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Final Report on the Safety Assessment of Glyoxal[1]

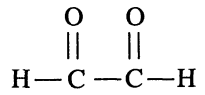
Abstract: The naturally occurring bialdehyde Glyoxal is used as a preservative in nail polishes and enamels. It is provided to formulators as a 40% aqueous solution because the nonhydrated form is highly reactive with water and other solvents. Reduced weight gain was seen in acute and subchronic animal studies, glyoxalase levels increased in the first 30 days, and hemorrhages of the mesenteric lymph nodes were found across a wide range of doses. Glyoxal readily forms DNA adducts at purine sites. Glyoxal is mutagenic in a wide range of systems, and oral studies indicate that it can act as a tumor promoter, but not an initiator. Clinical data indicate no evidence of sensitization. These data are insufficient to evaluate the safety of Glyoxal. Additional safety data are needed, including a dermal carcinogenesis study using the skin painting methods of the National Toxicology Program; impurities, especially with respect to selenium, chlorinated organic compounds, and the Glyoxal monomer; and current data on the types of products in which Glyoxal is used and at what concentrations. It is recognized that there are no reproductive or developmental toxicity data available to analyze—depending on the results of the studies described, additional data may be requested. It cannot be concluded that this ingredient is safe for use in cosmetic products until the listed safety data have been obtained and evaluated. **Key Words:** Glyoxal—Cosmetic use—Mutagenicity—Carcinogenicity—Safety.

The following is a summary of data available to the Cosmetic Ingredient Review (CIR) concerning the chemistry, cosmetic use, oral and dermal toxicity, genotoxicity, and carcinogenicity of Glyoxal.

CHEMISTRY

Definition and Structure

Glyoxal (CAS No. 107-22-2) is the bialdehyde that conforms to the following formula:



Other names for Glyoxal include biformal, biformyl, diformal, diformyl, ethane-

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

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dial, ethanedione, glyoxal aldehyde, glyoxaldehyde, odix, oxal, oxalaldehyde, oxaldehyde (Anonymous, 1987; Nikitakis et al., 1991). 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 autoxidized edible oils, such as sesame, safflower, and sardine oil (Hirayama et al., 1984). Drinking water will sometimes contain Glyoxal after ozonation (Ueno et al., 1991*b*).

Properties

Glyoxal, molecular weight 58.04, has a melting point of 15°C, a boiling point of 51°C (776 mg Hg), and a flash point of 220°C. The specific gravity is 1.14 at 20°C (National Research Council, 1981; Weast, 1982). It has a vapor pressure of 220.0 mm Hg at 20°C and a vapor density of 2.0 (National Research Council, 1981). The pH of a 40% aqueous solution of Glyoxal is 2.1–2.7. It appears as yellow prisms or a yellow liquid; it burns with a purple flame and emits green vapor. Glyoxal is soluble in water, alcohol, and ether (Weast, 1982; Windholz, 1983; Anonymous, 1987). 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 ≥ 1.4 *M*/atm (the intrinsic value is the apparent constant, corrected for the extent of hydration), and the hydration constant is 2.2×10^5 (Betterton and Hoffmann, 1988). Henry's Law constant is a ratio of the concentration of free, nonhydrated 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 nonhydrated 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 nonhydrated, dissolved aldehyde.

Chemical Reactivity

Glyoxal is highly reactive, polymerizing explosively with water. It also reacts explosively with air, chlorosulfonic acid, ethylene imine, HNO_3, oleum, and sodium hydroxide (Weast, 1982). The monomer can be restored by introducing the compound to an anhydrous environment and heating (Anonymous, 1987).

Method of Manufacture

Glyoxal is synthesized by the oxidation of acetaldehyde, either by nitric or selenious acid, or by the hydrolysis of dichlorodioxane (Weast, 1982). Commercially available Glyoxal is a 40% aqueous solution of many hydrated forms in equilibrium. Hydrated forms of Glyoxal are not volatile (Anonymous, 1987).

