Project Report 45-508

# Evaluation of the Dermal Carcinogenicity of AEROTEX® Glyoxal 40 and European Glyoxal 40 in Male C3H Mice

October 29, 1982 IR-407

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UNION CARBIDE

BUSHY RUN RESEARCH CENTER

R. D. 4, Mellon Road, Export, Pennsylvania 15632 Telephone (412) 327-1020

PROJECT REPORT 45-509



SAMPLE: AEROTEX® Glyoxal 40 and European Glyoxal 40

SUBJECT: Evaluation of the Dermal Carcinogenicity of AEROTEX® Glyoxal 40 and European Glyoxal 40 in Male C3H Mice



SPONSOR: American Cyanamid Company

MONITOR: Francis J. Koschier

DATE: October 29, 1982

# Evaluation of the Dermal Carcinogenicity of AEROTEX® Glyoxal 40 and European Glyoxal 40 in Male C3H Mice

Authors: Murray D. Woodside and Lionel R. DePass

Sponsor: American Cyanamid Company

## Summary

Groups of 40 C3H/HeJ male mice received 25 microliter (µl) applications of AEROTEX® glyoxal 40 or European glyoxal 40 as 1:8 dilutions in deionized water. A negative control group received deionized water only. The substances were applied to the skin of the back three times weekly for the lifetime of the animals. No skin neoplasms were found in any of the treated mice. While no subcutaneous neoplasms were found in the mice treated with AEROTEX® glyoxal 40, a fibrosarcoma was observed in one of the European glyoxal 40-treated mice. The occasional occurrence of fibrosarcoma in control C3H mice is supported by historical data in this laboratory. Consequently, the fibrosarcoma diagnosed in the treated mouse is considered to be of no biological importance. Furthermore, no increase in mortality rates attributable to treatment with the test substances was observed in the mice. Therefore, neither AEROTEX® glyoxal 40 nor European glyoxal 40 are considered to be tumorigenic when applied to the skin of C3H mice for their lifetime.

## Objective

These studies were designed to determine the dermal carcinogenic potential of AEROTEX® glyoxal 40 and European glyoxal 40.

## Samples

1. AEROTEX® glyoxal 40, Bushy Run Research Center (BRRC) sample number 41-400, labeled with the identification number R9195-160 was received as a one-gallon sample from American Cyanamid Company, Bound Brook, NJ on October 23, 1978. The material was a yellow liquid.

2. European glyoxal 40, BRRC sample number 41-429, labeled with the identification number R9516-43 was received as a one-gallon sample from American Cyanamid Company, Bound Brook, NJ on November 29, 1978. The material was a clear liquid.

Aliquots of the parent samples were shipped to the sponsor for stability and content analyses after treatment week 2 and four times during the studies. Furthermore, 1:8 dilutions of AEROTEX® glyoxal 40 and European glyoxal 40 in deionized water (CAS Number 77-32-18-5) as well as a sample of the deionized water used to prepare the dilutions were shipped to the sponsor for analysis 2 days after the start of treatment. The analytical results

demonstrated that the test samples retained stability for the duration of the study. Content analyses revealed that the concentrations of glyoxal found by analysis were quite similar to the expected concentrations. The analytical data received are included as Appendix II of this report.

## Procedures

The animals used in this study were received in two shipments from Jackson Laboratories, Bar Harbor, ME. The first shipment, received on January 31, 1979, contained a total of 763 C3H/HeJ male mice. The second shipment received on February 14, 1979, contained a total of 750 C3H/HeJ male mice. The mice were housed 5 per cage in stainless-steel suspended cages with dimensions of 8" x 9" x 6 3/4". Zeigler block feed (supplied by Zeigler Bros. Inc., Gardners, PA) and water (Municipal Authority of Westmoreland County, Greensburg, PA) from an automatic watering system were provided ad libitum. The mice were identified by toe-clip according to preassigned, unique identification numbers. The mice were weighed individually and as cagemates of 5. The weights of 5 cagemates were used to randomize the mice into test groups. One hundred twenty mice were assigned to this study while the remaining 1393 were culled, used as quality control sentinels or assigned to other skin carcinogenesis projects. Prior to the start of testing the fur was clipped from the backs of the mice and they were checked for general health status. The mice were housed for the duration of the study in Room 170 of the CHF building.

The dosing concentrations of AEROTEX® glyoxal 40 (1:8 dilution in deionized water) and European glyoxal 40 (1:8 dilution in deionized water) were determined in a two-week subacute test. This concentration was non-irritating and non-toxic to the mice in this subacute study. A summary of the data from this study is attached as Appendix III.

Testing began on March 12, 1979 when the mice were approximately 61 to 75 days of age. From that day each mouse received 25 µl of the appropriate material three times weekly (on Mondays, Wednesdays, and Fridays, excluding holidays) for its lifetime. On Tuesday or Thursday of each week the fur was clipped from the back of each mouse and the mice were moved to clean cages and racks. The test materials were applied by means of an Eppendorf automatic pipette with a clean disposable tip used for each material on each dosing day. The mice were observed daily for mortality and were carefully examined for lesions of the skin once per month.

A necropsy was performed on all dead mice and on moribund mice, which were sacrificed. A necropsy consisted of a careful examination of the skin and body cavities. All observations were recorded. The entire carcasses of all non-autolyzed mice that died after November 11, 1980 were fixed in 10% neutral buffered formalin. Sections were prepared and stained and were examined histologically (See Pathology Report for details).

Mortality was assessed by Kaplan-Meier methods (Gart et al., 1979; Mantel, 1966; Breslow, 1970).

All data, specimens and the final report generated in this study will be stored in the BRRC Archives.

## Results

The results of this study are summarized in Table I and a survival curve is presented as Figure I. In the group that received AEROTEX® glyoxal 40 as a 1:8 dilution in deionized water the mean survival time was statistically greater than that of the deionized water control group (580 versus 488 days) by one of the two statistical methods. While AEROTEX® glyoxal 40 caused skin irritation resulting in inflammation and necrosis in some mice, no skin or subcutaneous neoplasms were found in any mice treated with this material. The last surviving mouse in this group died on June 9, 1981.

The mean survival time (549 days) in the group of mice dosed with European glyoxal 40 was statistically greater than that of the deionized water control group. No skin neoplasms were found in the European glyoxal 40-treated mice. One mouse in this group had an infiltrative fibrosarcoma on the left lateral rib cage and axilla. Since this tumor type has been shown by BRRC historical pathology data to occur occasionally in control mice, it is considered to be of no biological importance. This tumor was observed after 496 days on study. The last surviving mouse in this group died on June 9, 1981.

In the deionized water control group the mean survival time of 488 days was the lowest of the groups. There were no tumors in this group and the last surviving mouse died on June 8, 1981.

## Conclusions

No skin tumors were found in any of the C3H/HeJ male mice treated for their lifetime with 25 µl of 1:8 dilutions of AEROTEX® glyoxal 40 or European glyoxal 40 in deionized water. Furthermore, no subcutaneous tumors were found in the AEROTEX® glyoxal 40-treated group. A subcutaneous tumor (fibrosarcoma) observed in one mouse in the European glyoxal 40-treated mice is considered to be of no biological importance. This conclusion is supported by BRRC historical data which document that fibrosarcoma has been encountered in control C3H male mice. Furthermore, mortality was not affected by treatment with either test material. Therefore, neither AEROTEX® glyoxal 40 nor European glyoxal 40 are considered to be tumorigenic by this testing method.

## References

Breslow, N. (1970). A Generalized Kruskal-Wallis Test for Comparing of Samples Subject to Unequal Patterns of Censorship. Biometrics 57: 579-594.

Gart, J. J., Chu, K. C. and Tarone, R. E., (1979). Statistical Issues in Interpretation of Chronic Bioassay Tests for Carcinogenicity. J. Natl. Cancer Inst. 62: 957-974.

Mantel, N. (1966). Evaluation of Survival Data and Two Rank Order Statistics Arising in its Consideration. Cancer Chemotherapy Reports 50: 163-170.

Reviewed and Approved by:

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WPC/rkk/0366A-2
08-13-82

Table I

AKROMEX® glycmal 40 and European glycmal 40
Summary of Results of a Lifetime Skin Carcinogenesis Test

| | AKROMEX® glycmal 40 | European glycmal 40 | Deionized water |
|---|---|---|---|
| Concentration | 1:8 dilution in deionized water | 1:8 dilution in deionized water | 100% |
| Volume (Microliters) | 25 | 25 | 25 |
| Mean Survival Time (Days) | 580* | 594** | 488 |
| Animals with Skin or Subcutaneous Tumors | 0 | 0 | 0 |

* Different from the control mean ($p < 0.05$) by the Mantel-Cox test.
** Different from the control mean ($p < 0.05$) by the Mantel-Cox and Breslow tests.

