Amended Safety Assessment of Glyoxal as Used in Cosmetics

Status:	Re-Review for Panel Review
Release Date:	May 19, 2017
Panel Meeting Date:	June 12-13, 2017

The 2017 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A. This report was prepared by Lillian C. Becker, Scientific Analyst/Writer.

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MEMORANDUM

To: CIR Expert Panel and Liaisons

From: Lillian C. Becker, M.S.
Scientific Analyst and Writer

Date: May 19, 2017

Subject: Re-review of Glyoxal as used in cosmetics

Attached is the re-review of Glyoxal as used in cosmetics. Glyoxal is the smallest dialdehyde. After a report in 1995 was published with an insufficient data conclusion, an amended report was published in 2000 with the conclusion that 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. [*glyoxa062017prev1,2*]

Most of the new data in this re-review come from other reports (e.g., ECHA, NICNAS, and WHO). The Panel should note that a SCCP report concluded that "…any risk to consumers when Glyoxal is present up to 100 ppm in cosmetic products is considered to be negligible."

According to the 2017 VCRP data, Glyoxal is only used in 2 cosmetic products (basecoats and undercoats and a face and neck product) and the Council survey reports no uses. [*glyoxa062017FDA; glyoxa062017data1*] There were no reported uses for this ingredient in 1998 (thus, no need for a use table in the report). However, the Council explains that Glyoxal is used to produce other compounds; for example, Glyoxal may be used as a cross-linking agent to make polycondensates. Therefore, Glyoxal may be present as a residue in finished products.

The Panel is now being asked to consider whether there is a reason to re-open the review, or should the original conclusion be reaffirmed, in which case the review would not be re-opened.

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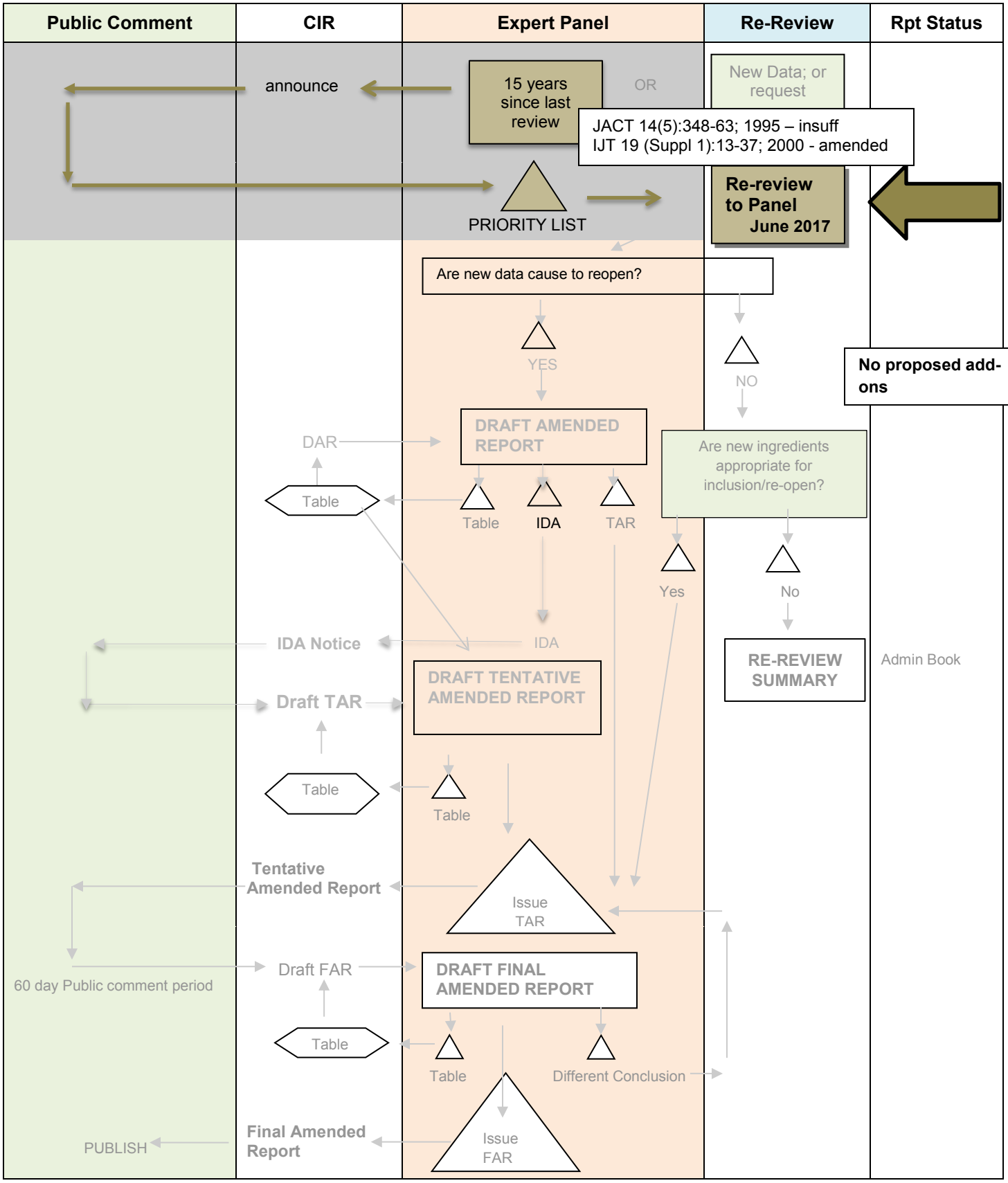
RE-REVIEW FLOW CHART

INGREDIENT/FAMILY Glyoxal

MEETING June 2017



*If Draft Amended Report (DAR) is available, the Panel may choose to review; if not, CIR staff prepares DAR for Panel Review.

History – Glyoxal

1995 – Safety assessment of Glyoxal published with the 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.

The data needs were:

(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.

2000 – After the submission of new data, a new report was published with the 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 ≤01.25%. The available data are insufficient to support the safety for other uses.

June, 2017 – The Panel re-reviews this safety assessment after 15 years.

Glyoxal Data Profile for ***June*, 2017**. Writer – Lill Becker																					
		ADME		Acute toxicity			Repeated dose toxicity			Irritation					Sensitization						
	Use	Log K_{ow}	Dermal Penetration	Oral	Dermal	Inhale	Oral	Dermal	Inhale	Ocular Animal	Ocular In Vitro	Dermal Animal	Dermal Human	Dermal In Vitro	Animal	Human	In Vitro	Repro/Devel	Genotoxicity	Carcinogenicity	Phototoxicity
Glyoxal	N	X	N	O N	O N	O N	O N		N	O N		O N			O N	O		O N	O N	O N	
Surrogate/analog																					
Glyoxal Trimeric Dihydrate							O											O			

O – Old data
N – New data
X-Any data

Ingredient Family Name

Ingredient	CAS #	InfoBase	SciFinder	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	FEMA	Web
Glyoxal	107-22-2	CRF	182/0	596/1	1073/1	Y	Y	N	Y/0	0	0	1	N	Old	Y	N	N	N

Search Terms:
Glyoxal and CAS No.

Search Strategy
PubMed – culled toxi*, 596, 1 useful
SciFinder – Toxicity, journal articles, 182/2 not useful.

Historic Minutes for Glyoxal

August, 1993

Glyoxal

Dr. Schroeter stated that Glyoxal is a preservative and that the report on this ingredient is insufficient.

The Panel voted in favor of issuing an Insufficient Data Announcement with the following data requests: (1) Concentration of use in cosmetics; (2) Impurities data, especially with respect to selenium and chlorinated organic compounds; (3) UV spectral analysis (if absorbance occurs in the UVA or UVB range, photosensitization data are needed); and (4) Dermal carcinogenesis using the methods of the National Toxicology Program's skin painting studies.

November, 1993

GLYOXAL

Dr. Bergfeld noted that Glyoxal is a Wyden ingredient, and that an insufficient data conclusion is included in the CIR report. She read the report conclusion as follows: 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.

The Panel agreed that there was no need for any further discussion of the report on Glyoxal, and voted in favor of issuing a Final Report on this ingredient.

June, 1996

Glyoxal

Dr. Belsito stated that Glyoxal was previously reviewed by the Panel at the November 22-23, 1993 Panel meeting, at which time the issuance of a Final Report with an insufficient data conclusion was approved. It was determined that the following data are needed in order for the Panel to complete its safety assessment: (1) Types of cosmetic products in which Glyoxal is used 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.

Dr. Belsito noted that a dermal carcinogenicity study had been received since the Final Report was issued, and that it was requested that the Panel reevaluate the safety of Glyoxal. Glyoxal was tested at a concentration of 4.5% in the dermal carcinogenicity study, and the results were negative.

Dr. Belsito also noted that his Team expressed concern over the potential for Glyoxal to be photoreactive. It was thought that in the absence of phototoxicity or photosensitization studies, it would be difficult to evaluate the safety of this ingredient. Therefore, the Belsito Team concluded that the existing data are insufficient for evaluating the safety of Glyoxal in cosmetics, and requested phototoxicity and photosensitization data at use concentrations.

Drs. Bergfeld and Andersen noted that new issues (phototoxicity and photosensitization) relating to the safety of Glyoxal in cosmetics were being raised by Dr. Belsito's Team, issues that were not raised initially during the review of this ingredient.

Dr. McEwen noted that the results of a UV spectral analysis on glyoxal trimeric dihydrate are included in the published Final Report on Glyoxal. There was no significant absorption in the UVA or UVB range.

Based on the results of the UV spectral analysis on glyoxal trimeric dihydrate, Dr. Belsito's Team withdrew its proposal for phototoxicity and photosensitization testing. Considering that phototoxicity and photosensitization are no longer concerns, Dr. Belsito's Team concluded that Glyoxal is safe for use in cosmetics at concentrations

up to 4.5%, based on the negative dermal carcinogenicity study conducted at this test concentration.

Dr. Schroeter asked why the concentration limit should not be based on the negative human skin sensitization study in which Glyoxal was tested at a concentration of 14.5%. He noted that his Team had chosen 14.5% as the concentration limit.

Dr. Belsito said that given all of the positive mutagenicity studies, there is much concern over the toxicity of Glyoxal. He noted that, to some extent, his Team was surprised by the negative results of the topical carcinogenicity study and established the concentration limit based on these negative results. Dr. Belsito described the carcinogenicity data as the "weakest link" with respect to determining the safety of Glyoxal.

Dr. McEwen noted that there are no reported uses of Glyoxal in cosmetics.

Dr. Andersen recalled that according to the Final Report published in 1995, Glyoxal was used in 33 nail polishes and enamels, and, apparently, concentration of use data were not made available.

Dr. Shank noted that 4.5% Glyoxal, the concentration limit chosen by the Belsito Team, was a necrotizing dose in the dermal carcinogenicity study. However, he also noted that his Team had determined that carcinogenicity was not considered to be an issue in the safety assessment of Glyoxal; the concentration that produced gross changes in the skin did not induce a carcinogenic response.

Dr. Belsito acknowledged that 4.5% Glyoxal induced necrosis in the dermal carcinogenicity study, and, thus, this is the highest concentration that could have been tested in that study. With this in mind, he wanted to know why Dr. Schroeter's Team had proposed a concentration limit as high as 14.5%, particularly in the absence of percutaneous absorption data. Dr. Belsito noted that his Team had predicted that Glyoxal would probably be highly absorbed.