Analytic Methods

The detection of total aldehydes is often obtained by solution-phase spectrophotometry (National Research Council, 1981). Steinberg and Kaplan (1984) re-

port a method that detected low-molecular-weight aldehydes, including Glyoxal, using reverse-phase liquid chromatography detection of 2,4-dinitrophenylhydrazone derivatives. Another method uses a fluorescent guanosine derivative to detect Glyoxal and other adduct-forming compounds on a high-performance liquid chromatography-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 use

Glyoxal is used in cosmetics as a preservative (Nikitakis, 1988). The combined chemical and trade name product formulation data indicated that Glyoxal was contained in 33 cosmetic formulations (Table 1), all of which were nail polishes or enamels (Food and Drug Administration, 1993).

Noncosmetic use

Glyoxal is used in the textile industry 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 (National Research Council, 1981).

BIOLOGY

Biochemical Reactivities

The effects of Glyoxal on collagen were investigated by Bowes and Cater (1968). Powdered collagen was soaked in water overnight. The excess water was removed, and 10% and 16% aqueous Glyoxal (20 ml/g collagen) was added. The pH was then adjusted to 7.5–8.0 with $NaHCO_3$. Samples were incubated at room temperature for 24 h 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 mol of amino acid groups reacted per 10^5 g of collagen for the 10% Glyoxal solution and 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 mol of amino acid groups per 10^5 g collagen

TABLE 1. *Product formulation data for Glyoxal*

Product category	Total no. of formulations in category	Number of formulations containing Glyoxal
Nail polishes and enamels	131	33
1993 Totals		33

were cross-linked. Glyoxal had little or no effect on skin shrinkage temperature or ultraviolet absorption (methods not reported).

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 showed that the adduct has a structure similar to ornithine. At pH 6–7, the adduct in solution was stable for at least 20 h. At pH 8–11.5, the adduct decomposed over a period of several hours.

ANIMAL TOXICOLOGY

Oral Toxicity

Acute Toxicity

The LD_{50} of aqueous Glyoxal was 2.02 g/kg in male Wistar rats and 0.76 g/kg in guinea pigs (Smyth et al., 1941).

Short-term Toxicity

Sprague-Dawley rats received drinking water containing 2,000, 4,000, or 6,000 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 decline 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 ratio of kidney to total body weight was also observed in rats of the high-dose group. Glyoxalase I and II concentrations were significantly higher in the liver, and erythrocytes also were higher 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 to controls. In the mid- and high-dose animals there 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., 1991*c*).

Subchronic Toxicity

Glyoxal, 6,000 mg/L, was administered to Sprague-Dawley rats ad libitum via drinking water for 180 days (Ueno et al., 1991*c*). Two control groups were used. The first group was given feed ad libitum (similar to dosed animals); the second group was given only the amount of diet measured to have been 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 6,000 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.

In an abstract, the National Toxicology Program (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 owing 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 groups. 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 consumption were observed in all dosed male mice and the two highest dose groups in female mice. The only histopathologic findings were changes of the salivary glands in dosed male mice (National Toxicology Program, 1992).

Dermal Toxicity

A modified Draize dermal study was conducted using 6 female New Zealand White rabbits (Cosmetic, Toiletry, and Fragrance Association, 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 h, the first application sites were scored. Then new patches were applied as before, and sites were scored at 24 and 48 h. No irritation to the product was observed.

Ocular Toxicity

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).

Phototoxicity

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).

GENOTOXICITY

Genotoxicity data are summarized in Table 2.

DNA Adducts

One mechanism by which Glyoxal may produce mutations is through chemical reaction with nucleosides to form DNA adducts. These adducts can increase the possibility of replication errors. Shapiro and Hachmann (1966) and Shapiro et al. (1986) have described the formation and the mechanism of formation of cyclic adducts with guanine nucleosides and Glyoxal. Broude and Budowdky (1971), using spectrophotometric methods, suggested a reaction for adenosine and cytosine with Glyoxal to form DNA adducts. Birnboim and Mitchel (1978) used [^{3}H]thymidine-incorporated DNA to study the effects of Glyoxal on DNA structure. The radioactive DNA was denatured and then incubated with Glyoxal for 3 min at 80°C at pH 9.8. The DNA was precipitated and then analyzed by thermal chromatography with hydroxyapatite. Glyoxal effectively prohibited G:C pairing in the DNA, interfering with the renaturation into the helical structure and greatly reducing the affinity of DNA to the hydroxyapatite.