WPC/rkk/0366A-6
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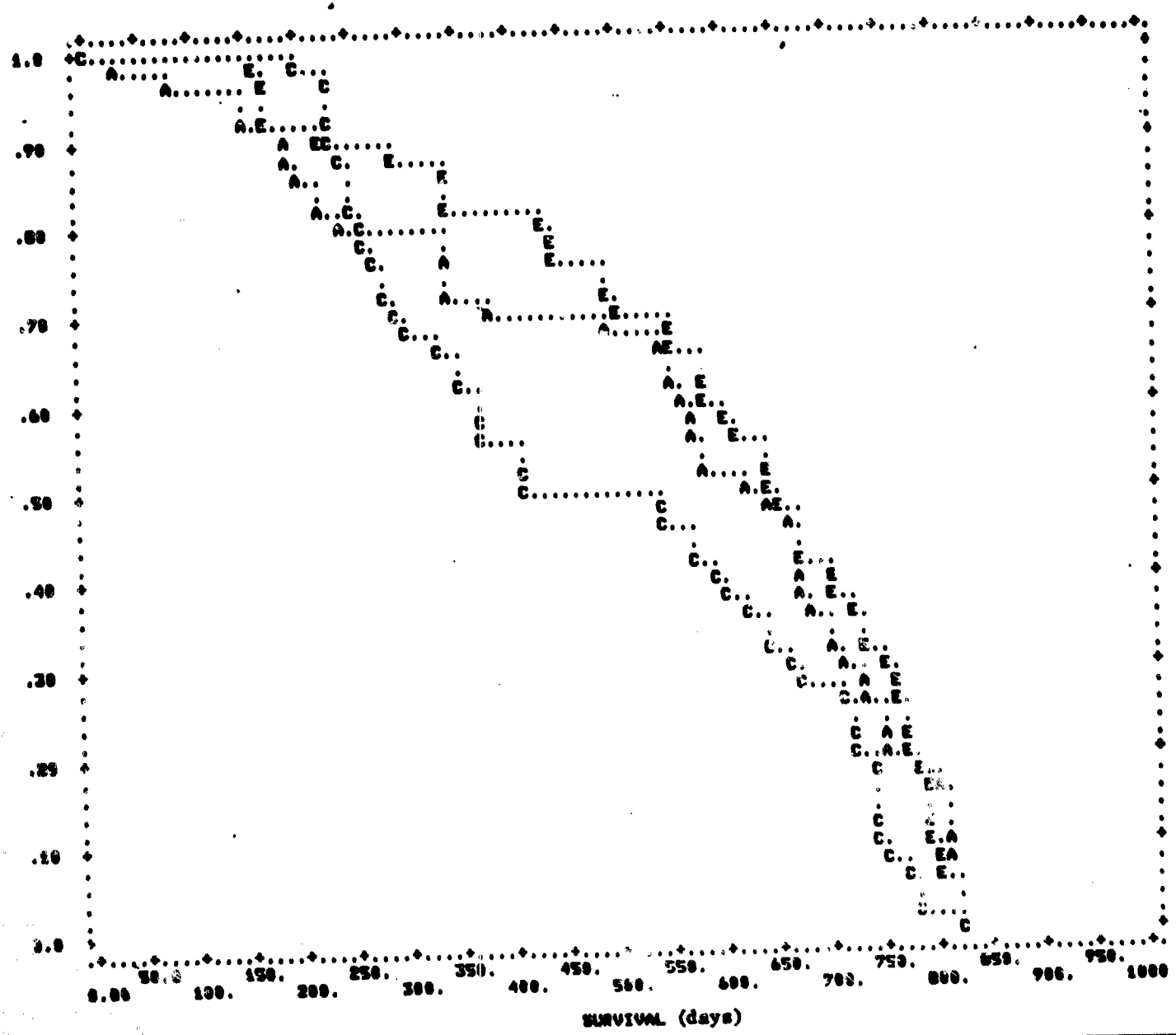
FIGURE

A = AERO  E = PYRO  C = CTL

CUMULATIVE PROPORTION SURVIVING



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## Quality Assurance Unit Study Inspection Summary

**Test Substance:** AEROTEX Glyoxal 40 and European Glyoxal 40

**Study:** Evaluation of the Dermal Carcinogenicity in Mice

**Study Director:** L. R. DePass

The Quality Assurance Unit of BRRC conducted the inspections listed below and reported the results to the study director and to management on the dates indicated. It is the practice of this Quality Assurance Unit to report the results of each inspection to both the study director and management.

| Inspection Date | Inspection Type | Date QAU Report Issued To Study Director | Date QAU Report Issued To Management |
|---|---|---|---|
| 2-23-79 | Protocol - (European) | 2-23-79 | 2-23-79 |
| 2-23-79 | Protocol - (AEROTEX) | 2-23-79 | 2-23-79 |
| 11-13 to 11-16-79 | In Progress | 11-16-79 | 11-28-79 |
| 2-13 to 3-3-80 | In Progress | 3-3-80 | 4-22-80 |
| 5-5 to 5-14-80 | In Progress | 5-14-80 | 5-21-80 |
| 10-6 to 10-9-80 | In Progress | 10-9-80 | 10-15-80 |
| 3-20 to 3-27-81 | In Progress | 3-27-81 | 4-1-81 |
| 3-19 to 5-5-82 | Final Data (AEROTEX) | 5-5-82 | 5-12-82 |
| 3-19 to 5-5-82 | Pathology Raw Data and Draft Pathology Report (AEROTEX) | 5-5-82 | 6-9-82 |
| 5-4 to 5-7-82 | Final Data - European) | 5-7-82 | 5-11-82 |
| 5-4 to 5-7-82 | Pathology Raw Data and Draft Pathology Report (European) | 5-7-82 | 6-9-82 |
| 10-5 to 10-7-82 | Final Report | 10-7-82 | 10-28-82 |

Daniel L. Geary 10/29/82
Quality Assurance Officer Date

LJC:acc

# Appendix I

## Pathology Report

29413

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APPENDIX I

# ANATOMIC PATHOLOGY REPORT

## DERMAL CARCINOGENESIS LIFETIME SKIN PAINTING IN MICE

## OF

## AEROTEX® GLYOXAL 40

Table of Contents



NOTE: This Pathology Report begins on page 29413 and ends on page 29501.

## Summary and Conclusions

Male C3H/HeJ mice were randomized into groups of 40 and treated three times weekly (holidays excepted) throughout their lifetime with a dilution of AEROTEX® glyoxal 40 which was applied to the clipped skin of their backs. A negative control group received similar treatments with deionized water alone.

No skin or subcutaneous neoplasms were found in any mice treated with AEROTEX® glyoxal 40 or deionized water. AEROTEX® glyoxal 40 did cause irritation of the skin in some mice which resulted in inflammation and necrosis.

Other lesions encountered in these mice were considered part of the spontaneous background of lesions seen in this mouse strain.

## Introduction

The purpose of this study was to determine the dermal neoplastic potential of AEROTEX® glyoxal 40 by applying it to the skin of male C3H/HeJ mice over the period of their lifetime and determining the gross and microscopic appearance of the resulting lesions.

## Methods

Male C3H/HeJ mice, 4-6 weeks of age were obtained from Jackson Laboratories, Bar Harbor, ME. The mice were randomized into groups of 40 animals. Each group of 40 male mice received either the test chemical or a control material three times per week, with the exception of holidays, applied by an Eppendorf pipette to the clipped skin of the back. The application of test chemical or control material was continued throughout the lifetime of the mice.

Following the death of each mouse a gross necropsy was performed. All body cavities were examined and all suspect internal tumors were fixed in 10% neutral buffered formalin (NBF) unless the degree of autolysis precluded saving the tissues for histologic examination. The dorsal skin of all mice, with or without skin lesions, was also fixed in 10% NBF for histologic examination unless the mouse was severely autolyzed. In addition, after November 11, 1980, the remainder of each mouse was fixed in 10% NBF and saved at the request of the sponsor.

Tissues fixed in 10% NBF were carefully trimmed, embedded, sectioned and stained with hematoxylin and eosin for examination by a pathologist. All neoplastic and non-neoplastic lesions discovered during the histopathologic examination were recorded and tabulated.

## Results and Discussion

The frequency of gross lesions are included in Table 1. Very few gross lesions were found in the skin or subcutis of either the AEROTEX® glyoxal 40-treated group or the negative controls. No nodules or masses were found in these tissues.

The gross lesions encountered in the other organs were all considered part of the spontaneous background lesions seen in mice of this strain used on lifetime studies.

The histologic findings are included in Table 2. No neoplasms were found in the skin or subcutis in any of the mice treated with AEROTEX® glyoxal 40 or deionized water. There was indication of the irritative nature of AEROTEX® glyoxal 40 as evidenced by the amount of inflammation and necrosis seen. However, there was evidence of notable epidermal hyperplasia in only 2 mice from the AEROTEX® glyoxal 40-treated group.

The following histological lesions encountered in these mice were not considered of biological importance and are part of the background of spontaneous neoplastic or inflammatory conditions found in this strain in lifetime studies.

The histologic changes observed in the adrenals reflect the gross observations, but it should be remembered that only adrenals with lesions observed at necropsy were examined histologically. Cortical hyperplasia/adenoma appeared in nearly all adrenals examined including those in the control group. Dunn (1970) considered the distinction between hyperplasia and neoplasia in mouse adrenals to be very difficult and somewhat arbitrary.

Low with the test and control groups, lesions seen in the male urogenital system were primarily related to the Proteus mirabilis infection which is frequently encountered in this strain from this vendor (Maronpot and Peterson, 1981).

Liver neoplasms are frequently encountered in this strain and therefore no significance can be attributed to their appearance in the treated or control mice.