Dr. McEwen said that if Glyoxal is not carcinogenic at the maximum tolerated dose (MTD), then it will not be carcinogenic when tested at higher concentrations.

Dr. Klaassen said that it is possible that Glyoxal could have induced cancer in this study at a concentration less than the MTD. Drs. Klaassen and Shank agreed that it could have been that 4.5% was such a high dose that it killed all of the transformed cells.

Dr. McEwen said that in a safety study, there is no reason to be concerned about the shape of the carcinogenesis induction curve, unless there is some indication (such as the structure of the molecule) that the system is being overloaded.

Dr. Slaga said that if a mutagenic compound is being tested at a high dose, and it is going to cause cancer, usually a few tumors will develop. Furthermore, necrosis, along with mutagenicity, can induce cancer in the skin. However, cancer was not observed when Glyoxal was tested in a lifetime dermal carcinogenicity study.

Dr. Klaassen expressed concern over the fact that 4.5% Glyoxal is a necrotizing dose and the potential adverse effects on the skin that may result from use of this concentration in cosmetics.

Dr. McEwen noted that 14.5% Glyoxal was tested under occlusion in a human repeated insult patch test (55 panelists; 15 days of induction followed by single challenge). No reactions were noted at challenge, and Glyoxal was considered a mild fatiguing agent at this concentration. In a second repeated insult patch test, 0.33% Glyoxal produced no reactions during induction or challenge in 55 panelists.

Dr. Carlton said that the concentration of Glyoxal used in cosmetics should be limited to 14.5% based on the negative human skin sensitization study. With respect to the dermal necrosis (mice) induced by 4.5% Glyoxal in the dermal carcinogenicity study, he noted that Glyoxal probably would not be as necrotizing in human skin; mouse skin is thin and fragile when compared to human skin. Furthermore, Dr. Carlton noted that because Glyoxal is used primarily as a biocide, it probably would never be used at concentrations as high as 14.5% in cosmetics.

Dr. Klaassen indicated that he had been convinced by the Panel's discussion that 14.5% Glyoxal is an acceptable concentration limit.

Dr. Schroeter said that the irritancy of Glyoxal and its corrosive effect at higher concentrations should be addressed in the report discussion. He said that it should be noted that Glyoxal is usually used as a biocide and a preservative, and that products will have to be formulated such that a minimum amount of skin irritation results.

Dr. Andersen said that given the expectation that Glyoxal is used in nail polishes and enamels, a statement

to this effect should be included in the report discussion.

Dr. Bergfeld recommended that it also be stated in the discussion that Glyoxal is used as a biocide.

The Expert Panel concluded that Glyoxal is safe for use in cosmetics at concentrations up to 14.5%, with a caveat in the report discussion qualifying the justification for this limitation as well as the limitation on formaldehyde (not to exceed 0.2% in the finished cosmetic and that it should not be used in products intended to be aerosolized).

Five Panel members voted in favor of the above conclusion, and there was one abstention (Dr. Belsito).

Dr. Bergfeld noted that the report discussion will contain the following points: (1) Glyoxal is an ingredient for use in nail enamels as a biocide; (2) the limitations on formaldehyde; and (3) glyoxal should be used at concentrations that will not produce severe irritation.

December 1996

Glyoxal

Dr. Schroeter noted that the following data on Glyoxal, received after the Tentative Amended Report was issued on June 4, 1996, have been incorporated into the current document (Amended Final Report): Information on the production/generation of Glyoxal, subchronic oral toxicity data, and Kligman maximization test data (human subjects). In the latter test, 10% Glyoxal induced sensitization in human subjects. In consideration of this finding, Dr. Schroeter recalled that the Panel concluded in the Tentative Amended Report that Glyoxal was safe for use at concentrations of 14.5% based on human sensitization data [This amended conclusion replaced the Panel's original insufficient data conclusion in the published Final Report on Glyoxal - *JACT* 14(5) 1995]. Thus, it was suggested that the Panel reconsider its 14.5% concentration limit.

Dr. Belsito agreed that the 14.5% concentration limit for Glyoxal should be retracted. In reviewing the available data to establish a more acceptable conclusion, the following points were made by Dr. Belsito's Team: (1) 1.25% was selected as the threshold for Glyoxal-induced sensitization; (2) negative results for 14.5% Glyoxal in human RIPT; (3) negative results for 4.5% Glyoxal in dermal carcinogenicity study; and (4) Industry is interested in Glyoxal primarily for use in nail products. The following conclusion was proposed based on these four observations: Based on the available information, Glyoxal is safe for use in products intended to be applied to the nail at concentrations up to 1.25%, and the data are insufficient for evaluating the safety of Glyoxal relative to uses other than nail application.

Dr. Belsito also said that the need to restrict the amount of formaldehyde in Glyoxal should be reflected in the report summary. He recalled that there are two commercially available forms of Glyoxal, one containing up to as high as 6% formaldehyde.

Dr. Bergfeld confirmed with the Panel that, as in previous CIR reports, the limitation on formaldehyde will be 0.2%.

Dr. McEwen noted that the results of a human RIPT indicated no effects following challenge with 14.5% Glyoxal. He then expressed concern over the applicability of the positive human maximization test results on Glyoxal relative to the actual use of cosmetic products containing this ingredient. He noted that the Kligman maximization test actually forces sensitization potential.

Dr. Shank suggested that the difference in test results (maximization test vs. human RIPT) may have been due to differences in formaldehyde content between the samples of Glyoxal tested.

Considering the possibility that some company may wish to demonstrate that Glyoxal can be used safely at a higher concentration, Dr. McEwen wanted to know if the Kligman maximization test is the only acceptable test for this ingredient. He noted that a standard human RIPT could very easily be used to show that Glyoxal is not a sensitizer.

Dr. Schroeter noted that sodium lauryl sulfate (SLS) was not used in the maximization test because of the primary irritant nature of Glyoxal. Therefore, the standard maximization procedure was not used.

Dr. McEwen said that based on the data that were presented, one does not know for sure whether or not SLS was used.

Dr. Andersen said that according to the published human maximization study, SLS was used to enhance the skin penetration of all test compounds, except for those that induced severe irritation. Thus, SLS was not used in the maximization test procedure for Glyoxal.

Dr. McEwen emphasized the aggressive nature of the maximization test that was performed. He noted that subjects were tested with irritating concentrations of Glyoxal in the maximization test.Dr. Belsito said that during the original discussions on Glyoxal, there was some concern over sensitization reactions to a trade mixture containing 1.3% Glyoxal and a 1.25% Glyoxal solution in guinea pigs. Furthermore, he said that some of the Panel members did not agree with a concentration limit as high as 14.5% initially.

Dr. McEwen wanted to know whether or not it is appropriate to conduct a sensitization test with a concentration of an irritant (Glyoxal) that is so aggressive, an ingredient so irritating that there is no need to pre-treat the skin of maximization test subjects.

Dr. Andersen recalled that Dr. Schroeter had expressed the need for a graded sensitization test to determine the threshold for sensitization.

Dr. Andersen read the proposed final conclusion as follows: On the basis of the information in the report, Glyoxal is safe for use in products intended to be applied to the nail at concentrations up to 1.25%. The data are insufficient to support safety for other uses. Dr. Andersen noted that human graded sensitization tests are the additional data that are needed.

Dr. Andersen also recalled the Panel's proposal to include in the Glyoxal report discussion its decision to restrict formaldehyde product concentrations to 0.2%, the limitation established in the CIR Final Report on Formaldehyde.

The Panel unanimously approved the preceding conclusion and the restriction on formaldehyde concentration that will be included in the report discussion.

Dr. Bergfeld stated that because the conclusion that was issued in the Tentative Report was changed at this meeting, the existing report will now have to be re-issued as a Tentative Report with the new conclusion that was approved. The announcement of this report will be followed by a 90-day comment period.

June 1997

Glyoxal

Dr. Belsito stated that at the December 16-17, 1996 Panel meeting, the Panel voted unanimously in favor of issuing a Tentative Amended Report with the following 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. This Tentative Amended Report is actually a re-issue of the Tentative Report (concentration limit of 14.5% determined for Glyoxal) that was announced at the June 3-4, 1997 Panel meeting.

Dr. Belsito noted that, compared to the old conclusion, a lower concentration limit for Glyoxal was determined based on comments regarding its sensitization potential that were made during the Panel's deliberations.

Dr. McEwen called the Panel's attention to the last sentence of the new conclusion, which reads as follows: The available data are insufficient to support the safety for other uses. He noted that, usually, when it is concluded that the data are insufficient for evaluating the safety of a cosmetic ingredient, the data needed for completion of the safety assessment are included in the report discussion. He said that the Final Report discussion should be modified to indicate the data that are needed for the Panel to evaluate the safety of Glyoxal in cosmetic products other than nail products.

Dr. Andersen said that Dr. McEwen's remark is potentially problematic because such a list of data requests has not been generated for uses of Glyoxal other than nail product uses. He noted that the discussion that led to the Panel's statement related to concern over the potential use of Glyoxal in products other than nail products; however, currently, there is no evidence of such uses.

Dr. McEwen said that, perhaps, the generation of a list of data requests relative to the safety assessment of

Glyoxal in products other than nail products could be considered at a future date.

Dr. Bergfeld asked whether the issue of use in other types of cosmetic products was related to the sensitization potential of Glyoxal.

Dr. Belsito said that this issue was based on concern about what appeared to be a highly reactive molecule, as well as concerns about absorption and metabolism of the molecule. He also noted that the Kligman studies were used to address the issue of sensitization potential.

Dr. Andersen said that the Panel could delete the last sentence of the conclusion and agree to consider the issue of insufficient data at a future Panel meeting. He noted that the Panel has reached a conclusion on the safety of Glyoxal in nail products, which is the current understanding of how this ingredient is used, and that the concentration limit is specified for that use.

Ms. Fise said that deletion of the statement on insufficient data from the report conclusion would imply that the Panel did not consider potential uses of Glyoxal in other types of cosmetic products in its deliberations.

Dr. McEwen suggested that, in the future, the Panel generate a list of the data needed for assessing the safety of Glyoxal in other types of cosmetic products (rinse-off, leave-on, etc.), which may be useful to anyone contemplating the use of Glyoxal in these products.

Dr. Bergfeld noted that the following two issues have been introduced: (1) long-term deliberations taking into consideration the possibility of other types of cosmetic products (other than nail products) containing Glyoxal in the future and (2) some activities of the Panel to establish the basis for the last statement in the report conclusion.

Dr. Bergfeld noted that the basis for statements such as the last sentence of the conclusion should be stated in the report discussion. She said that, in the future, the Panel should make sure that when a conclusion of safe, insufficient, etc., is reached, information supporting the conclusion is included in the report discussion.

The Panel voted unanimously in favor of issuing an Amended Final Report with the following conclusion: Based on the available data, the CIR Expert panel concludes that 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 safety for other uses.

Dr. Bergfeld said that the issue of safety of Glyoxal in products (other than nail products) that may be in use in the future will be referred back to Teams, such that a letter can be generated for submission to Dr. McEwen. The letter will contain the concerns raised by the Panel and the information/data that will be needed in order to expand the use of Glyoxal.