Brooks and Klamerth (1968) incubated DNA with 0.5% Glyoxal at pH 4.5 at 37°C for 16 h, removed excess Glyoxal by dialysis, and added deoxyribonuclease (DNase). DNA adducts formed in the initial reaction reduced by 70% the number of sites available for DNase cleavage. Hutton and Wetmur (1973) studied the connection between DNA's renaturation rate and its degree of glyoxalation. DNA was treated with concentrations of 1.1×10^{-3}–1.7×10^{-2} *M* Glyoxal at 80°C for 5 min, after which Glyoxal was removed gradually by dialysis. DNA samples were denatured in a water bath at 100°C for 2 min and then put at a lower temperature to renature. The inhibition of renaturation was roughly a linear function of the moles of Glyoxal bound to the DNA. Each of these results supports a role for DNA adducts in the mechanism of Glyoxal mutagenesis.

Bacteria

Salmonella typhimurium

The mutagenicity of Glyoxal has been tested by the method of Ames in a number of *S. typhimurium* strains. In general, Glyoxal is mutagenic in strains TA100, TA102, and TA104, but not TA97 and TA98. Metabolic activation tends to reduce the mutagenic effects of Glyoxal. Specific studies are described below.

TABLE 2. *Genotoxicity*

Strain/assay	Concentration	Results	References
DNA			
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, 1977
Renaturation		Inhibition of renaturation is a linear function of moles of bound Glyoxal; fully Glyoxalated DNA has a melting temperature depression of 12°C.	Hutton and Wetmur, 1973
Bacteria			
Salmonella typhimurium			
TA100	40 μg/plate	Mutagenic; with S-9 or catalase, mutagenicity is reduced	Yamaguchi and Nakagawa, 1983
TA98, TA100	10 μg–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 μg/plate	Mutagenic; with glyoxalase I and II, glutathione, 2,5-diphenylfuran, 2,5-dimethylfuran, and singlet O_2 scavengers, mutagenicity is reduced	Ueno et al., 1991*b*
TA102, TA2638	1,000 μg/plate	Mutagenic	Levin et al., 1982
TA104	NR (2,250 revertants/μmol)	Mutagenic	Marnett et al., 1985
TA100, TA102, TA104	5, 10, 50, 100, 500 μg/plate	Mutagenic with S-9	Shane et al., 1988
TA97, TA98, TA100, TA102, TA104	30, 60, 120 μg/plate	Mutagenic without S-9 in TA100, TA102, TA104; mutagenic with S-9 in TA100; not mutagenic in TA97, TA98	Sayato et al., 1987
μmμ assay (TA1535)	492.6 μg/ml	Slightly mutagenic without S-9; mutagenic with S-9	Ono et al., 1991
Escherichia coli			
Proliferation	10^{-3} *M*	Moderate reduction in proliferation; recovery over a period of hours	Együd, 1967
SOS chromotest (PQ37)	0.1, 0.3, 0.6 m*M* in DMSO	Mutagenic	Von der Hude et al., 1988
Bacillus subtilis			
Liquid rec-assay	NR	Strongly DNA damaging, with or without S-9	Matsui et al., 1989
Mammalian cell			
Chinese hamster ovary			
CHO AUXB1 revertants	NR	Dose-dependent increase in the number of revertants	Taylor and Wu, 1980
CHO AUXB1 SCEs and endoreduplicated cells	0.2–1.6 m*M*	Dose-dependent increase in SCEs and endoreduplicated cells	Tucker et al., 1989
Chinese hamster V79			
Chromosomal aberrations and mitotic activity	100–400 μg/ml	Increased chromosomal aberrations and decreased mitotic activity	Nishi et al., 1989