Edward H. Fowler, DVM, Ph.D.
Pathologist

9/28/82
Date

EHC/esk/0269A-3
09-28-82

## References

Dunn, T. B. Normal and Pathologic Anatomy of the Adrenal Gland of the Mouse, Including Neoplasms. J. Natl. Cancer Inst. 44: 1323-1389, 1970.

Marcopot, R. R. and L. C. Peterson. Spontaneous Proteus Nephritis Among Male C3H/HeJ Mice. Lab. Anim. Sci. 31: 697-700, 1981.

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09-24-82

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APPENDIX I

## Acknowledgements

| | |
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| | E. S. Kwasny |

TABLE 1

Frequency of Gross Findings Among C3H/HeJ Male Mice

Skin-Painted with AEROTEX® Givaual 40

| ORGANS/Findings | AEROTEX® Givaual 40 | Deionized Water (negative Control) |
|---|---|---|
| Total Number Examined Grossly | 40 | 40 |
| SKIN/SUBCUTIS, NCL | 35/40* | 39/40 |
| /Autolysis | 0/40 | 1/40 |
| /Surface alteration | 5/40 | 1/40 |
| THYROIDS, NCL | 39/40 | 40/40 |
| /Enlarged | 1/40 | 0/40 |
| ADRENALS, NCL | 19/40 | 22/40 |
| /Color change | 21/40 | 17/40 |
| /Enlarged | 2/40 | 0/40 |
| /Nodule/Mass | 1/40 | 3/40 |
| HEART, NCL | 38/40 | 40/40 |
| /Color change | 1/40 | 0/40 |
| /Enlarged | 1/40 | 0/40 |
| SPLEEN, NCL | 35/40 | 33/40 |
| /Color change | 1/40 | 2/40 |
| /Enlarged | 5/40 | 7/40 |
| MESENTERIC LYMPH NODE, NCL | 39/40 | 39/40 |
| /Color change | 1/40 | 1/40 |
| /Enlarged | 1/40 | 1/40 |
| RENAL LYMPH NODE, NCL | 39/40 | 40/40 |
| /Color change | 1/40 | 0/40 |
| /Enlarged | 1/40 | 0/40 |
| SUBLUMBAR LYMPH NODE, NCL | 40/40 | 39/40 |
| /Color change | 0/40 | 1/40 |
| /Enlarged | 0/40 | 1/40 |
| LUNGS, NCL | 30/40 | 28/40 |
| /Cannibalized | 0/40 | 1/40 |
| /Autolysis | 0/40 | 1/40 |
| /Color change | 9/40 | 10/40 |
| /Nodule/Mass | 1/40 | 2/40 |
| TESTES, NCL | 40/40 | 39/40 |
| /Smaller than normal | 0/40 | 1/40 |
| /Color change | 0/40 | 1/40 |
| SEMINAL VESICLES, NCL | 32/40 | 23/40 |
| /Autolysis | 0/40 | 7/40 |
| /Color change | 8/40 | 9/40 |
| /Enlarged/Firm/Mass | 8/40 | 9/40 |
| PREPUTIAL GLAND, NCL | 39/40 | 40/40 |
| /Enlarged | 1/40 | 0/40 |

(Continued)

TABLE 1
(Continued)

Frequency of Gross Findings Among C3H/HeJ Male Mice

Skin-Painted with AEROTEX® Glyoxal 40

| ORGANS/Findings | AEROTEX® Glyoxal 40 | Deoinized Water (Negative Control) |
|---|---|---|
| KIDNEYS, NGL | 29/40 | 20/40 |
| /Autolysis | 0/40 | 1/40 |
| /Cannibalized, partially | 0/40 | 1/40 |
| /Smaller than normal | 1/40 | 0/40 |
| /Cyst | 0/40 | 1/40 |
| /Color change | 9/40 | 14/40 |
| /Enlarged/Nodular | 3/40 | 3/40 |
| /Masses | 4/40 | 5/40 |
| URINARY BLADDER, NGL | 30/40 | 26/40 |
| /Autolysis | 0/40 | 2/40 |
| /Constriction | 0/40 | 1/40 |
| /Distended | 10/40 | 10/40 |
| /Color change | 0/40 | 2/40 |
| /Masses | 3/40 | 4/40 |
| LIVER, NGL | 26/40 | 22/40 |
| /Cannibalized, partially | 0/40 | 1/40 |
| /Autolysis | 1/40 | 6/40 |
| /Color change | 5/40 | 8/40 |
| /Enlarged/Nodular | 0/40 | 2/40 |
| /Mass | 3/40 | 4/40 |
| THORACIC CAVITY, NGL | 40/40 | 38/40 |
| /Fluid filled | 0/40 | 2/40 |
| ABDOMINAL CAVITY, NGL | 39/40 | 39/40 |
| /Fluid filled | 1/40 | 1/40 |
| FOOT, NGL | 40/40 | 39/40 |
| /Enlarged | 0/40 | 1/40 |

NGL = No gross lesions.

*Numerator equals number of mice with specified findings.
Denominator equals number of mice for which specified organ was examined.

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09-24-82

TABLE 2

Frequency of Histologic Findings Among C3H/Bai Male Mice

Skin-Painted with ARROTEX® Glycol 40

| ORGANS/Findings | ARROTEX® Glycol 40 | Deionized Water (Negative Control) |
|---|---|---|
| SKIN/SUBCUTIS, NEC. | 25/40* | 35/40 |
| /Dermatitis | 3/40 | 0/40 |
| /Ulcerative dermatitis | 5/40 | 3/40 |
| /Hyperkeratosis | 4/40 | 1/40 |
| /Adnexal atrophy | 1/40 | 0/40 |
| /Dermal fibrosis | 1/40 | 0/40 |
| /Epidermal necrosis | 10/40 | 0/40 |
| /Keratin necrosis | 0/40 | 1/40 |
| /Epidermal hyperplasia | 2/40 | 0/40 |
| PARATHYROIDS, NSL | 0/1 | - |
| /Parathyroid carcinoma | 1/1 | - |
| ADRENALS, NSL | 0/23 | 0/18 |
| /Autolysis | 1/23 | 0/18 |
| /Cortical cyst | 1/23 | 0/18 |
| /Cortical degeneration | 9/23 | 0/18 |
| /Cortical hyperplasia/Adenomas | 21/23 | 18/18 |
| /Pheochromocytoma | 2/23 | 0/18 |
| /Cortical carcinoma | 2/23 | 0/18 |
| HEART, NSL | 2/2 | - |
| SPLEEN, NSL | 1/4 | 0/7 |
| /Amyloidosis | 0/4 | 2/7 |
| /Extramedullary hematopoiesis | 2/4 | 7/7 |
| /Hemangiosarcoma | 1/4 | 0/7 |
| MESENTERIC LYMPH NODE, NSL | 0/1 | 0/1 |
| /Autolysis | 0/1 | 1/1 |
| /Mesenteric disease | 1/1 | 0/1 |
| SUBLUMBAR LYMPH NODE, NSL | - | 0/1 |
| /Plasmacytopoiesis | - | 1/1 |
| LUNGS, NSL | 5/9 | 1/11 |
| /Congestion | 3/9 | 8/11 |
| /Alveolar histiocytosis | 1/9 | 0/11 |
| /Pulmonary adenoma | 1/9 | 1/11 |
| /Pulmonary adenocarcinoma | 0/9 | 1/11 |
| /Hepatocellular carcinoma, metastatic | 0/9 | 1/11 |
| TESTES, NSL | 1/1 | 0/1 |
| /Mineralization | 0/1 | 1/1 |
| EPIDIDYMIDES, NSL | 2/2 | - |
| PROSTATE, NSL | 1/3 | 0/5 |
| /Inflammation | 0/3 | 2/5 |
| /Abscesses | 0/3 | 1/5 |
| /Prostatitis | 2/3 | 2/5 |

(Continued)

TABLE 2
(Continued)

Frequency of Histologic Findings Among C3H/HeJ Male Mice

Skin-Painted with AEROTEX® Glyoxal 40

| ORGANS/Findings | AEROTEX® Glyoxal 40 | Deoinized Water (Negative Control) |
|---|---|---|
| SEMINAL VESICLES | 8/9 | 4/8 |
| /Inflammation | 0/9 | 3/8 |
| /Seminal vesiculitis | 1/9 | 1/8 |
| PREPUTIAL GLAND, NSL | 1/2 | - |
| /Ulcerative dermatitis, skin | 1/2 | - |
| KIDNEYS, NSL | 4/12 | 1/19 |
| /Mineralization | 4/12 | 3/19 |
| /Interstitial nephritis | 1/12 | 2/19 |
| /Pyelonephritis | 3/12 | 13/19 |
| /Necropurulent nephritis | 2/12 | 0/19 |
| /Tubular proteinosis | 1/12 | 3/19 |
| /Interstitial fibrosis | 1/12 | 1/19 |
| /Tubular adenoma | 0/12 | 1/19 |
| /Malignant pheochromocytoma | 1/12 | 0/19 |
| URINARY BLADDER, NSL | 6/8 | 4/11 |
| /Autolysis | 0/8 | 3/11 |
| /Mineralization | 1/8 | 0/11 |
| /Cystitis | 1/8 | 3/11 |
| /Necrotic debris | 0/8 | 1/11 |
| /Epithelial necrosis | 0/8 | 1/11 |
| /Epithelial degeneration | 1/8 | 0/11 |
| LIVER, NSL | 3/17 | 6/12 |
| /Autolysis | 5/17 | 0/12 |
| /Congestion | 1/17 | 0/12 |
| /Vascular ectasia | 1/17 | 0/12 |
| /Amyloidosis | 0/17 | 1/12 |
| /Peliosis hepatis | 1/17 | 0/12 |
| /Hepatocellular carcinoma | 8/17 | 5/12 |
| EAR, NSL | 0/1 | - |
| /Otitis media | 1/1 | - |
| FOOT, NSL | 0/1 | 1/1 |
| /Hyperkeratosis | 1/1 | 0/1 |
| /Papillary hyperplasia | 1/1 | 0/1 |

NSL = No significant lesions.