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INTRODUCTION

The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) first published a Final Report on the Safety Assessment of Glyoxal in 1995; in that assessment, the Panel stated that 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.[1] The data needs were:

(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.

In an amended safety assessment published in 2000, the Panel reviewed additional information, including dermal carcinogenicity in mice and impurity data, and concluded that Glyoxal is safe for use in products intended to be applied to the nail at concentrations ≤1.25%. They also concluded that the available data are insufficient to support the safety for other uses.[2]

Because it has been 15 years since the last safety assessment of Glyoxal was published, the Panel is being asked to determine, based on data presented in this report, whether a re-review is warranted or if the amended conclusion that was published in 2000 can be reaffirmed.

According to the *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*), Glyoxal is reported to function as a fragrance ingredient and preservative in cosmetics.[3] However, Glyoxal is reported to be used to manufacture other compounds, including cosmetic ingredients; for example, Glyoxal may be used as a cross-linking agent to make polycondensates.[4]

An exhaustive search was conducted for additional safety test data that have entered the literature since the 2000 report was published. New safety data were discovered and presented in the appropriate sections.

The European Chemicals Agency (ECHA)[5] website and the Australian Government Department of Health National Industrial Chemicals Notification and Assessment Scheme (NICNAS)[6] website provide summaries of data generated by industry, and ECHA and NICNAS are cited as the sources of the summary data in this safety assessment as appropriate. Also referenced in this safety assessment are summary data found in reports published by the World Health Organization (WHO)[7] and the Scientific Committee on Consumer Products (SCCP).[8]

Summaries of data on Glyoxal and the Discussion sections from the original reports are included in the appropriate sections in *italics*. Glyoxal Trimeric Dihydrate was used as a surrogate/analog for Glyoxal in the original reports. Please see the original reports for details [http://www.cir-safety.org/ingredients].

CHEMISTRY

Definition and Structure

Glyoxal is a product of the decomposition of glucose when exposed to ionizing radiation[2] *It occurs naturally in heated coffee and in auto-oxidized edible oils, such as sesame, sunflower, and sardine oil. Drinking water will sometimes contain Glyoxal after ozonation.*

Glyoxal is the aliphatic dialdehyde that conforms to the formula in Figure 1.[3] Glyoxal (CAS No. 107-22-2) is the smallest dialdehyde.[9]

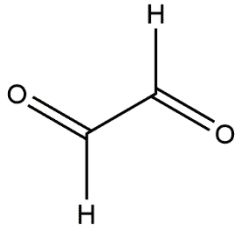

Figure 1. Glyoxal

Physical and Chemical Properties

Chemical and physical properties are cited in Table 1.

Anhydrous Glyoxal is a liquid at ambient temperature; it crystallizes at 15 °C in the form of yellow prismatic crystals.[8]

A 40% aqueous solution of Glyoxal is stable at room temperature for at least 6 months, when stored in the dark (content of dimer, trimer and other molecules was not described).[8]

The mean aerodynamic mass diameter of Glyoxal reported by one source was 0.8-1.2 μm with a mean geometric standard deviation of 1.5 to 1.7 μm.[8]

Method of Manufacture

Glyoxal may be synthesized by the oxidation of acetaldehyde, either by nitric or selenious acid, or by the hydrolysis of dichlorodioxane.[2]

Composition

Commercially available Glyoxal is a 40% aqueous solution of many hydrated forms in equilibrium.[2] 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 ≤1 M, the monomer predominates.

Anhydrous Glyoxal does not exist in a stable form at room temperature, and therefore it is commonly supplied in the form of an aqueous solution at 30%-40%.[6]

Another source states that Glyoxal is generally provided as an aqueous solution (typically containing 30%-50% Glyoxal) in which hydrated oligomers are present.[7]

Impurities/Constituents

Two commercial 40% Glyoxal solutions were analyzed before being used in a dermal carcinogenicity assay.[2] The standard for one sample 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 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.

The hydrated monomer (ethane *bis*-gemdiol) is the main form of Glyoxal in aqueous solution.[8] However this gemdiol tends to polymerize to acetals and semiacetals. The polymerization depends on both the pH and the concentration of Glyoxal in the solution. The main oligomeric forms are the dioxolane dimer and the *bis*(dioxolane) trimer. The equilibrium between monomer and dimer and trimer depends largely on the Glyoxal concentration in the aqueous solution. In a 5% solution, 39% of Glyoxal is present in the monomer form; in a 40% solution, the monomer content amounts to as little as 11% of Glyoxal, the dimer, and trimer forms being dominant.

The types of the impurities depends on the method of manufacture.[8] If the process used is the oxidation of acetaldehyde with nitric acid diluted in an aqueous medium, the main impurities are: <200 ppm formaldehyde, and formic acid, acetic acid, glyoxalin acid, and glycolic acid (approximately 1500 ppm total). If the method of manufacture is the oxidation of 1,2-ethanediol with oxygen in the presence of water, the impurities are mainly: 5000 ppm hydroxyacetaldehyde, 1500 ppm 1,2-ethanediol, and approximately 1000-2000 ppm organic acids. Former methods of manufacture yielded Glyoxal with acid contents of up to 2.1% total acids and 1000 ppm of formaldehyde.

USE

Cosmetic

The safety of the cosmetic ingredients included in this assessment is evaluated based on data received from the U.S. Food and Drug Administration (FDA) and the cosmetic industry on the expected use of these ingredients in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in FDA's Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted by cosmetic industry in response to surveys, conducted by the Personal Care Products Council (Council), of maximum reported use concentration by product category.

According to VCRP survey data received in 2017, Glyoxal is reported to be used in 2 formulations (basecoats and undercoats and a face and neck product).[10] In 1998, there were no used reported in the VCRP.[2]

The results of the concentration of use survey conducted by the Council in 2016 indicate that Glyoxal has no reported uses, and no concentrations of use were reported in 1998.[2,4]

The SCCP concluded that "on the basis of provided data any risk to consumers when Glyoxal is present up to 100 ppm in cosmetic products is considered to be negligible."[8]

Glyoxal is listed in the European Commission (EC) Cosmetic Ingredient (CosIng) Annex III (List of Restricted Substances) with a maximum concentration in ready for use preparations of 100 mg/kg.[11]

Glyoxal is used in the manufacture of other cosmetic ingredients; for example, Glyoxal is used as a cross-linking agent for compounds including cellulose, polyacrylamides, polyvinyl alcohol, and other polycondensates.[4] Therefore, in finished cosmetic products, Glyoxal may be present as a residue. The maximum concentration of Glyoxal found in cosmetic products is reported to be 100 ppm.

Non-Cosmetic

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.[2] 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.

In the United States, Glyoxal is permitted for use in adhesives that may come in contact with food (dry, aqueous, and fatty) and in packaging that may come in contact with food in single and repeated use. [21CFR 175.105, 21CFR 176.170, 21CFR 176.180]

Glyoxal is used as a chemical intermediate in the production of pharmaceuticals and dyestuffs, as a cross-linking agent in the production of a range of different polymers, as a biocide, and as a disinfecting agent.[7]

TOXICOKINETICS STUDIES

Absorption, Distribution, Metabolism, and Excretion (ADME)

[It was] reported that Glyoxal is produced by exposure of DNA to an oxygen radical-forming system (5 mM $FeS0_4$-EDTA at 37°C for 60 minutes).[2] The rate of Glyoxal generation was 17 times more efficient than that of 8-hydroxydeoxyguanosine. [It was] 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. Glyoxal is also generated in the Maillard reaction which is involved in meat mutagen formation.

There are limited qualitative and no quantitative data on the absorption and distribution of Glyoxal in humans and experimental animals.[7] Acute and subacute inhalation exposure resulted in local effects on eyes and respiratory organs, the extent of systemic absorption being unclear. After acute and chronic oral administration, there is evidence of systemic absorption, with distribution to erythrocytes, liver, lung, kidney, pancreas, and adrenal glands. There is some qualitative evidence that Glyoxal is absorbed after dermal exposure. Granular and vacuole degeneration in liver, kidney, and pancreas have been observed along with a distinct increase in blood glucose levels following dermal application. Further, data on skin sensitization provide supportive qualitative evidence that Glyoxal is absorbed across the skin.

In biological tissues, <10% of the Glyoxal present is in unbound forms in aqueous solution (free Glyoxal and hydrates), as most of the reactive carbonyl groups are reversibly bound to cysteinyl, lysinyl, and argininyl residues of proteins.[12] The endogenous concentrations of Glyoxal in human tissues and body fluids, as with other α-oxoaldehydes, are limited by the high catalytic efficiency of the glyoxalase system as well as by the rapid reaction of glyoxal with proteins.[12,13]

Elevated concentrations of Glyoxal have been measured in patients with certain pathological conditions (e.g., diabetes mellitus, uremia). The average concentration of Glyoxal in blood samples from normal human subjects was 0.21 ± 0.14 μmol/kg.[12] For blood plasma, a value of approximately 0.1 μmol/L was estimated for normal healthy subjects, which can double in diabetics.[14,15]

Animal

DERMAL EXPOSURE

Following a single dermal administration of ^{14}C-Glyoxal (0.2% w/w; 20 μg/cm^2 in water) under semi-occlusion for 8h, mean dermal absorption (3 separate runs of the experiment) of the clipped skin of Crl:WI (Han) rats (n=4) was 6.4% of the applied radioactivity.[5] In two runs of the experiment, total recovery of the radioactivity was 102% and 107% (the third was not reported).

ORAL EXPOSURE

Excretion of ^{14}C labelled-Glyoxal was studied under a range of conditions using male Wistar rats (n=4). Seven days after a single oral dosage of either 25 or 250 mg/kg, the amount excreted was 30%-32% (as CO_2) in exhaled air, 12%-13% in urine, and 24%-27 % in feces.[5] An additional group of rats was dosed with 250 mg/kg of non-labelled test material daily for 14 days followed by 250 mg/kg of labelled test material on day 15. Total radioactivity was measured in urine, feces, exhaled air (2 animals), and tissue distribution. The excreted amount of isotope was higher in urine (21.36%), lower in feces, and higher in exhaled air, compared with single doses, indicating changes in kinetics and pattern of Glyoxal metabolism after repeated exposure. In both single and repeated dose studies, comparable amounts of radioactivity (up to 30%) remained in the carcass as bound residues.

TOXICOLOGICAL STUDIES

Acute Dose Toxicity

Dermal

Glyoxal (10%) had a dermal LD_{50} of >5000 mg/kg in rabbits.[2] 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.

A mixture containing 1.3% Glyoxal was applied (2 g/kg body weight) to shaved and abraded skin sites on 10 albino rabbits (5/sex). The test material remained in contact with the skin for 24 h and then the skin was washed. Observations

were made for a 2-week period. Slight erythema and edema were visible at the 2- and 4-h observations. No treatment-related lesions were found at necropsy. The LD_{50} for the mixture was >2 g/kg.

A 40% Glyoxal solution (2000 mg/kg) was applied in a single 24-h semiocclusive patch to clipped sites on 10 rats (5/sex). Sites were rinsed after patch removal. Erythema was noted at the application sites during the 14-day observation period. No treatment-related lesions were noted at necropsy. The LD_{50} was >2000 mg/kg for the solution.