TC-SV40/INO hamster cell			
Unscheduled DNA synthesis	5×10^{-5} *M*	Increased conservative and semiconservative UDS	Cornago et al., 1989
Mouse lymphoma L5178Y/TK$^{\pm}$			
Forward mutation	NR	Mutagenic (without S-9)	Wangenheim and Bolcsfoldi, 1988
Alkaline unwinding and hydroxyapatite elution assays	0.462×10^{-3}–3.69×10^{-3} mol/L	Mutagenic above concentrations of 1.85×10^{-3} mol/L (without S-9)	Garberg et al., 1988
Thymidine kinase locus assay	0.479×10^{-3}–1.060×10^{-3} mol/L	Mutagenic for all concentrations	Wangenheim and Bolcsfoldi, 1988
Rat hepatocyte			
Single-strand DNA breaks	0.1, 0.3, or 0.6 mg/ml	Time and dose-dependent increase in single-strand DNA breaks	Ueno et al., 1991*a*
DNA cross-links	0.1, 0.3, or 0.6 mg/ml	No DNA cross-linking induced	Ueno et al., 1991*a*
Human fibroblasts			
[^{3}H]Thymidine and [^{3}H]uridine incorporation	Pretreatment of cells with 10–100 μg/ml	Time-dependent reduction in isotope incorporation into DNA; dose-dependent reduction in isotope incorporation into RNA	Klamerth, 1968
Thymidine kinase activity and concentration of DNA-dependent RNA polymerase	Pretreatment of cells with 50 μg/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			
Human peripheral lymphocytes	0.2–1.6 m*M*	Dose-dependent increase in SCEs, but no increase in endoreduplicated cells	Tucker et al., 1989
In vivo			
Rat			
Glandular stomach	150–400 mg/kg	Dose-dependent induction of ornithine decarboxylase and UDS, with peak activity at 16 h	Furihata et al., 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, 1,000 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 et al., 1991*a*
Drosophila melanogaster			
Recessive lethal	0.73 mg/ml	Increase in incidence of sex-linked recessive lethals	Mazar Barnett and Munoz, 1969

Yamaguchi and Nakagawa (1983) found that 40 μg/plate of Glyoxal was mutagenic to TA100, but the addition of PCB-induced S-9 or bovine liver catalase significantly reduced the number of revertants per plate. In an abstract, Sasaki and Endo (1978) reported that Glyoxal induced mutation in TA100, but not in TA98, and that its mutagenicity was reduced by metabolic activation.

The mutagenicity of Glyoxal, 50 or 100 μg/plate, in TA100 and TA104 was significantly reduced or inhibited by glyoxalase I and II, glutathione, 2,5-diphenylfuran, 2,5-dimethylfuran, and singlet O_2 scavengers, but not superoxide dismutase or D-mannitol. Catalase reduced the mutagenicity of Glyoxal in TA104, but not in TA100 (Ueno et al., 1991*b*). Glyoxal, 1 mg/plate, was strongly mutagenic in TA102 and weakly mutagenic in TA2638 (Levin et al, 1982). It was mutagenic at concentrations of <1 mg/plate in TA100, but was not mutagenic in TA98 (Bjeldanes and Chew, 1979). The mutagenicity of Glyoxal in TA104 was 2,250 revertants per μmol (Marnett et al., 1985). Shane et al. (1988) found that Glyoxal at concentrations of 5–500 μg/plate was mutagenic to TA100, TA102, and TA104. Metabolic activation by S-9 reduced the mutagenicity in TA100, increased the mutagenicity in TA102, and did not significantly change the mutagenicity in TA104.

Without S-9 metabolic activation, Glyoxal, in concentrations of 30, 60, and 120 μg/plate, was mutagenic in TA100, TA102, and TA104. With S-9, Glyoxal was still mutagenic in TA100. Glyoxal was not mutagenic in TA97 and TA98 with or without metabolic activation (Sayato et al., 1987). Ono et al. (1991) used *S. typhimurium* TA 135/pSK1002, in which the plasmid (pSK1002) carries the fused gene μmμC′-′lacZ, to evaluate Glyoxal mutagenesis. Expression of this gene, measured by β-galactosidase activity, is suggested by the author to indicate mutagenesis induced by either chemicals or radiation. This test indicated that Glyoxal was slightly mutagenic without S-9 and substantially mutagenic with S-9.