*Numerator equals number of mice with specified lesion.
Denominator equals number of mice for which specified organ was examined.

WPC/esk/0269A-3
09-24-82

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APPENDIX I

Test No.: 23 Test Name: Dermal Carcinogenesis

Sample No.: 41-400 Species: Mouse Animal No.: 79-2946

Sample Name: ARCOTEX Glycerol 40 Strain: C3H/HeJ

Treatment Level: 25 ul Sex: Male Age: Approx. 8 Mos.

Date: X 9 8 79 Date of Death: 09-17-79

Study Designation No.: 79-15

## Clinical History

## Gross Findings

Date of Necropsy 09-17-79 Time 8:40 a.m.

Skin - No gross lesions.
Area around penis red, encrusted, scabby.
Urinary bladder - Greatly distended, containing approximately 3 cc yellow fluid.
Liver - Pale.
Kidneys - Swollen, pale.
Lungs - Mottled salmon-pink and maroon.
Spleen - Enlarged to measure 22 x 4 x 3 mm.
Intestines - Autolytic.
Heart - Possibly enlarged.
No other gross lesions.

Prosector LGP

## Microscopic Findings

Skin - No significant lesion.
Liver - No significant lesion.
Spleen - Extramedullary hematopoiesis, diffuse, moderate.
Kidneys - Tubular mineralization, bilateral, multifocal, moderate to marked.
Heart - No significant lesion.
Lungs - No significant lesion.
Skin around prepuce - Ulcerative dermatitis, focal, moderate to marked.
Preputial gland - No significant lesion.
Urinary bladder - No significant lesion.

Pathologist 12-04-81 Date

S = Spontaneous Death E = Euthanized Ei = Euthanized when moribund

WC/ml/0765-1; 12-08-81

29423 PATHOLOGY RECORD REPORT 45-508

Test No.: 23 Test Name: Dermal Carcinogenesis APPENDIX I

Sample No.: 41-400 Species: Mouse Animal No.: 79-2947

Sample Name: AEROTEX Glyoxal 40 Strain: C3H/HeJ

Treatment Level: 25 ul Sex: Male Age: 24 Months

Fate: X D ___ E ___ EM Date of Death: 01-11-81

Study Designation No.: 79-15

## Clinical History

## Gross Findings

Date of Necropsy 01-12-81 Time 2:29 p.m.

Skin - Slight flakiness on dosing area (dorsal).
Adrenals - Tan punctate foci on both.
Kidneys and Lungs - No gross lesions.
Liver - Mass right lateral lobe approximately 20 x 9 x 7 mm, irregular surface.
No other gross lesions.

Prosector HLZ

## Microscopic Findings

Skin - Dermatitis, chronic, focal, moderate.
Epidermal necrosis, focal, mild.
Adrenals (only one adrenal present) - Cortical hyperplasia, focal, moderate.
Liver - Hepatocellular carcinoma, moderately differentiated, moderately large.

E Ftowler
Pathologist

12-04-81
Date

D = Spontaneous Death E = Euthanized EM = Euthanized when moribund

WPC/esk/0176A-1; 12-08-81

29424 [illegible] REPORT 45-508
APPENDIX I

Test No.: 33 Test Name: Dermal Carcinogenesis

Sample No.: 41-409 Species: Mouse Animal No.: 79-2948

Sample Name: AEROTEX Chrysal 40 Strain: C3H/HeJ

Treatment Level: 25 ul Sex: Male Age: Approx. 8 Mos.

Fate: X D ___ E ___ ME Date of Death: 10-01-79

Study Designation No.: 79-15

## Clinical History

## Gross Findings

Date of Necropsy 10-01-79 Time 3:30 p.m.

Skin and Urinary bladder - No gross lesions.
Kidneys - Bilaterally affected, patches of cream and tan discoloration on surfaces.
Accessory sex glands - Slightly enlarged, approximately 15 x 5 x 3 mm, yellow and red.
Liver - Autolyzed.
No gross lesions.
Lungs - Mottled pink to dark red. Some surfaces almost entirely dark red. This discoloration is still visible after inflation with fixative.
No other gross lesions.

Prosector JHC

## Microscopic Findings

Skin - Epidermal necrosis, focal, moderate.
Kidneys - Pyelonephritis, suppurative, bilateral, multifocal, moderate to marked.
Pelvic mineralization, unilateral, moderate.
Seminal vesicles - No significant lesion.
Epididymis - No significant lesion.
Lungs - No significant lesion.

Pathologist 12-04-81 Date

S = Spontaneous Death E = Euthanized ME = Euthanized when moribund

HJC/eak/01/6A-1; 12-08-81

29425 PATHOLOGY RECORD REPORT 45-508

APPENDIX I

Test No.: 23 Test Name: Dermal Carcinogenesis

Sample No.: 41-400 Species: Mouse Animal No.: 79-2949

Sample Name: AEROTEX® Glycerol 40 Strain: C3H/HeJ

Treatment Level: 25 ul Sex: Male Age: Approx. 11 Mos.

Fate: X D ___ E ___ EM Date of Death: 10-14-79

Study Designation No.: 79-15

## Clinical History

## Gross Findings

Date of Necropsy 10-15-79 Time 9:00 a.m.

Skin - Autolytic.
No gross lesions.
Feet and nose appear cherry red.
Penis is exposed, hard, red and black in color.
Urinary bladder - Distended, containing approximately 2 cc of dark red fluid with a black mass measuring approximately 2 mm in diameter contained within.
Kidneys - No gross lesions.
Liver - Soft to the touch.
No gross lesions.
Lungs - Mottled, dark black to red. Left lung still appears dark when inflated with fixative. Other lobes lost dark color.
Heart - Appears very dark in color.
No other gross lesions.

Prosector DRM

## Microscopic Findings

Skin - No significant lesion.
Kidneys - No significant lesion.
Heart - No significant lesion.
Lungs - No significant lesion.

Pathologist 12-04-81 Date

D = Spontaneous Death E = Euthanized EM = Euthanized when moribund

WPC/esk/0176A-1; 12-08-81

29426 LABORATORY REPORT REPORT 45-508

APPENDIX I

Test No.: 21 - Test Name: Dermal Carcinogenesis

Sample No.: 41-400 Species: Mouse Animal No.: 79-2950

Sample Name: AEROTEX® Glycosal 40 (U.S.) Strain: C3H/HeJ

Treatment Level: 25 µl Sex: Male Age: 18 months

Fate: X D ___ E ___ EM Date of Death: 8-6-79

Study Designation No.: 79-15

## Clinical History

## Gross Findings

Date of Necropsy 8-6-79 Time 1:50 PM

Skin - No gross lesions.
Lungs - No gross lesions.
Urinary bladder - Full of light yellow fluid. Also contained white masses compatible with protein concretion.
Spleen - Enlarged (approximately 20 x 5 x 3 mm) and red.
Liver - No gross lesions.
Kidneys - Bilaterally enlarged. Confluent and punctate white masses on surfaces (possible nephritis).
Accessory sex organs - No gross lesions.

No other gross lesions.

Prosector DR Meckley

## Microscopic Findings

Skin - Mild hyperkeratosis.
Seminal vesicles - Mild purulent seminal vesiculitis.
Prostate - Mild purulent prostatitis.
Epididymis, Testes - No significant lesions.
Urinary bladder - Marked purulent cystitis with associated bacterial colonies.
Kidneys - Moderate necropurulent nephritis with associated bacterial colonies.

RR Yancey 8-27-79
Pathologist Date sai

D = Spontaneous Death E = Euthanized EM = Euthanized when moribund

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REPORT 45-508
APPENDIX I

29427

# PATHOLOGY RECORD

Test No.: 23 Test Name: Dermal Carcinogenesis

Sample No.: 41-400 Species: Mouse Animal No.: 79-3016

Sample Name: AEROTEX® Glyoxal 40 Strain: C3H/HeJ

Treatment Level: 25 ul Sex: Male Age: Approx. 16 Mos.

Fate: X D ___ E ___ EM Date of Death: 07-11-80

Study Designation No.: 79-15

## Clinical History

## Gross Findings

Date of Necropsy 07-11-80 Time 11:20 a.m.

Skin, Kidneys, and Lungs - No gross lesions.
Abdominal cavity appears filled with blood.
Liver - All lobes appear light tan in color with areas of dark red on right median lobe.
No other gross lesions.