A modified Draize dermal study was conducted using six female rabbits. No irritation was observed during three applications of 0.5 mL of a 40% aqueous solution of a nail enamel containing 0.5% Glyoxal made under a topical dry patch to the clipped back or side of the animal and at 24 and 48 h after application.

Acute dermal studies are summarized in Table 2.

The dermal LD_{50} for Glyoxal was reported to be 800 mg/kg (active ingredient) in rats, 2000-12,700 mg/kg (active ingredient) in rabbits, and 5000-10,000 mg/kg (active ingredient) in guinea pigs.[8] Skin necrosis, congestion and hemorrhage of the lung, and congestion of the liver and kidneys were observed in rabbits exposed to 40% Glyoxal.

Oral

A range of oral LD_{50} values has been reported.[2] The LD_{50} of aqueous Glyoxal was 2.02 g/kg in male rats and 760 mg/kg in guinea pigs.[2] 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. Another rat study, using 10% Glyoxal, reported a value of >5000 mg/kg. Glyoxal (5%) had an LD_{50} value of 400 to 800 mg/kg in mice.

Acute oral studies are summarized in Table 2.

The oral LD_{50} for Glyoxal was reported to be 3300-4064 mg/kg (active ingredient) for mice, 2.20-5000 mg/kg in rats, 1700-3175 mg/kg (active ingredient) in rabbits, and 1700-3300 mg/kg (active ingredient) in cats.[8] Clinical signs included apathy, dyspnea, diarrhea, piloerection, and weakness. Macroscopic findings included reddened stomach mucosa, patchy liver, congestion of the abdominal viscera, intestinal inflammation, and kidney swelling.

Inhalation

In a time saturation test, rats (5/sex) received a single 7-h inhalation exposure to a 40% Glyoxal solution.[2] 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.

A group of rats (5/sex) received a single 4-h 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 µm in size. Irregular respiration, bloody tears, and bloody and crusted snouts were noted in all animals after 1 h 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 rats was > 1.3 mg Glyoxal/L air.

Acute inhalation studies are summarized in Table 2.

The inhalation LD_{50} for Glyoxal was reported to be 1300-2470 mg/m^3 (active ingredient) in rats.[8] Clinical signs irregular breathing, nasal secretion, partly closed eyes, and ruffled fur. Macroscopic findings included dark red lungs.

Short-Term Toxicity Studies

Dermal

No published short-term dermal toxicity studies were discovered and no unpublished data were submitted.

Oral

No new published oral short-term toxicity studies were discovered and no new unpublished data were submitted.

Groups of 12 rats (6/sex) were given drinking water containing 100, 300, or 1000 mg of a (40%) Glyoxal solution per kg/day for 28 days.[2] Male rats of the 300 mg/ kg/day and all rats of the 1000 mg/kg/day group had higher inorganic blood phosphorus concentrations. No treatment-related changes were noted at necropsy or microscopic examination. The no-toxic-effect dose was 100 mg/kg/day.

Rats received drinking water that contained 2000, 4000, or 6000 mg/L Glyoxal. 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. 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 serum enzymes.[2]

Inhalation

The short-term inhalation toxicity of Glyoxal was investigated in groups of Wistar rats (n=5/sex).[8] The rats were exposed to aerosolized Glyoxal (nominal concentrations 0, 0.4, 2.0 and 10 mg/m³) 6 h per day, 5 days per week over 29 days (nose only, 20 exposures total). The analytically-controlled concentrations amounted to 0.6 (± 0.2), 2.3 (± 0.8) and 8.9 (± 1.9) mg/m³, and the mean aerodynamic mass diameter was 0.8-1.2 µm with a mean geometric standard deviation of 1.5-1.7 µm. Behavior and state of health were observed daily. Body weights and food consumption were recorded twice weekly and water consumption once a week. Hematological examinations, clinical chemistry and urinalysis were performed at the end of the study. The rats were necropsied one day after the last exposure and all rats were investigated by gross pathology examination. Organs weights were determined and several organs were processed for histopathology examined.
The exposure was tolerated by all groups without any clinical signs of toxicity. There were no differences in body weight gain or food and water intake compared to the controls. There were no differences in hematological and clinical-chemical findings and results of the urinalysis. The necropsies showed no substance-related differences compared to the controls. The rats in the 2.0 and 10 mg/m³-dose groups showed a minimal squamous metaplasia of the epiglottal epithelium in the larynx that was accompanied by a minimal submucous lymphoid cell infiltration. No test substance-related changes were noted histopathologically in the rats of the 0.4 mg/m³ group. The no observed effect level (NOEL) for local effects was 0.4 mg/m³ air pure active ingredient. Since no signs of systemic intoxication were observed, the NOEL was >10 mg/m³ pure active ingredient.

Subchronic Toxicity Studies

Dermal

No published short-term dermal toxicity studies were discovered and no unpublished data were submitted.

Oral

Rats (10/sex) received feed containing 3.125, 12.5, or 25. mg Glyoxal/kg for 3 months.[2] One week after the start of dosing a fourth group of rats was started on a diet containing 6.25 mg Glyoxal/kg. (Glyoxal 40% was used, but reported doses are the amount of active agent Glyoxal received.) Liver weight, as a percentage of body weight, was increased in male rats of the highest dose group. The dose that "was without significant ill-effect" was 0.12 g/kg/day.

Beagle dogs were fed Glyoxal at doses of 3.1, 6.5, and 11.5 mg/kg/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".

In a drinking water study of G1yoxal Dihydrate on male and female rats and mice, the 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 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 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 mice were 93%, 90%, and 79% of weight gain in controls at the 4, 8, and 16 mg/mL dose concentrations, respectively. 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.

Glyoxal (40%) was administered in the diet to Harlan-Wistar rats (n=10) in a 90-day study.[8] Based on the feed consumption, the Glyoxal dosage rates were estimated to be 32.7, 63.2, 132 and 253 mg/kg/day Glyoxal for the males and 32, 63.2, 127 and 271 mg/kg/day for the females. No substance-related deaths occurred. There was no effect on the feed consumption observed. For the male animals of the highest dose group, a significant reduction in body weight gains was noted only during the first 2 weeks of the study. During the remainder of the study, the body weights were in the range of the controls. Moreover, significant increases in the relative liver and kidney weights were observed in the male rats in the highest dose group. Substance related macroscopic or histopathological organ changes (13 different organs) were not observed in any dose group. The authors derived a NOEL approximately 127 mg/kg for female rats and 132 mg/kg for males (pure active ingredient).

Inhalation

No published short-term inhalation toxicity studies were discovered and no unpublished data were submitted.

Chronic Toxicity Studies

Dermal

No published chronic dermal toxicity studies were discovered and no unpublished data were submitted.

Oral

Glyoxal (6000 mg/L) was administered to rats via drinking water for 180 days. 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. rats were killed for necropsy at 90 and 180 days. Two animals of the 6000 mg/L Glyoxal dose group died before 30 days due to hemorrhages in the glandular stomach. Rats had elevated organ-to-body weight ratios for the heart, liver, and kidneys at both 90 and 180 days. A reduction in total protein and a significant increase in the albumin-to-globulin ratio were seen at 180 days.

In a study conducted in accordance with Organisation for Economic Co-operation and Development test guidelines (OECD TG) 453 (combined chronic toxicity and carcinogenicity studies of up to 24 months), Glyoxal (0, 25, 75, and 300 mg/kg/day; test substance provided as 40% Glyoxal aqueous) was administered in drinking water of Crl:WI(Han) rats for 12 (n=10/sex) and 24 (n=50/sex) months.[5] Body weight gains in the males in the 300 mg/kg/day groups for both exposure durations were different from those of controls (direction not specified). There were no changes in feed consumption for any group [See Carcinogenicity section for detail on carcinogenic results].

Clinical pathological examination showed decreased alanine aminotransferase (ALT) activity in male and female rats in the 300 mg/kg/day groups. Neither any other clinical pathology alteration indicating microsomal-enzyme induction nor relevant liver weight increases were observed. The possible reasons for an ALT activity decrease include an effect by Glyoxal on pyridoxal 5'-phosphate levels. Lower cholesterol and globulin values in rats of both sexes in the 300 mg/kg/day group and in males in the 75 mg/kg/day group were most probably attributable to dysregulation of liver cell metabolism or decreased intestinal absorption of cholesterol combined with reduced synthesis of transport globulins. The reason for urinary excretion of higher ketone body and urobilinogen levels in males could not be elucidated by the authors.

Pathological examination showed that treatment with Glyoxal caused an 8% decrease in terminal body weights in males in the group exposed for 12 months to 300 mg/kg/day Glyoxal. Males and females of the 24-month 300 mg/kg/day group showed an 11% and 12% decrease, respectively, in terminal body weights. These reductions in the terminal body weights were regarded as manifestations of a systemic toxic effect. The reductions could not be attributed to changes in a specific organ, but was regarded to be adverse.

Microscopic examination of female animals of the 24-month groups showed an increase of erosions/ulcer in the glandular stomach, which was confirmed and an increased number were observed by histopathological examination. The erosions/ulcers in females of the 75 mg/kg/day and 300 mg/kg/day were regarded to be substance related and adverse in nature. All further pathological findings recorded were considered to be incidental in nature and not related to treatment.[5]

Inhalation

No published chronic inhalation toxicity studies were discovered and no unpublished data were submitted.

Risk Assessment

In a scenario compiled as a hypothesized high-end exposure for humans using a maximum daily intake of 10 mg Glyoxal via food, an estimated intake of 0.16 mg/kg/day was calculated. [7,8] This is slightly less than the tolerable intake of approximately 0.2 mg/kg/day for lifetime oral exposure to Glyoxal (based on a no-observed-adverse-effect level [NOAEL] of 100 mg/kg/day and an uncertainty factor of 100 and a factor of 5 for less-than lifetime exposure).

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY (DART) STUDIES

No published dermal or inhalation DART studies were discovered and no new unpublished data were submitted.

Oral Exposure

Glyoxal Trimeric Dihydrate was used as a surrogate/analog for Glyoxal in studies of oral developmental toxicity in rats and rabbits.[2] Glyoxal Trimeric Dihydrate was administered by gavage to rats at doses of 200, 800, 1200, 1600, and 2000 mg/kg on gestation days (GD) 6-15. 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 ≥8000 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.

Groups of rats were dosed by gavage with 50, 150, or 300 mg/kg/day Glyoxal Trimeric Dihydrate on GD 6-15. All rats were killed on GD 20 and the dams and fetuses were examined. Pregnancy rates were comparable to the rate for

untreated controls. Maternal body weight gain was decreased in dams of the 300 mg/kg/day group compared to control dams. 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 of ≥300 mg/kg/day was established. At that dose mild maternal toxicity was indicated by reduced maternal body weight and feed consumption.

Glyoxal Trimeric Dihydrate, at doses of 200, 800, 1000, 1200, and 1500 mg/kg/day, was administered by gavage on GD 6 to 19 to pregnant rabbits. Maternal mortality was 100% at doses ≥800 mg/kg/day. One of seven rabbits of the 200 mg/kg/day group delivered prior to GD 30. No other adverse effects were noted.