Escherichia coli

Együd (1967) studied the effect of various aldehydes, including Glyoxal, on cell division in *E. coli*. Glyoxal, 10^{-3} *M*, was added to agar plates containing *E. coli* and incubated for set lengths of time. Photographic papers were used to measure inhibition. During the first 2 h, Glyoxal moderately inhibited the proliferation of the cells. Afterward, however, the cells gradually recovered to nearly control levels of growth. The mutagenicity of Glyoxal was studied using the SOS chromotest (Von der Hude et al., 1988). In this *E. coli* assay, the *sfiA* gene (SOS-gene) controls the β-galactosidase *lacZ* gene. A colorimetric assay to determine the concentration of β-galactosidase determines the extent of DNA damage. Glyoxal concentrations of 0.1, 0.3, and 0.6 m*M* in dimethyl sulfoxide (DMSO) were considered to be mutagenic by this assay.

Bacillus subtilis

A liquid rec-assay using a *B. subtilis*/microsome system was employed to determine the mutagenicity of Glyoxal (Matsui et al., 1989). The two strains, rec^- and rec^+, were grown and then added to varying concentrations of Glyoxal and/or

S-9 and incubated for 1 h. This preparation was then added to a nutrient broth and incubated for a set period of time. A turbidity meter measured the growth within the cultures. The DNA-damaging potential was measured as a ratio of the 50% survival concentrations for the rec^- and rec^+ strains (R50). The R50 for Glyoxal without S-9 was 3.70; with S-9 it was 2.08. Glyoxal was considered to be strongly DNA-damaging with or without S-9 metabolic activation.

Mammalian Cell

Chinese Hamster Ovary

Glyoxal induced as much as a 50-fold increase in the incidence of revertants in reversion assays using Chinese hamster ovary triple auxotroph cells (CHO AUXB1), reportedly increasing as a function of dose (Taylor and Wu, 1980). Using the same CHO AUXB1 line exposed to concentrations of Glyoxal ranging from 0.2 to 1.6 m*M* in the culture media, a dose-dependant increase in sister chromatid exchanges (SCEs) and endoreduplicated cells (ERCs) was seen (Tucker et al., 1989).

Chinese Hamster V79

Glyoxal, at concentrations of 100–400 μg/ml, significantly increased the incidence of chromosome aberrations (at all concentrations tested) and significantly reduced the mitotic activity (at all but the lowest concentration tested) in Chinese hamster V79 cells (Nishi et al., 1989).

TC-SV40/INO Hamster

Cornago et al. (1989) studied the effect of Glyoxal on the unscheduled DNA synthesis (UDS) of TC-SV40/INO hamster cells. Cells were treated with 5×10^{-5} *M* Glyoxal for 1 h and then centrifuged to remove excess Glyoxal. Some cell samples received hydroxyurea (an inhibitor of semiconservative DNA synthesis), while others received 25 Gy of irradiation (an inductor of UDS); [^{3}H]thymidine was then added. Cell samples were incubated for 40 min. Aliquots of 1 ml were removed every 5 min. Glyoxal inhibited semiconservative DNA synthesis between 26.9 and 34.9% and increased UDS by 10-fold in this assay.

Mouse Lymphoma

The mutagenicity of Glyoxal was studied in a mouse lymphoma L5178Y/$TK^{+/-}$ thymidine kinase locus assay (Wangenheim and Bolcsfoldi, 1988). Concentrations of 4.790×10^{-4}–10.600×10^{-4} mol/L Glyoxal, without metabolic activation, increased the incidence of mutations in a significant, dose-dependent manner. Garberg et al. (1988) performed alkaline unwinding and hydroxyapatite elution assays on mouse lymphoma L5178Y/$TK^{+/-}$ cells that had been treated with concentrations of 4.62×10^{-4}–36.9×10^{-4} mol/L Glyoxal in order to assess the

DNA-damaging capacity of the compound. Glyoxal was considered to be mutagenic in concentrations of $\geq 18.5 \times 10^{-4}$ mol/L.