Prosector RAB

## Microscopic Findings

Skin - Dermatitis and epidermal necrosis, focal, moderate.
Liver - Autolysis, superficial, moderate.
No apparent significant lesion.

Pathologist 12-04-81 Date

D = Spontaneous Death E = Euthanized EM = Euthanized when moribund

WPC/esk/0176A-1; 12-08-81

29428 REPORT 45-508

APPENDIX I

Test No.: 23 Test Name: Dermal Carcinogenesis

Sample No.: 41-400 Species: Mouse Animal No.: 79-3017

Sample Name: AEROTEX® Glyoxal 40 Strain: C3H/HeJ

Treatment Level: 25 ul Sex: Male Age: Approx. 26 Mos.

Fate: X D ___ E ___ Mi Date of Death: 03-27-81

Study Designation No.: 79-15

## Clinical History

## Gross Findings

Date of Necropsy 03-27-81 Time 11:05 a.m.

Skin - No gross lesions.
Liver - All lobes are dark.
Adrenals - Both are dark with creamy-tan punctate foci on all surfaces.
Kidneys - Both are dark.
Lungs - All lobes are mottled, pink, dark and maroon in color. Outer margin of right lung is green in color. Color remains upon inflation with fixative.
No other gross lesions.

Prosector DRM

## Microscopic Findings

Skin - Ulcerative dermatitis, focal, moderate.
Epidermal necrosis and hyperkeratosis, focal, moderate.
Adrenals - Cortical adenomas, bilateral, multifocal, marked.
Lungs - No significant lesion.
Liver - Autolysis, superficial, moderate.
Kidneys - Autolysis, moderate.
No apparent significant lesion.

Pathologist 12-04-81 Date

D = Spontaneous Death E = Euthanized Mi = Euthanized when moribund

WPC/eak/0176A-1; 12-08-81

# PATHOLOGY RECORD

Test No.: 23 Test Name: Dermal Carcinogenesis

Sample No.: 41-400 Species: Mouse Animal No.: 79-3018

Sample Name: AEROTEX® Oyo.al 40 Strain: C3H/HeJ

Treatment Level: 25 ul Sex: Male Age: 22 Months

Fate: X D ___ E ___ EM Date of Death: 11-20-80

Study Designation No.: 79-15

## Clinical History

## Gross Findings

Date of Necropsy 11-20-80 Time 1:20 p.m.

Skin - No gross lesions.
Adrenals - Singular and confluent foci (tan) present.
No other gross lesions.

Prosector RAB

## Micrcscopic Findings

Skin - No significant lesion.
Adrenals - Cortical adenomas, bilateral, multifocal, marked.

Pathologist 12-04-81 Date

D = Spontaneous Death E = Euthanized EM = Euthanized when moribund

WPC/esk/0176A-1; 12-08-81

REPORT 45-508
APPENDIX I

# 29235 ANATOMIC PATHOLOGY REPORT

## DERMAL CARCINOGENESIS LIFETIME SKIN PAINTING IN MICE

## OF

## EUROPEAN GLYOXAL-40

Table of Contents



NOTE: This Pathology Report begins on page 29235 and ends on page 29323.

29236

## Summary and Conclusions

Male C3H/HeJ mice were randomized into groups of 40 and treated three times weekly (holidays excepted) throughout their lifetime with European glyoxal-40 which was applied to the clipped skin of their backs. A negative control group received similar treatments with deionized water alone.

No skin neoplasms were found in the European glyoxal-40-treated mice or in the deionized water-treated control mice. A fibrosarcoma was diagnosed on the ventral thorax of a European glyoxal-40-treated mouse, but this is not considered biologically important as tumors of this type are occasionally encountered in control mice.

Other gross and histologic lesions found in these mice were considered part of the background of spontaneous lesions found in this strain of mice used in lifetime studies.

## Introduction

The purpose of this study was to determine the dermal neoplastic potential of European glyoxal-40 by applying it to the skin of male C3H/HeJ mice over the period of their lifetime and determining the gross and microscopic appearance of the resulting lesions.

## Methods

Male C3H/HeJ mice, 4-6 weeks of age were obtained from Jackson Laboratories, Bar Harbor, ME. The mice were randomized into groups of 40 animals. Each group of 40 male mice received either the test chemical or a control material three times per week, with the exception of holidays, applied by an Eppendorf pipette to the clipped skin of the back. The application of test chemical or control material was continued throughout the lifetime of the mice.

Following the death of each mouse a gross necropsy was performed. All body cavities were examined and all suspect internal tumors were fixed in 10% neutral buffered formalin (NBF) unless the degree of autolysis precluded saving the tissues for histologic examination. The dorsal skin of all mice, with or without skin tumors, was also fixed in 10% NBF for histologic examination unless the mouse was severely autolyzed. In addition, as of November 11, 1980, the remainder of each mouse was fixed in 10% NBF and saved at the request of the sponsor.

Tissues fixed in 10% NBF were carefully trimmed, embedded, sectioned and stained with hematoxylin and eosin for examination by a pathologist. All neoplastic and non-neoplastic lesions discovered during the histopathologic examination were recorded and tabulated.

29237

## Results and Discussion

The frequency of gross lesions is included in Table 1. A nodule was tentatively diagnosed on the skin of one mouse receiving the European glyoxal-40 treatments. Skin surface alterations were present in a few of the mice receiving European glyoxal-40 and in only one mouse receiving the deionized water. A subcutaneous mass was present over the sternal area extending from one axillary area to the other and up the ventral cervical area in one of the European glyoxal-40-treated mice.

The gross lesions encountered in the other organs were all considered part of the spontaneous background lesions seen in mice of this strain used on lifetime studies.

The histologic findings are included in Table 2. The skin nodule identified grossly in the European glyoxal-40-treated mouse was found not to be a significant lesion. The subcutaneous mass in the ventral thoracic and cervical area of another European glyoxal-40-treated mouse was diagnosed as a fibrosarcoma. Fibrosarcomas have occasionally been found in control mice of this strain and therefore cannot be considered biologically important when an occasional one is encountered.

European-glyoxal-40 caused some mild reaction in the skin as evidenced from the appearance of hyperkeratosis and epidermal necrosis in a few of the mice.

Other histological lesions encountered in these mice, including the neoplasms, were not considered of biological importance as they are part of the background of spontaneous neoplastic or inflammatory conditions found in this strain in lifetime studies.

The histologic changes observed in the adrenals reflect the gross observations, but it should be remembered that only adrenals with lesions observed at necropsy were examined histologically. Cortical hyperplasia/adenoma appeared in nearly all adrenals examined. Dunn (1970) considered the distinction between hyperplasia and neoplasia in mouse adrenals to be very difficult and somewhat arbitrary.

Lesions seen in the male urogenital system are primarily related to the Proteus mirabilis infection which is frequently encountered in this strain from this vendor (Marompot and Peterson, 1981).

Liver neoplasms are frequently encountered in this strain and therefore no significance can be attributed to their appearance in the European-glyoxal-40-treated or control mice.

Edward H. Fowler 8/20/82

Edward H. Fowler, DVM, Ph.D. — Date
Pathologist

WPC/esk/0311A-2
06-07-82

0104

29238

## References

Dunn, T. B. Normal and Pathologic Anatomy of the Adrenal Gland of the Mouse, Including Neoplasms. J. Natl. Cancer Inst. 44: 1323-1389, 1970.

Maronpot, R. R. and L. G. Peterson. Spontaneous Proteus Nephritis Among Male C3H/HeJ Mice. Lab. Anim. Sci. 31: 697-700, 1981.

WPC/esk/0311A-2
06-07-82

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APPENDIX I

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## Acknowledgements

| | |
|---|---|
| Pathologist | E. H. Fowler, DVM; Ph.D. EHF |
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| Secretaries | M. Dubich MD |
| | R. K. Kephart RKK |
| | E. S. Kwasny ESK |

TABLE 1

Frequency of Gross Findings Among C3H/HeJ Male Mice

Skin-Painted with European Glyoxal-40

| ORGANS/Findings | European Glyoxal-40 | Deionized Water (Negative Control) |
|---|---|---|
| TOTAL NUMBER EXAMINED GROSSLY | 39 | 40 |
| SKIN/SUBCUTIS, NGL | 34/39* | 39/40 |
| /Autolysis | 0/39 | 1/40 |
| /Surface alteration | 4/39 | 1/40 |
| /Nodule(s) | 1/39 | 0/40 |
| ADRENALS, NGL | 13/38 | 22/40 |
| /Color change | 25/38 | 17/40 |
| /Cyst | 1/38 | 0/40 |
| /Enlarged | 2/38 | 0/40 |
| /Nodule/Mass | 1/38 | 3/40 |
| SPLEEN, NGL | 34/38 | 33/40 |
| /Color change | 2/38 | 2/40 |
| /Enlarged | 4/38 | 7/40 |
| MEDIASTINAL TISSUE/THYMUS, NGL | 37/38 | 40/40 |
| /Color change | 1/38 | 0/40 |
| /Enlarged | 1/38 | 0/40 |
| MESENTERIC LYMPH NODE, NGL | 36/38 | 39/40 |
| /Color change | 2/38 | 1/40 |
| /Enlarged/Mass | 2/38 | 1/40 |
| SUBLUMBAR LYMPH NODE, NGL | 38/38 | 39/40 |
| /Color change | 0/38 | 1/40 |
| /Enlarged | 0/38 | 1/40 |
| LUNGS, NGL | 35/38 | 28/40 |
| /Cannibalized | 0/38 | 1/40 |
| /Autolysis | 0/38 | 1/40 |
| /Color change | 3/38 | 10/40 |
| /Nodule/Mass | 0/38 | 2/40 |
| TESTES, NGL | 38/38 | 39/40 |
| /Smaller than normal | 0/38 | 1/40 |
| /Color change | 0/38 | 1/40 |
| SEMINAL VESICLES, NGL | 34/38 | 23/40 |
| /Autolysis | 1/38 | 7/40 |
| /Color change | 4/38 | 9/40 |
| /Enlarged/Firm/Mass | 4/38 | 9/40 |
| KIDNEYS, NGL | 28/38 | 20/40 |
| /Cannibalized, partially | 0/38 | 1/40 |
| /Autolysis | 0/38 | 1/40 |
| /Cyst | 0/38 | 1/40 |
| /Color change | 8/38 | 14/40 |
| /Enlarged/Firm/Nodular | 2/38 | 3/40 |
| /Masses | 2/38 | 5/40 |