Pregnant rabbits received 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. Two of 10 animals of this group aborted prior to necropsy. Maternal weight gain and corrected weight gain were significantly decreased compared to controls.

Rabbits were administered 50 mg/kg/day Glyoxal Trimeric Dihydrate on GD 6 to 19. Rabbits were killed on GD 30. 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 were 50 mg/kg/day.

In a study conducted in accordance with OECD GL 414 (Prenatal Developmental Toxicity Study), Glyoxal (0, 5, 25, and 125 mg/kg; 40% in water) was administered to female Wistar rats (n=19-24) by gavage on days 6 through 19 after mating.[5,7] Signs of maternal toxicity observed in the 125 mg/kg/day group were reduced feed consumption and lower corrected body weight gain. No test substance-related effects were observed on gestational parameters or fetuses. The NOAELs were 25 mg/kg/day for maternal toxicity and 125 mg/kg/day for embryotoxicity.

In a study conducted in accordance with OECD GL 416 (Two-Generation Reproduction Toxicity Study), Glyoxal (0, 5, 25, 100, and 400 mg/kg; originally supplied in a 40% solution) was administered to male and female Wistar rats (n=25) in drinking water beginning 1 week before mating and continuing through parturition.[5] The test substance was then administered at 50% of the dose calculated in the last week of pregnancy through weaning (post-natal day [PND] 21). The adults were then killed and necropsied. The experiment was then repeated with the offspring (F_1 generation), which were then killed necropsied.

The parental rats in the 100 and 400 mg/kg/day groups showed clinical signs of a test-substance-related effect. The most common finding in clinical pathology was decreased ALT activity in male and female rats of the 100 and 400 mg/kg/day dose groups of the F_0 and the F_1 generations. No other clinical pathology alteration indicating a microsomal enzyme induction was observed. Therefore, other reasons for the ALT-activity decrease, including an effect on the pyridoxal 5'-phosphate levels, cannot be excluded.

One female rat in the 25 mg/kg/day group died during delivery. All the other test rats survived treatment and were killed at scheduled dates. The reduction of the terminal body weights of the males in both generations (F_0 or F_1 parental) in the 400 mg/kg/day was regarded as treatment-related effect. There were no indications from clinical, gross, or histopathology examinations that the test substance adversely affected the fertility or reproductive performance of the F_0 or F_1 parental rats at the highest dosage tested (400 mg/kg/day). For all live-born pups of the F_0 and F_1 parents, no test substance-induced signs of developmental toxicity were noted at dosage rates as high as 100 mg/kg/day. Postnatal survival and post-weaning development of the offspring of these test groups until sexual maturity were unaffected by the test substance. Furthermore, clinical and/or gross necropsy examinations of the F_1 and F_2 pups revealed no adverse findings. The developmental toxicity NOAEL was determined to be 400 mg/kg/day; 100 mg/kg/day for maternal toxicity.[5]

GENOTOXICITY STUDIES

In Vitro

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/I0T1/2 cell transformation assay, and the in vivo mouse micronucleus assay.*[2]

Summaries of in vitro genotoxicity studies of Glyoxal are provided in Table 3.

Glyoxal had positive results in Ames tests for both *Salmonella typhimurium* and *Escherichia coli* (lowest concentration tested was10 µg/plate active ingredient).[8] Mitotic recombinations were observed in *Saccharomyces cerevisiae* exposed to 1.9-15.2 µg/mL of Glyoxal (active ingredient). There was no genotoxicity observed when mouse lymphoma cells were exposed to up to 250 µg/mL Glyoxal (active ingredient) with metabolic activation and up to 375 µg/mL (active

ingredient) without metabolic activation. There was no genotoxicity observed when a plasmid was exposed to up to 150 µg/100 µL (active ingredient) with metabolic activation.

In Vivo

Glyoxal was mutagenic in most assays. Glyoxal inhibited the effect of DMN in a short-term oral study in rats.[2]

In vivo genotoxicity tests are summarized in Table 4.

Negative results for Glyoxal were observed in a micronucleus test (up to 225 mg/kg active ingredient) using mice and in two unscheduled DNA synthesis test using rats (up to 2000 mg/kg active ingredient).[8]

CARCINOGENICITY STUDIES

No new published dermal carcinogenicity studies were discovered and no new unpublished data were submitted.

Dermal

Groups of mice were treated three times weekly throughout their lifetime with one of two Commercial 40% Glyoxal solutions (1:8 dilution in water; effective concentration was 4.5%) to the clipped skin of the back.[2] *Treated mice had longer mean survival times than did the controls. 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; this neoplasm type occasionally occurs in control mice. No neoplasms were observed in control mice.*

Mice were shaved and painted with 500 µmol 40% aqueous Glyoxal for 5 weeks. Half of these mice were painted with a known tumor promoter, 12-0-tetradeconaylphorbol-13-acetate (TPA). There was no induction of neoplasms in mice treated with Glyoxal and TPA when compared to mice treated with Glyoxal alone, DMSO and TPA, or TPA alone.

Rats were dermally treated with 100 mg/L N *-methyl-*N′ *-nitro-*N*-nitrosoguanidine (MNNG) and 10% sodium chloride via drinking water for 8 weeks. Other rats were given drinking water for 8 weeks. After this, animals were dosed with 0.5% Glyoxal via the drinking water for 32 weeks, and then killed for necropsy. Animals dosed with Glyoxal after initiation had an 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

An 8-week liver bioassay was used to detect potential hepatocarcinogenic activity in the constituents of coffee.[2] *One group of 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 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. 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 GSTP-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 as compared to control rats. Absolute weight of the liver was lower in DMN-initiated Glyoxal-treated rats as compared to control. The absolute liver weight for non-DMN-initiated Glyoxal-treated rats was not statistically significant. Glyoxal had an inhibitory effect both in the number and areas of GSTP-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. No foci were detected in samples from noninitiated Glyoxal-treated rats.

In a study conducted in accordance with OECD TG 453 (combined chronic toxicity and carcinogenicity studies of up to 24 months), Glyoxal (0, 25, 75, and 300 mg/kg/day active ingredient; test substance provided as 40% Glyoxal) was administered in drinking the water of Crl:WI(Han) rats for 12 (n=10/sex) and 24 (n=50/sex) months.[5] There were no substance-related organ weight changes, gross lesions, and non-neoplastic findings in male and female rats after 12 and 24 months of treatment respectively. No substance-related neoplastic lesions were observed either in males or females after 12 and 24 months of treatment with Glyoxal 40%. All further pathological findings recorded were considered to be incidental in nature and not related to treatment [See Subchronic Toxicity Studies section for detail on toxicity results].[5]

Inhalation

No published inhalation carcinogenicity studies were discovered and no unpublished data were submitted.

Tumor Promotion

C57BL/6J wild type female mice (46 total, n/group was not clear) were mated with heterozygous B6 multiple intestinal neoplasia (MIN) males (heterozygous for wild type or MIN).[16] Four days after mating and throughout gestation and lactation, the pregnant dams were exposed to Glyoxal (0.0125%, 0.025%, 0.05%, or 0.1%, assumed active ingredient, in drinking water) or regular tap water. Litters were weighed and evaluated on PND 7. Female (n=8-19) and male (n=12-20) offspring were housed separately on PND21 and the same treatment was continued. One group was only exposed to 0.1% Glyoxal from postnatal day (PND) 21. After weaning, the F0 females were mated again with the MIN males; the dams in were assigned to different treatment groups to minimize the cage-effect. There was no difference in the number of intestinal tumors between control and treatment groups. However, exposure to 0.1% Glyoxal starting in utero and at to PND21 caused an increase in tumor size in the small intestine for both male and female mice in comparison with respective control groups. The authors suggest that Glyoxal has tumor growth promoting properties in the small intestine in MIN mice.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

In Vitro

No published in vitro irritation studies were discovered and no unpublished data were submitted.

Animal

In dermal studies, Glyoxal powder was not an irritant whereas 40% Glyoxal solution produced negative to moderate irritation in rabbits.

Two different grades of Glyoxal ("pure" 30% or 40% aqueous and "raw" at 40% aqueous; no further information/characterization was provided) were administered to the shaved back skin of white rabbits (n not specified) and covered with cotton patches (approximately 2.5 cm^2) in a patch test.[8] The patches were left in place for 1, 5, and 15 min and 20 h. After the three shorter exposure periods, the treated skin was washed with undiluted polyethylene glycol 400 and then with a 50% aqueous polyethylene glycol 400 solution. The test site was not washed after the 20-h treatment period. In addition, the ear of each rabbit was treated with the test substance for 20 h. For an application period of 1 and 5 min, no or slight erythema with a yellowing of the skin could be observed 24 h after exposure for all three test substances. For the treatment period of 15 minutes, a mild edema was also noted for all test substances at all concentrations. Eight days after treatment, a yellowing and scaling of the skin at the test site was observed noted for all test substances at all concentrations. The 20-h exposure caused a slight, in some cases also a strong, erythema and edema formation for all test substances at all concentrations. After 8 days, a scaling of the skin, scab formation and a superficial necrosis were observed noted at all test concentrations. The 20-h exposure of the rabbit ear caused erythema and inflammation as well as minor skin defects 24 h after the application (no further details were provided). After 8 days, scab formation and a slight necrosis were observed for all test substances at all concentrations. No significant differences could be found between the 30% and 40% aqueous solution of "pure" Glyoxal and a 40% aqueous solution of "raw" Glyoxal; slight to pronounced irritation was observed, depending on the application period, not grade of material.

Glyoxal (40%) was administered to the shaved back skin (5 cm x 7 cm) of adult white rabbits (n not specified); the duration of treatment was not specified.[8] Beginning on the third day, a strong reddened inflammation followed by necrosis with tissue demarcation was observed. The inflammation and necrosis were resolved at 30 days. The histopathological examination showed severe necrotic skin on the 4th day. These changes were less pronounced on the 9th day, and a regeneration of the epidermis was observed on the 18th day.

The application of Glyoxal (29.2% aqueous solution; 10 µL) to the depilated abdominal skin of a rabbit caused a slight irritation as indicated by minor hyperemia and an irritation index of 2 on a scale of 10.[8] No further details were provided.

A single occlusive administration of Glyoxal (40% aqueous; 1.57 mL; 798 mg/kg) to the shaved skin (dorsal, dorso-lateral) of Wistar rats (n=5/sex) for 24 h caused erythema in all of the rats.[8]

Human

No published human irritation studies were discovered and no unpublished data were submitted.

Sensitization

In Vitro

No published in vitro irritation studies were discovered and no unpublished data were submitted.

Animal

In a guinea pig study using the Magnusson-Kligman protocol, a trade mixture containing 1.3% Glyoxal (tested at 0.00065%) induced sensitization and was rated a "moderate" sensitizer.[2] A guinea pig study using the Ritz-Buehler technique found a threshold level of sensitization with 1.25% aqueous Glyoxal.