Rat Hepatocytes

Hepatocytes from male Sprague-Dawley rats were incubated with 0.1, 0.3, or 0.6 mg/ml Glyoxal for between 1 and 12 h (Ueno et al., 1991*a*). Some cell cultures were then exposed to methyl methanesulfonate. Cells were stained with trypan blue to determine viability. An alkaline elution was then performed to determine the amount of single-strand breaks in the DNA. Glyoxal induced a time-dependent and dose-dependent increase in single-strand breaks of the DNA. Data from the methyl methanesulfonate indicated that Glyoxal did not induce DNA cross-links.

Human Fibroblasts

Klamerth (1968) studied the effect of 30% aqueous Glyoxal on DNA synthesis in human fibroblasts in four separate pulse-labeling experiments. Pretreatment with 50 μg Glyoxal led to a decrease in the incorporation of tritiated thymidine in a time-dependent manner ranging from 100% of control at 1 h to 7% of control at 5 h. Pretreatment with 10, 50, and 100 μg/ml Glyoxal 1 h before exposure to tritiated uridine lessened the uptake of the uridine 95, 93, and 67%, respectively, as compared with the control, although the sedimentation rate of the RNA in Glyoxal-treated cells was similar to the control, as measured at 260 nm. Thymidine kinase activity, as measured by the incorporation of [^{14}C]thymidine, was lower at 1 h, higher at 5 h, and lower again at 10 h of incubation with 50 μg/ml Glyoxal. Concentrations of DNA-dependent RNA polymerase, as measured by the uptake of tritiated uridine triphosphate, were decreased compared with the control at 1-h incubation, but increased somewhat thereafter.

Human Peripheral Lymphocytes

As part of the earlier study by Tucker et al., (1989), human peripheral lymphocytes were exposed to the same levels of Glyoxal to which CHO cells had been exposed. With the lymphocytes, however, there was only an increase in SCEs, not an ERC increase.

In Vivo Effects

Rat

Male Fischer F344 rats, five in each group, were given a single 0.5-ml aqueous dose of 120–400 mg/kg body weight Glyoxal by gastric intubation (Furihata et al., 1985). After 24 h on a restricted diet, the animals were killed at 0, 4, 7, 16, 24, and 48 h postdose, and the pyloric mucosa of the stomach was removed. Ornithine decarboxylase (ODC) activity was measured and UDS was determined by the incorporation of [^{3}H]thymidine into cultured cells. The induction of ODC activity was dose-dependent. The ODC activity in the 400 mg/kg Glyoxal-treated rats increased sharply between hours 7 and 16, to a maximum of 100 times that of the

control, and decreased somewhat thereafter. The results of the UDS followed closely that of the ODC activity, with a strong increase in induction between 7 and 16 h and a sharp decline thereafter. Only doses of ≥300 mg/kg significantly increased the UDS.

The effect of Glyoxal on UDS and total DNA synthesis (TDS) on the pyloric mucosa of the glandular stomach was investigated by Furihata and Matsushima (1987). Groups of five Fischer F344 rats were given a single gastric intubation dose of 240, 360, or 400 mg/kg Glyoxal after a 24-h period of a reduced diet. In the presence of hydroxyurea, the TDS in test animals increased significantly at 2 h over controls. The increase in UDS was dose-dependent. The UDS in the high-dose rats was significantly greater than controls.

Furihata et al. (1989) again studied the effect of Glyoxal on UDS in the pyloric mucosa of the stomach of rats. Male Fischer F344 rats, five in each group, were given a single 1.0-ml aqueous dose of 5, 50, 500, or 550 mg/kg body weight Glyoxal by gastric intubation after a 24-h period on a restricted diet. After 2 h the animals were killed and the stomachs removed. The pyloric mucosa was removed with a razor blade. Alkaline elution was performed on 5-mg samples of pyloric mucosa. A dose-dependent increase in the elution rate constant was observed after treatment with Glyoxal and the positive control, *N*-methyl-*N'*-nitro-*N*-nitroguanidine (a stomach carcinogen). The increase was significant at 500 and 550 mg/kg Glyoxal.