(Continued)

29241

TABLE 1
(Continued)

Frequency of Gross Findings Among C3H/HeJ Male Mice

Skin-Painted with European Glyoxal 40

| ORGANS/Findings | European Glyoxal-40 | Deionized Water (Negative Control) |
|---|---|---|
| URINARY BLADDER, NGL | 26/38 | 26/40 |
| /Autolysis | 1/38 | 2/40 |
| /Constriction | 2/38 | 3/40 |
| /Distended | 1/38 | 10/40 |
| /Color change | 1/38 | 2/40 |
| /Granular concretion | 1/38 | 0/40 |
| /Masses | 2/38 | 4/40 |
| LIVER, NGL | 27/38 | 22/40 |
| /Cannibalized, partially | 0/38 | 1/40 |
| /Autolysis | 0/38 | 6/40 |
| /Color change | 1/38 | 8/40 |
| /Enlarged/Nodular | 4/38 | 2/40 |
| /Mass | 6/38 | 4/40 |
| PANCREAS, NGL | 37/38 | 40/40 |
| /Mass | 1/38 | 0/40 |
| RIB CAGE/AXILLARY AREA, NGL | 37/38 | 40/40 |
| /Mass | 1/38 | 0/40 |
| THORACIC CAVITY, NGL | 38/38 | 38/40 |
| /Fluid filled | 0/38 | 2/40 |
| ABDOMINAL CAVITY, NGL | 38/38 | 39/40 |
| /Fluid filled | 0/38 | 1/40 |
| FOOT, NGL | 37/38 | 39/40 |
| /Swollen/Enlarged | 1/38 | 1/40 |

NGL = No gross lesions.

*Numerator equals number of mice with specified finding.
Denominator equals number of mice for which specified organ was examined.

WPC/esk/0311A-1
06-07-82

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TABLE 2

Frequency of Histologic Findings Among C3H/HeJ Male Mice

Skin-Painted with European Glyoxal-40

| ORGANS/Findings | European Glyoxal-40 | Deionized Water (Negative Control) |
|---|---|---|
| SKIN/SUBCUTIS, NSL | 31/39* | 35/40 |
| /Ulcerative dermatitis | 0/39 | 3/40 |
| /Hyperkeratosis | 6/39 | 1/40 |
| /Keratin necrosis | 0/39 | 1/40 |
| /Epidermal/Dermal necrosis | 2/39 | 0/40 |
| /Dermal fibrosis | 2/39 | 0/40 |
| ADRENALS, NSL | 1/26 | 0/18 |
| /Cortical cyst | 1/26 | 0/18 |
| /Brown degeneration | 16/26 | 0/18 |
| /Cortical hyperplasia/Adenoma | 25/26 | 18/18 |
| /Cortical carcinoma | 1/26 | 0/18 |
| SPLEEN, NSL | 0/4 | 0/7 |
| /Amyloidosis | 1/4 | 2/7 |
| /Extramedullary hematopoiesis | 1/4 | 7/7 |
| /Lymphoid hyperplasia | 1/4 | 0/7 |
| /Reticulum cell sarcoma, type B | 1/4 | 0/7 |
| MEDIASTINAL TISSUE, NSL | 0/1 | - |
| /Hemangiosarcoma | 1/1 | - |
| MESENTERIC LYMPH NODE, NSL | 0/2 | 0/1 |
| /Autolysis | 0/2 | 1/1 |
| /Mesenteric disease | 1/2 | 0/1 |
| /Plasmacytoma | 1/2 | 0/1 |
| PANCREATIC LYMPH NODE, NSL | 0/1 | - |
| /Reticulum cell sarcoma, type A | 1/1 | - |
| SUBLUMBAR LYMPH NODE, NSL | - | 0/1 |
| /Plasmacytopoiesis | - | 1/1 |
| LUNGS, NSL | 1/4 | 1/11 |
| /Congestion | 2/4 | 8/11 |
| /Alveolar hemorrhage | 1/4 | 0/11 |
| /Pulmonary adenoma | 1/4 | 1/11 |
| /Pulmonary adenocarcinoma | 0/4 | 1/11 |
| /Hepatocellular carcinoma, metastatic | 0/4 | 1/11 |
| TESTES, NSL | - | 0/1 |
| /Mineralization | - | 1/1 |
| PROSTATE, NSL | 0/1 | 0/5 |
| /Inflammation | 0/1 | 2/5 |
| /Abscess | 0/1 | 1/5 |
| /Prostatitis | 1/1 | 2/5 |
| SEMINAL VESICLES, NSL | 4/4 | 4/8 |
| /Inflammation | 0/4 | 3/8 |
| /Seminal vesiculitis | 0/4 | 1/8 |

(Continued)

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TABLE 2
(Continued)

Frequency of Histologic Findings Among C3H/HeJ Male Mice

Skin-Painted with European Glyoxal-40

| ORGANS/Findings | European Glyoxal-40 | Deionized Water (Negative Control) |
|---|---|---|
| COAGULATING GLAND, NSL | 1/1 | - |
| KIDNEYS, NSL | 1/11 | 1/19 |
| /Mineralization/Tubular calcification | 2/11 | 3/19 |
| /Interstitial nephritis | 1/11 | 2/19 |
| /Pyelonephritis | 6/11 | 13/19 |
| /Necropurulent nephritis | 1/11 | 0/19 |
| /Tubular proteinosis | 0/11 | 3/19 |
| /Interstitial fibrosis | 0/11 | 1/19 |
| /Tubular hyperplasia | 1/11 | 0/19 |
| /Tubular adenoma | 0/11 | 1/19 |
| /Reticulum cell sarcomas, metastatic | 1/11 | 0/19 |
| URINARY BLADDER, NSL | 6/10 | 4/11 |
| /Autolysis | 5/10 | 3/11 |
| /Cystitis | 1/10 | 3/11 |
| /Necrotic debris | 0/10 | 1/11 |
| /Epithelial necrosis | 0/10 | 1/11 |
| /Transitional cell carcinoma | 1/10 | 0/11 |
| LIVER, NSL | 1/12 | 6/12 |
| /Mineralization | 1/12 | 0/12 |
| /Amyloidosis | 0/12 | 1/12 |
| /Thrombosis | 1/12 | 0/12 |
| /Infarction/Hemorrhage | 3/12 | 0/12 |
| /Coagulation necrosis | 2/12 | 0/12 |
| /Hepatocellular pleomorphism | 1/12 | 0/12 |
| /Hepatocellular degeneration | 1/12 | 0/12 |
| /Hepatocellular adenoma | 1/12 | 0/12 |
| /Hepatocellular carcinoma | 6/12 | 5/12 |
| RIB CAGE/AXILLA, NSL | 0/1 | - |
| /Fibrosarcoma | 1/1 | - |
| FOOT, NSL | 0/1 | 1/1 |
| /Pododermatitis | 1/1 | 0/1 |

NSL - No significant lesions.

*Numerator equals number of mice with specified finding.
Denominator equals number of mice for which specified organ was examined.

WPC/esk/0311A-2
06-07-82

REPORT 45-508
APPENDIX I

# PATHOLOGY RECORD

28244

Test No.: 23 Test Name: Dermal Carcinogenesis

Sample No.: 41-629 Species: Mouse Animal No.: 79-3286

Sample Name: GLYCOKAL 40 (European Process) Strain: C3H/HeJ

Treatment Level: 25 ul Sex: Male Age: Approx. 12 Mos.

Fate: X D ___ E ___ EM Date of Death: 12-22-79

Study Designation No.: 79-16

## Clinical History

## Gross Findings

Date of Necropsy 12-23-79 Time 2:45 p.m.

Internal organs too autolytic to note any gross lesion or cause.
No other gross lesions.

Prosector DRM

## Microscopic Findings

Skin - No significant lesion.