Female Pirbright-White guinea pigs (n=20) were used in a Magnusson and Kligman sensitization maximization test of Glyoxal (40%).[8] For induction the guinea pigs were intradermally injected with the test substance (20%; 0.1 mL; effective concentration of Glyoxal 8%) into the shoulder region. One week after intradermal injection, an occlusive epicutaneous application of approximately 300 mg of a 40% solution of the test substance was applied. The challenge was an occlusive epicutaneous application of the test substance (10%; 150 mg; effective concentration of Glyoxal 4%) on days 19 and 26 after intradermal induction (1st and 2nd challenge) on the shaved flank. One guinea pig died after the intradermal induction for unknown reasons. The intradermal induction of the test substance formulation in Freund's adjuvant/distilled water (1:1) caused necrotic skin changes and edema. After the first challenge, 1/19 guinea pigs showed a slight erythema and 6/19 guinea pigs showed distinct erythema (one of which additionally had a slight edema); a positive response was observed in a total of 7/19 guinea pigs. After the second challenge, a positive skin response occurred in 11/19 guinea pigs in total, where 7/19 guinea pigs showed a slight erythema and 4/19 guinea pigs showed a distinct erythema (one of which additionally had a slight edema). No skin reactions were observed in the control group at any time point. Glyoxal was shown to have a sensitizing effect on the skin of guinea pigs in the Maximization test.

Human

No new published sensitization studies were discovered and no new unpublished data were submitted.

Glyoxal was tested according to the Kligman maximization test. During induction, 48-hour occlusive patches containing 10% Glyoxal were applied to 24 subjects.[2] Panelists were challenged on the back with a 48-hour occlusive patch containing 2% Glyoxal in petrolatum. Sensitization was noted in all 24 panelists and Glyoxal was classified as an "extreme" sensitizer.

A human repeat insult patch test (HRIPT; n=55) was conducted using patches containing 14.5% Glyoxal (in a mixture), that were applied to the upper arm for 24 h every other day for a total of 15 applications. A 24-h 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 HRIPT using the same protocol, 0.33% Glyoxal produced no reactions during induction or challenge in 55 panelists.

In an HRIPT (n=155), 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. The challenge was two consecutive 48-h patches adjacent to the induction site. Seven of the subjects had responses to the challenge phase. However, upon retest, none of these were reactions to Glyoxal.

OCULAR IRRITATION STUDIES

In Vitro

No published ocular irritation studies were discovered and no unpublished data were submitted.

Animal

A solution of 32.8% aqueous Glyoxal caused grade 5, out of 20, injury to the eyes of rabbits.[2]

Glyoxal (30%) caused moderate irritation in the rabbit eye that cleared within 48 h without permanent injury.

When Glyoxal (40%) was instilled into one conjunctival sac of each of three rabbits, no changes in the iris or corneal opacity were noted. Well-defined redness and chemosis of the conjunctiva was noted in all three treated eyes at the 1-h observation. The reactions were scored as slight at the 72-h reading and as normal at the day 8 observation.

Glyoxal powder was a severe ocular irritant when instilled into the conjunctival sac of rabbits. Reactions produced had an average 1-h maximum score of 65.6 out of a maximum possible 110. Glyoxal powder was classified as a slight ocular irritant with an average 1-h maximum score of 6.6.

In an ocular irritation study performed in accordance with OECD GL 405 (Acute Eye Irritation/Corrosion) using rabbits, Glyoxal (40%) caused reversible reddening and chemosis of the conjunctiva within 8 days and thus showed an irritating effect.[8]

The instillation of Glyoxal (30% or 40% aqueous; 0.05 mL) into the conjunctival sac of rabbits caused a slight to strong reddening, mild edemas, and inflammation as well as a hazy clouding of the cornea depending on the concentration.[8] These effects were resolved within 1 or 2 weeks.

In a comparative study between "pure" Glyoxal (40% aqueous; 0.05 mL) and "raw" Glyoxal (40% aqueous; 0.05 mL; no data on the impurities) on the eyes of rabbits, the instillation into the conjunctival sac caused a clear reddening and very strong inflammation on the conjunctiva.[8] After installation of pure Glyoxal, a temporary, hazy corneal clouding developed, while a milky clouding of the cornea and scarification on the upper eyelid resulted after instillation of raw

Glyoxal. Slight reddening and inflammation (pure product) and reddening, corneal clouding and scarification (raw product) were still observed 8 days after the administration.

CLINICAL STUDIES

Retrospective and Multicenter Studies

Of 14 workers who had contact with Glyoxal (40%), 9 exhibited a contact dermatitis with localizations mainly on the lower arms and fingers.[7] Patch tests with Glyoxal (20%) resulted in a positive reaction in 7 of 9 workers.

In a multicenter study of dermal sensitivity, the records of 31,849 health care workers from 24 allergy departments between 1992 and 1995 were evaluated; 4.2% of the 774 female patients working in the medical profession were found to show positive reactions to patch tests of Glyoxal, whereas only 1.4% of the control group (1895 persons not in the medical profession) were found to be positive.[7]

In a continuation of this multicenter study, between 1997 and 1999, patients (2689) were patch tested with Glyoxal (trimer; 1% in petrolatum).[7] Positive reactions were observed in 1.6% of the patients, irritation in 0.3%, and questionable (i.e., non-allergic) in 0.6%.

SUMMARY OF NEW DATA

In a safety assessment published in1995, the CIR Panel concluded that the data were insufficient to determine the safety of Glyoxal as used in cosmetic products. In an amended safety assessment published in 2000, the Panel concluded that Glyoxal is safe for use in products intended to be applied to the nail at concentrations ≤1.25%; however, the available data were still insufficient to support the safety for other uses.

According to the *Dictionary*, Glyoxal was reported to function as a fragrance ingredient and preservative in cosmetics.

Anhydrous Glyoxal does not exist in a stable form at room temperature, and therefore it is commonly supplied in the form of an aqueous solution at 30%-50%. In a 5% solution, 39% of Glyoxal is present in the monomer form; in a 40% solution, the monomer content amounts to as little as 11% of Glyoxal, the dimer, and trimer forms being dominant.

According to the VCRP survey data received in 2017, Glyoxal is reported to be used in 2 formulations (basecoats and undercoats and a face and neck product). In 1998, there were no used reported in the VCRP. The results of the concentration of use survey conducted by the Council in 2016 indicate that Glyoxal has no reported uses and no concentrations of use were reported in 1998.

SCCP concluded that on the basis of provided data any risk to consumers when Glyoxal is present up to 100 ppm in cosmetic products is considered to be negligible.

In finished cosmetic products, Glyoxal may be present as a residue from the manufacture of other ingredients (i.e., cellulose, polyacrylamides, polyvinyl alcohol, and other polycondensates). The maximum concentration of Glyoxal found in cosmetic products is reported to be 100 ppm.

In a scenario compiled as a hypothesized high-end exposure for humans using a maximum daily intake of 10 mg Glyoxal via food, an estimated intake of 0.16 mg/kg/day was calculated. The tolerable intake for lifetime oral exposure to Glyoxal is approximately 0.2 mg/kg/day.

Dermal administration of ^{14}C-Glyoxal to the clipped skin of rats resulted in a dermal absorption of 6.4%.

Excretion of single oral dose of ^{14}C labelled-Glyoxal was 30%-32% (as CO_2) in exhaled air, 12%-13% in urine, and 24%-27 % in feces. After daily doses of Glyoxal for 14 day, excretion of and additional oral dose of ^{14}C labelled-Glyoxal was higher in urine (21.36%), lower in feces, and higher in exhaled air, compared with single doses, indicating changes in kinetics and pattern of Glyoxal metabolism after multiple dosing.

The dermal LD_{50} for Glyoxal was reported to be 800 mg/kg (active ingredient) in rats, 2000-12,700 mg/kg (active ingredient) in rabbits, and 5000-10,000 mg/kg (active ingredient) in guinea pigs. Skin necrosis, congestion and hemorrhage of the lung, congestion of the liver and kidneys were observed at 40% Glyoxal in rabbits.

The oral LD_{50} for Glyoxal was reported to be 3300-4064 mg/kg (active ingredient) for mice, 2.20-5000 mg/kg (active ingredient) in rats, 1700-3175 mg/kg (active ingredient) in rabbits, and 1700-3300 mg/kg (active ingredient) in cats. Clinical signs included apathy, dyspnea, diarrhea, piloerection, and weakness. Macroscopic findings included reddened stomach mucosa, patchy liver, congestion of the abdominal viscera, intestinal inflammation, and kidney swelling.

The inhalation LD_{50} for Glyoxal was reported to be 1300-2470 mg/m^3 (active ingredient) in rats. Clinical signs irregular breathing, nasal secretion, partly closed eyes, and ruffled fur. Macroscopic findings included dark red lungs.

In a short-term inhalation toxicity study of Glyoxal in rats, no test substance-related changes were noted histopathologically in the rats of the 0.4 mg/m³ active ingredient group. The NOEL for local effects was 0.4 mg/m³ air active ingredient. Since there were no signs of systemic intoxication, the NOEL was >10 mg/m³ active ingredient.

In a 90-day feeding study in rats, the NOEL was approximately 127 mg/kg for female rats and 132 mg/kg for males (active ingredient).

Glyoxal administered in drinking water for 12 and 24 months to rats resulted in a decreased alanine ALT activity, and lower cholesterol and globulin values in male and female rats at 300 mg/kg/day (active ingredient). Rats of both sexes had decreased body weights at the end of the experiment at 300 mg/kg/day (active ingredient). Microscopic examination of

female animals of the 24-month groups showed an increase of erosions/ulcer in the glandular stomach, which was confirmed and an increased number were observed by histopathological examination.

When Glyoxal (up to 125 mg/kg active ingredient; 40% in water) was administered to female rats by gavage on days 6 through 19 after mating, signs of maternal toxicity observed in the 125 mg/kg/day group were reduced feed consumption and lower corrected body weight gain. No test substance-related effects were observed on gestational parameters or fetuses. No test substance-related effects were observed on gestational parameters or fetuses. The NOAELs were 25 mg/kg/day active ingredient for maternal toxicity and 125 mg/kg/day active ingredient for embryotoxicity.

Glyoxal (up to 400 mg/kg) was administered to male and female rats in drinking water beginning 1 week before mating through parturition and then administered at 50% of the last week of pregnancy through weaning PND 21. The experiment was then repeated with the offspring (F_1 generation). There was decreased ALT activity in male and female rats of the 100 and 400 mg/kg/day dose groups of the F_0 as well as the F_1 generation. The males in both generations had reduced body weight gains in the 400 mg/kg/day group. For all live-born pups of the F_0 and F_1 parents, no test substance-induced signs of developmental toxicity were noted at dose levels as high as 100 mg/kg/day. Postnatal survival as well as post-weaning development of the offspring of these test groups until sexual maturity were unaffected by the test substance. Furthermore, clinical and/or gross necropsy examinations of the F_1 and F_2 pups revealed no adverse findings. The developmental toxicity NOAEL was determined to be 400 mg/kg/day and 100 mg/kg/day for maternal toxicity.

Glyoxal had positive results in Ames tests for both *S. typhimurium* and *E. coli* (lowest concentration tested was10 µg/plate (active ingredient). Mitotic recombinations were observed in *S. cerevisiae* exposed to 1.9-15.2 µg/mL of Glyoxal. There was no genotoxicity observed when mouse lymphoma cells were exposed to up to 250 µg/mL Glyoxal with metabolic activation and up to 375 µg/mL Glyoxal without metabolic activation. There was no genotoxicity observed when a plasmid was exposed to up to 150 µg/100 µL with metabolic activation.