Male Sprague-Dawley rats fasted overnight and then were given doses of 200, 500, or 1,000 mg/kg by gastric intubation (Ueno et al., 1991*a*). Animals were then killed 1–24 h after exposure to Glyoxal. The livers were perfused with saline, removed, and placed into ice-cold buffer. Nuclei were extracted. An alkaline elution assay was performed on the nuclei of the liver, along with nuclei extracted from the kidney, spleen, pancreas, and lung. DNA single-strand breaking in the nuclei of the liver was first seen at 2 h, reached a peak at 9 h, and returned to near-control levels at 24 h. This induction was dose-dependent. Little or no induction of single-strand breaks was found in the other tissues.

Drosophila melanogaster

A single dose of Glyoxal was injected into the abdomen of male Oregon R *D. melanogaster*. Males were then mass-mated with Basc females for 24 h. Standard recessive lethal assays were performed on all F1 females. A slight but significant increase in the number of sex-linked recessive lethals was seen in the progeny of the Glyoxal-dosed males (Mazar Barnett and Munoz, 1969).

CARCINOGENICITY

Female CD-1 mice, 20 per group, were shaved and painted with 500 μmol of 40% aqueous Glyoxal for 5 weeks. The Glyoxal was dissolved in 0.1 ml DMSO per 50 μmol Glyoxal. In addition, half of these mice were painted with a known tumor promoter, 12-O-tetradecanoylphorbol-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 induction of neoplasms in mice treated with Glyoxal and TPA when compared with 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 (Miyakawa et al., 1991).

Groups of 30 Wistar rats were dosed 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. Afterward, 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 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 (Takahasi et al., 1989), suggesting that Glyoxal may act as a promotor, not as an initiator.

CLINICAL ASSESSMENT OF SAFETY

A repeated-insult patch test (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 Monday, Wednesday, and Friday for 3 weeks. A 2-week nontreatment period followed, after which two consecutive 48-h patches adjacent to the induction site were applied. These challenge sites were read at 48 and 96 h. Seven of the panelists had responses to the challenge phase. However, upon retest, none of them were reactions to Glyoxal (Cosmetic, Toiletry, and Fragrance Association, 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 used in cosmetics as a preservative in nail polishes and enamels. Glyoxal is provided to formulators in a 40% solution, since the nonhydrated form is highly reactive with water and other solvents. In oral toxicity studies, doses of 4,000 mg/L of drinking water suppressed body weight gain. Animals receiving 16,000 mg/L were killed owing to moribundity. Increases in glyoxalase in the kidneys and liver were observed at 30, but not at 60 or 90 days. Other serum parameters also were affected by Glyoxal. A cosmetic product containing 0.5% Glyoxal tested at 40% was nonirritating in a modified Draize dermal study.

Glyoxal readily forms multiple, nonspecific adducts with purine but not pyrimidine nucleic acids. These adducts can inhibit C:G bonding. In general, Glyoxal is

mutagenic in bacterial strains, mammalian cells, and in vivo assays; metabolic activation tends to reduce the mutagenicity. Short-term carcinogenesis studies indicated that Glyoxal can act as a promoter but not as an initiator. An RIPT study using 155 panelists did not induce sensitization reactions to Glyoxal.

DISCUSSION

Section 1, paragraph (p) of the CIR Procedures states that "a lack of information about an ingredient shall not be sufficient to justify a determination of safety." In accordance with Section 30(j)(2)(A) of the Procedures, the Expert Panel informed the public of its decision that the data on Glyoxal were not sufficient for determining whether the ingredient, under relevant conditions of use, was either safe or unsafe. The Panel released a Notice of Insufficient Data on March 26, 1993, outlining the data needed to assess the safety of Glyoxal. Comments regarding the UV spectral analysis requested were received during the 90-day public comment period. Additional data needed to make a safety assessment are: (1) types of cosmetic products Glyoxal is used in and the typical concentrations of use for each of these products; (2) impurities, especially with respect to selenium and chlorinated organic compounds and the Glyoxal monomer; (3) dermal carcinogenesis using the methods of the National Toxicology Program's skin-painting studies. It is recognized that there are no reproductive or developmental toxicity data available to analyze—depending on the results of the studies described, additional data may be requested.

CONCLUSION

On the basis of the available data, the CIR Panel cannot conclude that Glyoxal is safe for use in cosmetic products until the appropriate safety data have been obtained and evaluated.

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