Pathologist 02-11-82 Date

D = Spontaneous Death E - Euthanized EM = Euthanized when moribund

UPC/esk/0266A-1; 02-22-82

REPORT 45-508
APPENDIX I

# 29323 PATHOLOGY RECORD

Test No.: 23 Test Name: Dermal Carcinogenesis

Sample No.: Fresh Species: Mouse Animal No.: 79-4600

Sample Name: Deionized Water Strain: C3H/HeJ

Treatment Level: 25 ul Sex: Male Age: Approx. 14 Mos.

Note: X D E EM Date of Death: 03-19-80

Study Designation No.: 79-34

## Clinical History

## Gross Findings

Date of Necropsy 03-19-80 Time 1:25 p.m.

Skin and Lungs - No gross lesions.
Spleen - Enlarged to 20 x 6 x 4 mm and dark.
Liver - Dark.
Kidneys - Both kidneys affected by singular, punctate, slightly raised dots on all surfaces. These dots are tan in color.
Seminal vesicles - Right has a yellow mass inside near junction with left seminal vesicle measuring 5 x 5 x 6 mm.
Lumbar lymph node - Right is enlarged to 4 x 7 x 3 mm and dark. Left appears grossly normal.
No other gross lesions.

Prosector DRM

## Microscopic Findings

Skin - No significant lesion.
Liver - No significant lesion.
Spleen - Extramedullary hematopoiesis, diffuse, moderate.
Kidneys - Pyelonephritis, subacute, bilateral, multifocal, severe.
Prostate - Prostatitis, suppurative, diffuse, marked.
Lumbar lymph node - Plasmacytopoiesis, reactive, diffuse, marked.

C. H. Fowler
Pathologist

10-05-81
Date

D = Spontaneous Death E = Euthanized EM = Euthanized when moribund

WPC/esk/0128A-1; 10-16-81

# Appendix II

## Analytical Data

# CARNEGIE-MELLON INSTITUTE OF RESEARCH

CHEMICAL HYGIENE FELLOWSHIP
Bushy Run Laboratories

Mailing Address:
4400 Fifth Avenue
Pittsburgh, Pennsylvania 15213
(412) 327-1020

March 14, 1979

Dr. R. B. Toothill
American Cyanamid Company
Bound Brook, New Jersey 08805

Dear Dr. Toothill:

As per the protocols for the lifetime dermal carcinogenesis testing of Aerotex Glyoxal 40 and European Glyoxal 40 I am shipping to you, under separate cover, 40 ml of a 1:8 dilution of Aerotex Glyoxal 40 in deionized water, 40 ml of a 1:8 dilution of European Glyoxal 40 in deionized water, and a sample of the deionized water used to prepare the dilutions. These samples should be analyzed for content of test agent upon their arrival, after one week, two weeks, and one month. Please inform us of the results of these analyses as well as the pre-shipment analyses of the parent material. The CHF will also need a statement concerning the analytical methodology employed.

If you have any questions concerning this please contact me at the above telephone number.

Sincerely,

L. G. Peterson
Research Technologist

LGP:acc

CC: D. R. Brown - American Cyanamid
L. R. DePass - CHF
C. S. Weil - CHF

CYANAMID

AMERICAN CYANAMID COMPANY
DYES AND CHEMICALS DEPARTMENT
P. O. BOX 10536, CHARLOTTE, NO. CAROLINA 28237
AREA CODE 704 394-4361

April 6, 19[illegible]

Mr. L. G. Peterson
Carnegie-Mellon Institute of Research
4400 Fifth Ave.
Pittsburg, Pa. 15213

Dear Mr. Peterson:

As you requested the enclosed table gives the first two assays of the samples of glyoxal which we received from you 3/28/79.

The acid, glyoxal and formaldehyde are assayed by acid-base titrations. The glycolaldehyde and ethylene glycol are assayed by gas chromatography. A copy of the assay procedure can be furnished on request.

Yours very truly,

AMERICAN CYANAMID COMPANY
COLOR, TEXTILE AND INTERMEDIATE CHEMICALS DEPARTMENT

Rita S. Farmer
Rita S. Farmer
Chemist

RSF/mp

Attachment

CENTRAL FILE



CYANAMID

AMERICAN CYANAMID COMPANY
DYES AND CHEMICALS DEPARTMENT
P. O. BOX 10538, CHARLOTTE, NO. CAROLINA 28237
AREA CODE 704 394-4861

April 27, 1979

Aerotex Glyoxal 40

Mr. L. G. Peterson
Carnegie-Mellon Institute of Research
4400 Fifth Ave.
Pittsburg, Pa. 15213

Dear Mr. Peterson:

As you requested the enclosed table includes the last two assays of the samples of glyoxal which we received from you 3/28/79.

Yours very truly,

AMERICAN CYANAMID COMPANY
COLOR, TEXTILE AND INTERMEDIATE CHEMICALS DEPARTMENT

Rita S. Farmer

Rita S. Farmer
Chemist

RSF/ps

Attachment

# CARNEGIE-MELLON INSTITUTE OF RESEARCH

CHEMICAL HYGIENE FELLOWSHIP
Bushy Run Laboratories

Mailing Address:
4400 Fifth Avenue
Pittsburgh, Pennsylvania 15213
(412) 327-1020

June 26, 1979

Dr. Steve Haworth
EG&G Mason Research Institute
1530 Jefferson Street
Rockville, Maryland 20852

Dear Dr. Haworth:

As per your request, a 150 ml sample of both AEROTEX® Glyoxal 40 (R9195-160) and European Glyoxal 40 (R9193-65-1) is being sent to you via motor freight.

Sincerely,

Daniel R. Meckley

Daniel R. Meckley
Technologist

DRM/bjw

cc: D. R. Brown, Amer. Cyn.
L. R. DePass, CMU
W. A. Fead, Amer. Cyn.
J. L. Kozinski, Amer. Cyn.
L. C. Peterson, CMU
R. B. Toothill, Amer. Cyn.

CYANAMID

AMERICAN CYANAMID COMPANY
DYES AND CHEMICALS DEPARTMENT
P. O. BOX 31707 CHARLOTTE, NO. CAROLINA 28232
AREA CODE 704 394-4361

RECEIVED
JAN 24 1980
L. G. PETERSON

JANUARY 21, 1980

MR. L. G. PETERSON
CARNEGIE-MELLON INSTITUTE OF RESEARCH
4400 FIFTH AVENUE
PITTSBURG, PA. 15213

DEAR MR. PETERSON:

AS YOU REQUESTED THE ENCLOSED TABLE INCLUDES ALL OF THE ASSAYS OF THE SAMPLES OF GLYOXAL WHICH WE RECEIVED FROM YOU 12/18/79.

YOURS VERY TRULY,

AMERICAN CYANAMID COMPANY
TEXTILES & INTERMEDIATE CHEMICALS DEPARTMENT

Rita S. Farmer

RITA S. FARMER
CHEMIST

RSF:CME

ENCLOSURE

CYANAMID

AMERICAN CYANAMID COMPANY
P. O. BOX 32787. CHARLOTTE, NO. CAROLINA 28232
AREA CODE 704 394-4261

May 1, 1980

Mr. L. G. Peterson
Carnegie-Mellon Institute of Research
4400 Fifth Avenue
Pittsburg, Pennsylvania 15213

Dear Mr. Peterson:

As you requested, the enclosed table includes all of the assays of the samples of glyoxal which we received from you 4/1/80.

Yours very truly,

AMERICAN CYANAMID COMPANY
INTERMEDIATE & TEXTILE CHEM. DEPT.

Rita S. Farmer

Rita S. Farmer
Chemist

RSF:aw

Enclosure

## ASSAYS OF GLYOXAL

| | Expected | | | Found | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 3-28-79 | | | 4-4-79 | | |
| | Deionized Water 79-34 | U.S. Glyoxal 79-15 41-400 | EUR Glyoxal 79-16 41-429 | Deionized Water 79-34 | U.S. Glyoxal 79-15 41-400 | EUR Glyoxal 79-16 41-429 | Deionized Water 79-34 | U.S. Glyoxal 79-15 41-400 | EUR Glyoxal 79-16 41-429 |
| Acid | 0.00 | 0.08 | 0.01 | 0.00 | 0.06 | 0.00 | 0.00 | 0.07 | 0.01 |
| Glyoxal | 0.00 | 4.44 | 4.49 | 0.00 | 5.13 | 4.86 | 0.00 | 4.97 | 4.64 |
| Formaldehyde | 0.00 | 0.66 | 0.08 | 0.14 | 0.65 | 0.17 | 0.07 | 0.68 | 0.08 |
| Glycolaldehyde | 0.00 | 0.03 | 0.002 | 0.00 | 0.10 [a] | 0.08 [a] | 0.00 | 0.12 [a] | 0.09 [a] |
| Ethylene glycol | 0.00 | 0.10 | - | 0.00 | 0.07 | 0.00 | 0.00 | 0.07 | 0.00 |

Probable inaccuracies due to dilution of samples and sensitivity of gas chromatograph used.