Negative results were also observed in a micronucleus test (up to 225 mg/kg active ingredient) using mice and in two unscheduled DNA synthesis test using rats up to 2000 mg/kg Glyoxal.

In a study where up to 0.1% Glyoxal was administered in drinking water to mice starting 1 week prior to mating through PND21, there was no difference in the number of intestinal tumors between control and treatment groups. However, treatment caused an increase in tumor size in the small intestine for both male and female mice in comparison with respective control groups. The authors suggest that Glyoxal has tumor growth promoting properties in the small intestine in MIN mice.

Glyoxal, up to 300 mg/kg/d, administered in the drinking water of rats for 12 and 24 months caused no substance-related neoplastic lesions in either males or females after 12 and 24 months of treatment.

When two different grades of Glyoxal ("pure" 30% or 40% aqueous and "raw" at 40% aqueous) were administered to the shaved back skin of white rabbits for 1 and 5 min, no or slight erythema with a yellowing of the skin was observed 24 h after exposure. For the treatment period of 15 minutes, a mild edema was also noted. Eight days after treatment, a yellowing and scaling of the skin at the test site was observed. A 20-h exposure caused a slight, in some cases a strong, erythema and edema formation. After 8 days, a scaling of the skin, scab formation and a superficial necrosis were observed. A 20-h exposure caused a slight, in some cases also a strong, erythema and edema formation. Eight days after treatment, a scaling of the skin, scab formation and a superficial necrosis were observed. After the same concentrations were exposed to the rabbit's ear for 20-h, Glyoxal caused erythema and inflammation as well as minor skin defects 24 h after the application; after 8 days, scab formation and a slight necrosis were observed. No differences could be found between the 30% and 40% aqueous solution of "pure" Glyoxal and a 40% aqueous solution of "raw" Glyoxal; slight to pronounced irritation was observed, depending on the application period, not grade of material.

Glyoxal at 40%, administered to the shaved backs of rabbits caused a strong reddened inflammation, followed by a necrosis with tissue demarcation. The inflammation and necrosis were resolved at 30 days. The histopathological examination showed severe necrotic skin on the 4th day. These changes were less pronounced on the 9th day, and a regeneration of the epidermis was observed on the 18th day.

The application of Glyoxal (29.2% aqueous solution) to the depilated abdominal skin of a rabbit caused a slight irritation as indicated by minor hyperemia and an irritation index of 2 on a scale of 10.

A single occlusive administration of Glyoxal (40% aqueous; 1.57 mL; 798 mg/kg) to the shaved skin of rats for 24 h caused erythema in rats.

In a Magnusson and Kligman sensitization maximization test, guinea pigs were intradermally injected with Glyoxal (20%; effective concentration of Glyoxal 8%) followed an occlusive epicutaneous application of approximately 300 mg of a 40% solution of the test substance one week later. Challenge (10%; 150 mg; effective concentration of Glyoxal 4%) was administered on days 19 and 26. After the first challenge, a positive response was observed in a total of 7/19 guinea pigs. After the second challenge, a positive skin response occurred in 11/19 guinea pigs in total, where 7/19 guinea pigs showed a slight erythema and 4/19 guinea pigs had a distinct erythema (one of which additionally had a slight edema). No skin reactions were observed in the control group at any time point. Glyoxal was shown to have a sensitizing effect on the skin of guinea pigs.

In an ocular irritation study using rabbits, Glyoxal (40%) caused reversible reddening and chemosis of the conjunctiva within 8 days and thus showed an irritating effect. The instillation of Glyoxal (30% or 40% aqueous) into the conjunctival sac of rabbits caused a slight to strong reddening, mild edemas, an inflammation as well as a hazy clouding of the cornea depending on the concentration; these effects were resolved within 1 or 2 weeks.

In a comparative study between "pure" Glyoxal (40% aqueous) and "raw" Glyoxal (40% aqueous) on the eyes of rabbits, the instillation into the conjunctival sac caused a clear reddening and very strong inflammation on the conjunctiva. Pure Glyoxal caused a temporary, hazy corneal clouding developed, while a milky clouding of the cornea and scarification on the upper eyelid resulted after instillation of raw Glyoxal. Slight reddening and inflammation (pure product) and reddening, corneal clouding and scarification (raw product) were still observed 8 days after the administration.

Of 14 workers who had contact with Glyoxal (40%), 9 exhibited a contact dermatitis with localizations mainly on the lower arms and fingers. Patch tests with Glyoxal (20%) resulted in a positive reaction in 7 of 9 workers.

In a multicenter study of dermal sensitivity, the records of 31,849 health care workers from 24 allergy departments were evaluated; 4.2% of the 774 female patients working in the medical profession were found to show positive reactions to patch tests of Glyoxal, whereas only 1.4% of the control group (1895 persons not in the medical profession) were found to be positive. In a continuation of this multicenter study, patients (2689) were patch tested with Glyoxal (trimer; 1%); positive reactions were observed in 1.6% of the patients, irritation in 0.3%, and questionable (i.e., non-allergic) in 0.6%.

DISCUSSION FROM 1995 REPORT[1]

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 30G)(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.

DISCUSSION FROM THE 2000 REPORT[2]

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/J0TI/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.

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TABLES

Table 1. Chemical and physical properties of Glyoxal.

Property	Value	Reference
Physical Form	Liquid	2
	Powder	8
Color	Yellow	2
	Colorless	8
Odor	Mild	6
Molecular Weight g/mol	58.04	2,6
Density 20°C	1.14	2
20°C	1.27	5
Vapor pressure mmHg @ 20°C	220.0	2
Vapor Density mmHg	2.0	2
Melting Point °C	15	2,7
	-25	5
	-15	5
Boiling Point °C	51	2
	103.6	5
Water Solubility g/L	20.2	5
	600[a]	8
	Soluble	2
Other Solubility g/L		
Alcohol	Soluble	2
Ether	Soluble	
log K_{ow} @ 20°C	-1.15	5
Disassociation constants		
Log P_{ow}	0.85	8

Table 2. Acute dermal, oral, and inhalation tests on animals.[8]

Species	Concentration/ dose tested	LD_{50}/LC_{50}	Comments/effects	Observation period
Dermal				
Wistar rat (n=5/sex)	40% Glyoxal for 24 h under semi-occlusion	Both sexes, >2000 mg/kg (>800 mg/kg active ingredient)	Comparable to OECD GL 402 (Fixed Dose Method). No macroscopic findings	14 days
Male rabbit (n not specified)	30% Glyoxal for 24 h	>20 mL/kg	Mortality: 1/4	14 days
New Zealand White Rabbit (n=5/sex)	40% Glyoxal (2000 mg/kg active ingredient) for 24 h under semi-occlusion	Both sexes, >2000 mg/kg active ingredient	Comparable to OECD GL 402; applied to abraded skin No deaths occurred. Slight erythema and edema (score=1) was visible at the 2 and 4 h observation period. No macroscopic findings	14 days
Rabbit (n not specified)	40% Glyoxal occlusive patch	10 mL/kg (12,700 mg/kg active ingredient)	Skin necrosis, congestion and hemorrhage of the lung, congestion of the liver and kidneys	No data
Rabbit (n not specified)	Unknown	6600 mg/kg active ingredient	N/A	No data
Guinea pig (n not specified)	Unknown	5000-10,000 mg/kg active ingredient	N/A	No data
Oral				
Mouse (n not specified)	29.2% Glyoxal	~5.0 mL/kg active ingredient	Clinical signs: apathy, reeling, dyspnea Necropsy of mice that died before 7 days: irritation of the gastrointestinal tract (forestomach bleeding and congestion in the gastrointestinal tract), lungs, kidneys and adrenal glands.	7 days
Mouse (n not specified)	30% Glyoxal	3300 mg/kg active ingredient	Disturbances of balance, apathy	No data
Mouse (n not specified)	40% Glyoxal	~3.2 mL/kg (4064 mg/kg active ingredient)	Apathy, reeling, dyspnea	7 days
Sprague-Dawley rat (n not specified)	20% Glyoxal	1680 mg/kg active ingredient	Clinical signs: diarrhea, weakness. Macroscopic findings: hemorrhagic lung, liver, heart; inflamed gastrointestinal tract.	No data

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Table 2. Acute dermal, oral, and inhalation tests on animals.[8]

Species	Concentration/ dose tested	LD_{50}/LC_{50}	Comments/effects	Observation period
Male Carworth-Farms rat (n not specified)	29.2% Glyoxal	7.46 mL/kg (2.200 mg/kg active ingredient)	8 mL/kg macroscopic findings: congestion of lungs, gastrointestinal tract and adrenal glands, patchy livers, pale kidneys	14 days
Rat (n not specified)	30% Glyoxal	~4700 mg/kg	disturbances of balance, apathy	No data
Wistar rat (n not specified)	40% Glyoxal	Overall: 3300 mg/kg; males, 3660 mg/kg; females, 2960 mg/kg active ingredient	Clinical signs: decreased spontaneous activity increased respiratory rate. Macroscopic findings: reddened stomach mucosa, patchy liver, dark discolored adrenal glands, increased pulmonary hyperemia in deceased rats.	14 days
Female Wistar rat (n not specified)	40% Glyoxal	0.5-0.6 mL/kg (640-770 mg/kg active ingredient)	Mortality at 0.5 mL/kg 0/20; 0.7 mL/kg 10/10. Clinical signs: comatose state, piloerection, and chromodacryorrhea.	14 days
Male Harlan-Wistar rat (n not specified)	40% Glyoxal (aqueous)	3.08 mL/kg (3912 mg/kg active ingredient)	None	No data
Male and female rat (n not specified)	40% Glyoxal (aqueous)	Males, 7.07 mL/kg (8.98 mg/kg active ingredient); females, 6.16 mL/kg (7.82 mg/kg active ingredient)	No clinical signs. Macroscopic findings: congestion of the abdominal viscera, intestinal hemorrhage	No data
Wistar rat (n=5/sex)	40% Glyoxal (aqueous); (2000 and 5000 mg/kg active ingredient	Males, >2 000; females <5000 mg/kg (active ingredient)	Mortality at 2000, 0/10; 5000 10/10 within 10 days of dosing Clinical signs: transient diarrhea, unspecified signs of intoxication until death. Macroscopic findings: died due to treatment-irritation of the stomach and discolored livers with broadened lobes. Survived treatment-no gross pathologic alterations in the tissues and organs examined.	14 days
Wistar rats (n not specified)	40% Glyoxal (aqueous)	Both sexes, >5000 mg/kg active ingredient	Ataxia, hypersensitivity to external stimuli	14 days
Rabbit (n not specified)	30% Glyoxal (aqueous)	~1700 mg/kg	Clinical signs: lethal within 8 days, proteinuria, erythrocytes and leukocytes in sediment. Macroscopic findings: intestinal inflammation, kidney swelling.	No data
Rabbit (n not specified)	40% Glyoxal (aqueous)	~2.5 mL/kg (3175 mg/kg active ingredient)	None reported	8 days
Cat (n not specified)	30% Glyoxal (aqueous)	1700-3300 mg/kg active ingredient	Mortality at 1700 mg/kg, 1/1; 3000 mg/kg, 1/2. Clinical signs: protein, erythrocytes and leukocytes found in urine within 5 days. Macroscopic findings: gastritis, enteritis, follicular hyperplasia of spleen, kidney swelling	No data

