 0.7–1.0 mg/plate but value not within defined range for (+) result; cytotoxic at 1.0 mg/plate | Société Française Hoechst 1986a |
| CHO sister chromatid exchange (SCE) | Without S9: 10–250 $\mu$g/ml<br>With S9: 10–100 $\mu$g/ml | Dose-related response. Without S9, statistically significant response at 200 $\mu$g/ml (not enough cells at 250 $\mu$g/ml to be reliable). With S9, statistically significant response at ≥50 $\mu$g/ml | Pharmakon Research Intl. Inc. 1982c |
| CHO AUXB1 SCEs and endoreduplicated cells | 0.2–1.6 mM | Dose-dependent increase in SCEs and endoreduplicated cells | Tucker et al. 1989 |
| CHO chromosomal aberrations | 50–500 $\mu$g/ml (± S9 metabolic activation) | Dose-dependent increase in aberrations ± activation | Henkel 1990 |
| CH V79 Chromosomal abberations and mitotic activity | 100–400 $\mu$g/ml | Increase chromosomal abberations and decreased mitotic activity | Nishi, Miyakawa, and Kato 1989 |
| Unscheduled DNA synthesis in TC-SV40/ INO hamster cells | $5 \times 10^{-5}$ M | Increased conservative and semiconservative UDS | Cornago et al. 1989 |
| C3H/10T1/2 mouse embryo cell transformation | 0.0013–0.0098 $\mu$l/ml media | Negative | EG&G Mason Research Institute 1980a |
| C3H/10T1/2 mouse embryo cell transformation | 0.0049–0.039 $\mu$l/ml media | Negative | EG&G Mason Research Institute 1980b |
| C3H/10T1/2 mouse embryo cell transformation | 0.0025–0.195 $\mu$l/ml media | Negative | EG&G Mason Research Institute 1980c |
| Mouse lymphoma L5178Y/TK$^{+/-}$ forward mutation | 0.479–1.060 $\times 10^{-3}$ mol/L | Dose-dependent mutagenesis (without S-9) | Wangenheim and Bolcsfoldi 1988 |
| Mouse lymphoma L5178Y/TK$^{+/-}$ alkaline unwinding and hydroxyapatite elution assays | 0.462–3.69 $\times 10^{-3}$ mol/L | Mutagenic above concentrations above $1.85 \times 10^{-3}$ mol/L (without S-9) | Garberg, Aaekerblom, and Bolcsfoldi 1988 |
| DNA repair in rat hepatocytes | 0.03, 1.0, 3.0 mg/ml (cytotoxic at 3.0 mg/ml) | Mutagenic at 0.03 and 1.0 mg/ml | Pharmakon Research Intl. Inc. 1982b |
| Single-strand DNA breaks in rat hepatocytes | 0.1, 0.3, or 0.6 mg/ml | Time and dose dependent increase in single-strand DNA breaks | Ueno et al. 1991a |

*(Continued on next page)*

**TABLE 1**
Glyoxal genotoxicity in vitro *(Continued)*

| Strain/assay | Concentration[a] | Results | References |
|---|---|---|---|
| DNA cross-links in rat hepatocytes | 0.1, 0.3, or 0.6 mg/ml | No DNA cross-linking induced | Ueno et al. 1991a |
| $^3$H-thymidine and -uridine incorporation in human fibroblasts | Pretreatment of cells with 10–100 $\mu$g/ml | Time dependent reduction in isotope incorporation into DNA; dose dependent reduction in isotope incorporation into RNA | Klamerth 1968 |
| Thymidine kinase and DNA-dependent RNA polymerase in human fibroblasts | Pretreatment of cells with 50 $\mu$g/ml | Thymidine kinase activity down at 1 h pretreatment, up at 5 h, and down at 10 h; polymerase levels down at 1 h, but increased some at 5 and 10 h. | Klamerth 1968 |
| Human peripheral lymphocytes | 0.2–1.6 mM | Dose-dependent increase in SCEs, but no increase in endoreduplicated cells | Tucker et al. 1989 |

[a]Unless otherwise noted, studies tested various 40% Glyoxal solutions.
NR, not reported.

induction of neoplasms in mice treated with Glyoxal and TPA when compared to mice treated with Glyoxal alone, DMSO and TPA, or TPA alone. There were no neoplasms in any control group mice. All of the DMBA-treated mice had neoplasms.

In another study, Takahasi et al. (1989) dosed groups of 30 Wistar rats with 100 mg/L *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and 10% sodium chloride via the drinking water for 8 weeks. Groups of 10 Wistar rats were given nondosed drinking water for 8 weeks. After this, animals were dosed with 0.5% Glyoxal via the drinking water for 32 weeks, then killed for necropsy. The stomachs were removed for macroscopic examination, fixed with 10% formalin, and then prepared for microscopic examination. Animals dosed with Glyoxal after initiation had a significant increase in hyperplasia and carcinoma of the pyloric region and hyperplasia of the fundic region of the stomach. Neither hyperplasia nor carcinomas were seen in animals that were not treated with MNNG, suggesting Glyoxal may act as a promotor not as an initiator.

### Oral

Hasegawa et al. (1995) used an 8-week liver bioassay to detect potential hepatocarcinogenic activity in the constituents of coffee. One group of 12 male F344 rats was given a single IP injection of 200 mg/kg body weight dimethylnitrosamine (DMN) to initiate hepatocarcinogenesis. Following a 2-week recovery period, the animals were given 0.2% (*w/v*) Glyoxal in the drinking water for the next 6 weeks. A two-thirds partial hepatectomy (PH) was done at week 3. Feed and water were available ad libitum. A second group of nine rats was treated using the same protocol without DMN pretreatment. A control group of 13 rats received DMN and PH, but was given untreated drinking water.

Body weights were recorded weekly; feed and water consumption were measured during the first 2 days of each treatment week. All surviving animals were killed at week 8; the livers were excised and samples obtained for immunohistochemical examination of the glutathione *S*-transferase placental form (GST-P). The assay measures the numbers and areas of GST-P–positive foci >0.2 mm in diameter as indicators of carcinogenicity.

Body weight was slightly reduced in all rats after PH. Animals treated with Glyoxal had reduced water consumption (15.2 ml/day/rat) as compared to animals of the control group that received untreated tap water (23.2 ml/day/rat). No marked differences were observed in feed or water consumption between Glyoxal-dosed animals of the diethylnitrosamine (DEN)-initiated and noninitiated groups. Rats treated with Glyoxal had significantly lower final body weights (235 g) as compared to control rats (255 g) ($p < .001$; Student's or Welch's $t$ test). Absolute weight of the liver was lower in DMN-initiated Glyoxal-treated rats (5.92 g) as compared to control rats (6.38 g) ($p < .05$). The absolute liver weight for non–DMN-initiated Glyoxal-treated rats was 5.77 g and not statistically significant. Glyoxal had an inhibitory effect both in the number and areas of GST-P–positive foci. The control group (treated with DMN) had 7.83 foci/cm$^2$ with an area of 0.55 mm$^2$/cm$^2$. The DMN-initiated Glyoxal group had 6.06 foci/cm$^2$ with an area of 0.40 mm$^2$/cm$^2$ ($p < .05$). No foci were detected in samples from noninitiated Glyoxal-treated rats (Hasegawa et al. 1995).

## CLINICAL ASSESSMENT OF SAFETY

### Maximization Test

Glyoxal was tested according to the Kligman maximization test. During induction, 48-hour occlusive patches containing

**TABLE 2**
Glyoxal genotoxicity in vivo

| Assay | Concentration[a] | Results | References |
|---|---|---|---|
| | | **Mouse** | |
| Micronucleus | Groups of eight mice were IP injected with either a single or double dose of 400 mg/kg; erythrocytes harvested at 30–72 h after dosing and analyzed for micronuclei | Negative | Pharmakon Research Intl. Inc. 1982a |
| Micronucleus | Groups of ten mice received 1000 mg/kg by oral gavage; bone marrow erythrocytes harvested at 24, 48, and 72 h | Negative; value at 24 h significant compared to concomitant control but not to historic control | Société Française Hoechst 1986b |
| | | **Rat** | |
| Glandular stomach | 150–400 mg/kg | Dose-dependent induction of ornithine decarboxylase and UDS, with peak activity at 16 h | Furihata, Yoshida, and Matsushima 1985 |
| Glandular stomach | 240, 360, 400 mg/kg | Increased UDS in a dose-dependent manner. Significant increase at high-dose | Furihata and Matsushima 1987 |
| Glandular stomach | 5, 50, 500, 550 mg/kg | Increased alkaline elution of DNA in a dose-dependent manner | Furihata et al. 1989 |
| Liver, kidney, spleen, pancreas, and lung | 200, 500, 1000 mg/kg | Single-strand breaks in liver tissue within 2 h, returning almost to control levels by 24 h; no single-strand breaks seen in other tissues | Ueno 1991a |
| | | **Fruit fly** | |
| Recessive lethal | 0.73 mg/ml | Increase in frequency of sex-linked recessive lethals | Mazar-Barnett and Munoz 1969 |
| Sex-linked recessive | Two routes of male exposure: oral dose of 10,000 ppm for 3 days prior to mating, or injected (at base of the halteres) with 4500 ppm, 24 h prior to mating | Feed studies negative; injection studies, positive results in one of three runs, but combined total mutations from the three runs were not significantly different from concomitant or historical controls | American Cyanamid 1983 |