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Table 2. Acute dermal, oral, and inhalation tests on animals.[8]

Species	Concentration/ dose tested	LD_{50}/LC_{50}	Comments/effects	Observation period
		Inhalation		
Albino rat (n not specified)	29.2% Glyoxal (aqueous) for 8 h	N/A	Inhalation hazard test-Mortality: 0/6 (male/female)	14 days
Rat (n not specified)	30% Glyoxal (aqueous) for 8 h	N/A	Inhalation hazard test at atmosphere saturated 20°-no findings at necropsy	No data
Wistar rat (n=5/sex)	40.1% Glyoxal (aqueous) (2200, 2600 and 2700 mg/m^3 active ingredient) nose only aerosol for 4 h	Both sexes, 2440 mg/m^3; males, 2470; females, 2410 mg/m^3 (active ingredient)	Mortality at 2200 mg/m^3, 2/10; 2600 mg/m^3, 5/10; 2700 mg/m^3, 10/10. Clinical signs: irregular breathing, nasal secretion, partly closed eyes, ruffled fur, dizziness, lying on abdomen. Macroscopic findings: dark-red lungs	14 days
Wistar rat (n not specified)	40% Glyoxal (aqueous) for 7 h	N/A	Inhalation hazard test with an atmosphere enriched at 20°C-mortality 0/10 (males and females); irregular breathing rate; no findings at necropsy.	14 days
Rat (n not specified)	40% Glyoxal (aqueous) for 8 h	N/A	Inhalation hazard test with an atmosphere enriched at 20°C-mortality 0/10; no findings at necropsy.	No data
Rat (n not specified)	40% Glyoxal (aqueous) as dust or fog; time unspecified	N/A	Clinical signs: dyspnea, partly closed eyes, sneezing, blood-colored lacrimation, piloerection, flanks that were drawn in, lying on the abdomen. Necropsy of rats that died before end of observation period (number not specified): hyperemia and foamy secretion in the lung.	At least 9 days
Wistar rats (n not specified)	80% Glyoxal (aqueous) dust for 4 h	Both sexes, >1300 mg/m^2 active ingredient	Clinical signs: irregular breathing, irritations. Macroscopic findings: none.	No data

Table 3. In vitro genotoxicity assays of Glyoxal.[8]

Test system/species	Test concentrations/conditions	Results/remarks
Ames test. *S. typhimurium* (strains TA98, TA100, TA102, TA1535, TA1537)	~40% Glyoxal in water with and without metabolic activation: 52-13,000 µg/plate active ingredient	Positive in TA100 and TA102 with and without metabolic activation
Ames test. *S. typhimurium* (strains TA98, TA100, TA1537, TA1538)	With and without metabolic activation: 10-500 µg/plate active ingredient	Positive in TA100 with and without metabolic activation
Ames test. *S. typhimurium* (strain TA100)	With and without metabolic activation: up to 500 µg/plate active ingredient	Positive in TA100 with and without metabolic activation
Ames test. *S. typhimurium* (strain TA100)	30 % Glyoxal in water Without metabolic activation: 116-929 µg/plate (~2-16 µmol/plate active ingredient; 30% Glyoxal)	Positive
Ames test. *S. typhimurium* (strains TA98, TA100, TA1537, TA1538)	With and without metabolic activation: 10-10,000 µg/plate active ingredient	Positive in TA100 with and without metabolic activation
Ames test. *S. typhimurium* (strains TA98, TA7006)	With and without metabolic activation: 20-100 µg/plate active ingredient	Positive in TA98 and TA7006 with and without metabolic activation
Ames test. *S. typhimurium* (strain TA100)	Without metabolic activation: 58-325 µg/plate (~1-5.6 mM)	Positive
Ames test. *S. typhimurium* (strains TA98, TA100, TA102)	Without metabolic activation: 14.5-580 µg/plate active ingredient (~0.25-10 µM)	Positive in TA100 and TA102 with and without metabolic activation
Ames test. *S. typhimurium* (strains TA98, TA100, TA104) and *E. coli* (strain WP2uvrA/pKM101)	No data	Positive in TA98, TA100, TA104 and with and WP2uvrA/pKM101 without metabolic activation
Saccharomyces cerevisiae	40% Glyoxal 1.5-12 µL/mL (corresponding to 1.9-15.2 µg/mL active ingredient). Incubation temperatures of 28°C or with cold-shock treatment	Mitotic recombinations and chromosome losses in a small number of colonies.
Chromosome aberration, CHO cells	40% Glyoxal With metabolic activation: 190.1-580 µg/mL active ingredient Without metabolic activation: 25.51-580 µg/mL active ingredient	Positive with and without metabolic activation
Mouse lymphoma test, L5178TK+/- cells	40% Glyoxal in water. With metabolic activation: 25-250 µg/mL active ingredient Without metabolic activation: 25-375 µg/mL active ingredient	Negative with metabolic activation. Positive without metabolic activation.
Co S-7- Plasmid pMy 189	With metabolic activation: 0-150 µg/100 µL active ingredient plasmid solution	Cytotoxicity and mutation frequency increased

CHO-Chinese hamster ovary

Table 4. In vivo genotoxicity studies of Glyoxal.[8]

Test system/species	Test conditions	Results/remarks
Micronucleus test, mouse (CD-1), 6 males/group; 1000 polychromatic erythrocytes (bone marrow)/mouse examined	56.25, 112.5, 225 mg/kg i.p., once daily on 2 consecutive days, Glyoxal (40%; doses related to active ingredient). Killed 24 h after administration.	Negative (maximum tolerated dose tested)
Unscheduled DNA synthesis test, male Wistar rats, primary hepatocytes	Autoradiographic Unscheduled DNA synthesis test (net grain count). 1000 and 2000 mg/kg Glyoxal (40%; doses related to active ingredient) oral. Killed 2-4 or 12-14 h after administration (doses related to active ingredient)	Negative (maximum tolerated dose tested)
Unscheduled DNA synthesis test, male Wistar rats, primary hepatocytes.	Autoradiographic Unscheduled DNA synthesis test (net grain count). 100, 500, 1000 mg/kg Glyoxal (40%) oral. Killed 2 and 16 h after administration	Negative (No toxicity up to 1 000 mg/kg)

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Final Report on the Safety Assessment of Glyoxal[1]

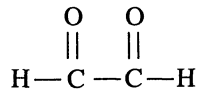
Abstract: The naturally occurring bialdehyde Glyoxal is used as a preservative in nail polishes and enamels. It is provided to formulators as a 40% aqueous solution because the nonhydrated form is highly reactive with water and other solvents. Reduced weight gain was seen in acute and subchronic animal 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-

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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

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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

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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,

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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.

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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

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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

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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

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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

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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

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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

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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

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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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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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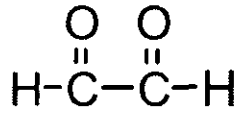

FIGURE 1
Chemical formula for Glyoxal.

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 ≥ 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, 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 ≤ 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

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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).

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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 μm 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

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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

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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

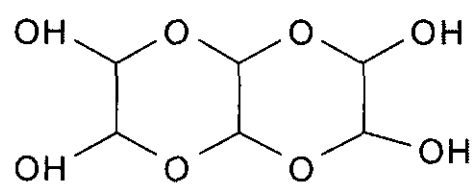

FIGURE 2
Chemical structure of Glyoxal Trimeric Dihydrate (NTP [lab supplement] 1993, 1994).

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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 μl) 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 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*-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

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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	1.1×10^{-3}–1.7×10^{-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 μg/plate	Mutagenic; with S-9 or catalase, mutagenicity reduced	Yamaguchi and Nakagawa 1983
TA98, TA100	10 μg/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 μg/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 μg/plate	Mutagenic	Levin et al. 1982
TA104	NR (2250 revertants/μmol)	Mutagenic	Marnett et al. 1982
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, 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 μg/plate ± S9 (three doses)	Without S9: toxic at 100 and 1000 μg/plate With S9: dose-dependent positive in TA100; upon retest with lower doses, positive results were noted in TA100 at $\geq$10μg/plate	American Cyanamid 1977
TA100, TA1535, TA1537, TA1538, TA98, *E. coli* WP2uvrA	4–5000 μg/plate ± S9 (six doses)	Without S9: dose-dependent increase in TA100 With S9: increased revertants in TA100 and TA1535	Hoechst AG 1984
TA102	4–5000 μg/plate ± S9 (six doses)	Without S9: dose-dependent increase in revertants With S9: "relevant" increase in revertants	Hoechst AG 1986
TA1535, TA1537, TA1538, TA98, TA100	100–10,000 μg/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

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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 μg/ml ± S9 activation (cytotoxic at 1000 μg/ml)	Increased mutation at 600 μg/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 μg/ml With S9: 10–100 μg/ml	Dose-related response. Without S9, statistically significant response at 200 μg/ml (not enough cells at 250 μg/ml to be reliable). With S9, statistically significant response at ≥50 μg/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 μg/ml (± S9 metabolic activation)	Dose-dependent increase in aberrations ± activation	Henkel 1990
CH V79 Chromosomal abberations and mitotic activity	100–400 μg/ml	Increase chromosomal abberations and decreased mitotic activity	Nishi, Miyakawa, and Kato 1989
Unscheduled DNA synthesis in TC-SV40/ INO hamster cells	5×10^{-5} M	Increased conservative and semiconservative UDS	Cornago et al. 1989
C3H/10T1/2 mouse embryo cell transformation	0.0013–0.0098 μl/ml media	Negative	EG&G Mason Research Institute 1980a
C3H/10T1/2 mouse embryo cell transformation	0.0049–0.039 μl/ml media	Negative	EG&G Mason Research Institute 1980b
C3H/10T1/2 mouse embryo cell transformation	0.0025–0.195 μl/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 1.85×10^{-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)

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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 μg/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 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	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

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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

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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

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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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2017 VCRP Data for Glyoxal

08A - Basecoats and Undercoats	GLYOXAL	1
12C - Face and Neck (exc shave)	GLYOXAL	1
		2

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Lillian Gill, D.P.A.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: CIR Science and Support Committee of the Personal Care Products Council

DATE: July 6, 2016

SUBJECT: Concentration of Use: Glyoxal

There were no reports of intentional addition of Glyoxal to cosmetics in response to the Council's November 2015 concentration of use survey.

Glyoxal is used to produce other compounds, such as use as a cross-linking agent for compounds including cellulose, polyacrylamides, polyvinyl alcohol and other polycondensates. Therefore, in finished cosmetic products, Glyoxal may be present as a residual. The maximum concentration of Glyoxal found in cosmetic products is 100 ppm. This level is consistent with the 2005 Scientific Committee on Consumer Products (SCCP) Opinion on Glyoxal[1] and the European Cosmetic Regulations which limits Glyoxal in finished cosmetic products to 100 ppm (entry 102 of Annex III).

[1]Found at
http://ec.europa.eu/health/archive/ph_risk/committees/04_sccp/docs/sccp_o_023.pdf

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