[a]Unless otherwise noted, studies tested various 40% Glyoxal solutions.

10% Glyoxal (in petrolatum) were applied to the forearm or calf of 24 panelists, most of whom were African American. A total of five induction patches were applied with a 24-hour nontreatment period between applications. Panelists were challenged on the back with a 48-hour occlusive patch containing 2% Glyoxal in petrolatum. (Note: the protocol specified pretreatment with a 24-hour occlusive patch containing 5% aqueous sodium lauryl sulfate [SLS] prior to each induction and challenge exposure. However, it does not appear that panelists were pretreated prior to Glyoxal exposure.) Sensitization was noted in all 24 panelists and Glyoxal was classified as an "extreme" sensitizer (Kligman 1966).

### Repeat Insult Patch Test

A human repeat insult patch test (RIPT) was conducted by Food and Drug Research Labs (1969a) using 55 panelists. During induction, patches containing 14.5% Glyoxal (in a mixture) were applied to the upper arm for 24 hours' contact, every other day for a total of 15 applications. Following a 2-week nontreatment period, a 24-hour challenge patch was applied to the original contact site. Isolated reactions were noted in 16 panelists at various evaluations during the induction period. Most of the reactions were slight; however, five panelists had at least one reaction scored as "marked erythema" prompting application of the subsequent patch on a different site. No reactions were noted

at challenge. Glyoxal at 14.5% was considered a mild fatiguing agent.

In a second RIPT using the same protocol, 0.33% Glyoxal produced no reactions during induction or challenge in 55 panelists (Food and Drug Research Labs 1969b).

An RIPT was performed using 155 volunteers (44 male, 111 female). A topical dry occlusive patch was impregnated with a 40% aqueous solution of a nail enamel containing 0.5% Glyoxal. Patches were applied on Mondays, Wednesdays, and Fridays for 3 weeks. A 2-week nontreatment period followed. Then, two consecutive 48-hour patches adjacent to the induction site were applied. These challenge sites were read at 48 and 96 hours. Seven of the panelists had responses to the challenge phase. However, upon retest, none of these were reactions to Glyoxal (CTFA 1992).

### Case Report

A 27-year-old woman who had been working with fiberglass wrapped with a polyvinyl resin emulsion (containing Glyoxal) had dry eczema on the dorsal area of both hands. Patch testing elicited a strong sensitization reaction to 10% aqueous Glyoxal (Hindson and Lawlor 1982).

## SUMMARY

Glyoxal is a naturally occurring bialdehyde. It was used historically as a preservative in nail polishes and enamels, but there are currently no reported uses in cosmetic formulations. Glyoxal itself is a powder but is commercially supplied as a 40% solution. Glyoxal in aqueous solution is a mixture of the fully hydrated monomer (predominant species), dimer, and trimer. Residual chemicals that may be found in commercial solutions of Glyoxal include formaldehyde, glycolaldehyde, acetic acid, and a trace of ethylene glycol.

A wide range of oral, dermal and intraperitoneal $LD_{50}$ values have been reported. A 28-day drinking water study noted significantly suppressed water intake and significantly reduced terminal body weight in rats which received >100 mg/kg/day and >300 mg/kg/day, respectively. Three-month feeding studies on rats and dogs reported a no-effect level of approximately 100 mg/kg/day.

Ocular irritation studies conducted with Glyoxal solutions (30% and 40%) produced slight to moderate injury. Results are conflicting for Glyoxal powder; one study suggested severe damage whereas another found only slight damage.

In dermal studies, Glyoxal powder was not an irritant whereas 40% Glyoxal solution produced negative to moderate irritation. In a guinea pig study using the Magnusson-Kligman protocol, a trade mixture containing 1.3% Glyoxal (tested at 0.00065%) induced sensitization. A guinea pig study using the Ritz-Buehler technique found a threshold level of sensitization with 1.25% aqueous Glyoxal.

In developmental toxicity studies, the NOAEL for Glyoxal Trimeric Dihydrate was ≥300 mg/kg/day in rabbits (with reduced maternal body weight) and 50 mg/kg/day for rabbits (though maternal toxicity was noted at this dose).

Glyoxal was mutagenic in most assays. Glyoxal inhibited the effect of DMN in a short-term oral study in rats. In a 5-week dermal study in mice, Glyoxal was not carcinogenic nor did it promote the action of DMBA. In a 32-week dermal study, Glyoxal was not itself carcinogenic, but did cause an increase in hyperplasia and carcinoma in the pyloric stomach and hyperplasia in the fundic stomach in animals treated with MNNG. In a life-time dermal assay in mice, however, two separate commercially available Glyoxal solutions were not carcinogenic. In this study, 4.5% Glyoxal did produce cutaneous inflammation and necrosis in mice.

In clinical studies, 10% Glyoxal was a sensitizer when tested using the Kligman-maximization protocol but 14.5% Glyoxal was not a sensitizer in a RIPT, but was a fatiguing agent. There is one case report of a positive patch test to 10% Glyoxal in an individual occupationally exposed to Glyoxal in a manufacturing plant.

## DISCUSSION

The list of data needs cited in the CIR Expert Panel's original safety assessment emphasized the Panel's concerns regarding potential carcinogenic action. These concerns primarily arose from genotoxicity studies in which Glyoxal was found to be mutagenic. A significant number of additional studies were provided by industry, including clinical safety tests, additional genotoxicity studies, and a life-time dermal carcinogenicity assay conducted using mice. While noting that the lifetime dermal carcinogenicity study was not performed to NTP standards, the Panel was of the opinion that the study adequately addressed their concerns. Neither dermal nor subcutaneous neoplasms were found in mice treated with 4.5% Glyoxal (in one of two commercial solutions). The toxicologists on the Panel noted that the development of necrosis at the application site in one fourth of the mice treated with one solution indicated that the 4.5% dose was at or approached the maximum tolerated dose (MTD). Thus, no carcinogenic response was produced in the presence of gross changes in the skin. The MTD may have killed all transformed cells, thus producing false-negative results; however, the lack of dermal fibrosarcomas that are less sensitive to dose supported the negative findings. Further, whereas Glyoxal was a mutagen in several assays, it was negative in the C3H/10T1/2 cell transformation assay.

The Panel also focused on two clinical sensitization studies that produced conflicting results. In one study, 10% Glyoxal induced sensitization in all 24 panelists when tested under a maximization protocol. In the second study, 14.5% Glyoxal did not induce sensitization in any of 55 panelists when tested under the conditions of an RIPT. The Panel noted that these different findings could be explained by the differences in protocol or by differences in the Glyoxal samples tested. The impurities section of this report noted that the formaldehyde content of

two commercial Glyoxal solutions differed by almost an order of magnitude. Neither sensitization study gave sufficient information regarding the sample tested, and the Panel was unable to interpret the results. A guinea pig study using the Ritz-Buehler technique indicated a threshold concentration of 1.25%. Of 15 guinea pigs, none responded to challenges of ≤0.1% Glyoxal and only one responded to the 0.3% challenge dose. The Panel elected to use this study in setting a concentration limit. Industry is alerted that if a higher limit is desired, the results of a graded clinical sensitization study with chemical characterization of the Glyoxal tested will be needed.

Suppliers should take steps to limit the concentration of the free formaldehyde impurity to 0.2%, consistent with the 1984 CIR evaluation of formaldehyde (Elder 1984). Also, as stipulated in that evaluation, the safety of aerosol products containing formaldehyde has not been substantiated. Although there are no current reported uses of Glyoxal, it is expected that this ingredient would be used in nail polishes and enamels as historically reported. The Panel expects that its function as a preservative will preclude its use at concentrations that would produce severe irritation. The Expert Panel expressed a willingness to discuss additional data needs for other uses of this ingredient should the need arise.

## CONCLUSION

Based on the available data, the CIR Expert Panel concludes Glyoxal is safe for use in products intended to be applied to the nail at concentrations ≤1.25%. The available data are insufficient to support the safety for other uses.

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