## ASSAYS OF GLYOXAL

| | Expected | | | Found | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 4-11-79 | | | 4-25-79 | | |
| | Deionized Water 79-34 | U. S. Glyoxal 79-15 41-400 | EUR Glyoxal 79-16 41-429 | Deionized Water 79-34 | U. S. Glyoxal 79-15 41-400 | EUR Glyoxal 79-16 41-429 | Deionized Water 79-34 | U. S. Glyoxal 79-15 41-400 | EUR Glyoxal 79-16 41-429 |
| Acid as Acetic | 0.00 | 0.08 | 0.01 | 0.00 | 0.05 | 0.01 | 0.00 | 0.04 | 0.00 |
| Glyoxal | 0.00 | 4.44 | 4.49 | 0.00 | 4.91 | 4.69 | 0.00 | 5.10 | 4.78 |
| Formaldehyde | 0.00 | 0.66 | 0.08 | 0.00 | 0.80 | 0.09 | 0.00 | 0.71 | 0.14 |
| Glycolaldehyde | 0.00 | 0.03 | 0.002 | 0.00 | 0.11* | 0.10* | 0.00 | 0.10* | 0.08* |
| Ethylene glycol | 0.00 | 0.10 | — | TRACE | 0.09 | Trace | 0.00 | 0.08 | 0.00 |

* Probable inaccuracies due to dilution of samples and sensitivity of gas chromatograph used.

# ASSAYS OF GLYOXAL#1

| | | EXPECTED | 12-19-79 | 12-26-79 | 1-8-80 | 1-16-80 #3 |
|---|---|---|---|---|---|---|
| EUROPEAN GLYOXAL 40<br>CHF SAMPLE #41-429<br><br>UNDILUTED, AS RECEIVED<br>30 ML<br>DATE SHIPPED 11/26/79 | % ACID (CALC. AS ACETIC) | 0.1 | 0.25 | 0.20 | 0.26 | 0.23 |
| | % GLYOXAL | 40.4 | 40.36 | 40.40 | 40.27 | 40.21 |
| | % FORMALDEHYDE | 0.7 | 0.56(0.20) #2 | 0.77(0.39) | 0.71(0.34) | 0.60(0.24) |
| | % GLYCOLALDEHYDE | 0.2 | 0.19 | 0.13 | 0.14 | 0.19 |
| | % ETHYLENE GLYCOL | TR. | 0.00 | 0.00 | 0.00 | 0.00 |
| | | | | | | |
| AEROTEX GLYOXAL 40 (US)<br>CHF SAMPLE #41-400<br><br>UNDILUTED, AS RECEIVED<br>30 ML<br>DATE SHIPPED 11/26/79 | % ACID (CALC. AS ACETIC) | 0.7 | 0.75 | 0.69 | 0.72 | 0.73 |
| | % GLYOXAL | 40.0 | 39.65 | 40.08 | 39.78 | 39.54 |
| | % FORMALDEHYDE | 5.9 | 6.17(6.07) | 6.28(5.83) | 6.54(5.97) | 6.05(5.66) |
| | % GLYCOLALDEHYDE | 0.3 | 0.27 | 0.21 | 0.27 | 0.27 |
| | % ETHYLENE GLYCOL | 0.9 | 0.95 | 0.75 | 0.90 | 0.81 |
| | | | | | | |

#1 ALL ASSAYS AVERAGE OF 2 BY R. S. FARMER OR G. L. WILKES

#2 ALL NUMBERS IN PARENTHESES CORRECTED BY BLANK

#3 EUROPEAN GLYOXAL 40 PRECIPITATE NOTICED

## ASSAYS OF GLYOXAL *1

| | | EXPECTED | 4-2-80 | 4-9-80*3 | 4-16-80 | 4-30-80 |
|---|---|---|---|---|---|---|
| European Glyoxal 40<br>R-9514-43<br>CHF Sample No. 41-429<br>50 ml Undiluted (as received)<br>Sample Drawn:3-5-80 | % Acid (Calc. As Acetic) | 0.1 | 0.30 | 0.24 | 0.19 | 0.21 |
| | % Glyoxal | 40.4 | 39.74 | 40.13 | 39.96 | 39.78 |
| | % Formaldehyde | 0.7 | 0.40 (0.03)*2 | 0.64 (0.00) | 0.59 (0.11) | 0.46 (0.00) |
| | % Glycolaldehyde | 0.2 | 0.26 | 0.33 | 0.40 | 0.34 |
| | % Ethylene Glycol | Tr.-0.0 | 0.00 | 0.00 | 0.00 | 0.00 |
| AEROTEX Glyoxal 40<br>(Lot 9-745)<br>R-9195-160<br>CHF Sample No. 41-400<br>50 ml Undiluted (as received)<br>Sample Drawn 3-5-80 | % Acid (Calc. As Acetic) | 0.7 | 0.77 | 0.75 | 0.73 | 0.74 |
| | % Glyoxal | 40.0 | 39.16 | 39.30 | 39.68 | 39.53 |
| | % Formaldehyde | 5.9 | 5.97 (5.48) | 6.01 (5.36) | 5.88 (5.40) | 5.93 (5.22) |
| | % Glycolaldehyde | 0.3 | 0.34 | 0.37 | 0.36 | 0.34 |
| | % Ethylene Glycol | 0.9 | 0.85 | 0.85 | 0.79 | 0.82 |

*1 All Assays Average of 2 by R. S. Farmer.
*2 All Numbers in Parentheses Corrected by Blank.
*3 European Glyoxal 40 Precipitate Noticed.

## ASSAYS OF GLYOXAL #1

| | | EXPECTED | 11-11-80 | 11-17-80 | 11-25-80 | 12-9-80 |
|---|---|---|---|---|---|---|
| ropean Glyoxal 40<br>C No. 41-429<br>ml Undiluted (as eived)<br>ple Drawn: 10/16/80 | % Acid (Calc. As Acetic) | 0.1 | 0.30 | 0.26 | 0.28 | 0.31 |
| | % Glyoxal | 40.4 | 39.77 | 40.11 | 39.82 | 39.94 |
| | % Formaldehyde | 0.7 | 0.25 (0.03)#2 | 0.48 (0.15) | 0.52(- ) | 0.49 (0.4 |
| | % Glycolaldehyde | 0.2 | 0.13 | 0.13 | 0.15 | 0.11 |
| | % Ethylene Glycol | Tr.-0.0 | 0.00 | 0.00 | 0.00 | 0.00 |
| NOTEX Glyoxal 40<br>RC No. 41-400<br>ml Undiluted (as ceived)<br>ple Drawn 10/16/80 | % Acid (Cal. As Acetic) | 0.7 | 0.71 | 0.71 | 0.71 | 0.75 |
| | % Glyoxal | 40.0 | 39.34 | 39.51 | 39.43 | 39.37 |
| | % Formaldehyde | 5.9 | 5.73 (5.44) | 5.90 (5.58) | 5.91(-) | 5.84 (5.25 |
| | % Glycolaldehyde | 0.3 | 0.18 | 0.19 | 0.25 | 0.19 |
| | % Ethylene Glycol | 0.9 | 0.75 | 0.75 | 0.72 | 0.77 |

#1 All Assays Average of 2 by R. S. Farmer
#2 All Numbers in Parentheses Corrected by Blank

F/lr

## ASSAYS OF GLYOXAL #1

| | | EXPECTED | 7-22-81 | 7-28-81 | 8/4/81 | 8/18/81 |
|---|---|---|---|---|---|---|
| ropean Glyoxal 40<br>HF No. 41-429<br>ml Undiluted (as eived)<br>ple Drawn: 4/24/81<br>R-9516-43 | % Acid (Calc. As Acetic) | 0.1 | 0.33 | | 0.50 | 0.34 |
| | % Glyoxal | 40.4 | 39.99 | | 39.54 | 39.77 |
| | % Formaldehyde | 0.7 | 0.58(0.00)#2 | | 0.52(0.00) | 0.34(0.0 |
| | % Glycolaldehyde | 0.2 | 0.10 | | 0.12 | 0.12 |
| | % Ethylene Glycol | Tr.-0.0 | 0.00 | | 0.00 | 0.00 |
| OTEX Glyoxal 40<br>F. No. 41-400<br>ml Undiluted (as eived)<br>ple Drawn 4/24/81<br>R-9195-160 | % Acid (Cal. As Acetic) | 0.7 | 0.78 | | 0.79 | 0.78 |
| | % Glyoxal | 40.0 | 39.47 | | 39.99 | 39.32 |
| | % Formaldehyde | 5.9 | 6.31(5.74) | | 5.75(5.28) | 5.64(5.2 |
| | % Glycolaldehyde | 0.3 | 0.14 | | 0.19 | 0.18 |
| | % Ethylene Glycol | 0.9 | 0.74 | | 0.75 | 0.71 |

#1 All Assays Average of 2 by R. S. Farmer
#2 All Numbers in Parentheses Corrected by Blank

'lr

# Appendix III

**Carnegie-Mellon University**

**INTER-OFFICE CORRESPONDENCE**

**To:** L. G. Peterson, L. R. DePass, C. S. Weil
J.H.C. M.J.C.

**From:** J. H. Coleman, M. J. Cardella

**Date:** February 7, 1979

**Subject:** Results of Preliminary Mouse Skin Painting Tests

---

Groups of five C3H/HeJ male mice were dosed once daily for ten days on the clipped skin of the back with 25 microliters of either a 1:4 dilution of U.S. process glyoxal in distilled water, a 1:8 dilution of U.S. process glyoxal in distilled water, a 1:4 dilution of European process glyoxal in distilled water or a 1.8 dilution of European process glyoxal in distilled water. A negative control group was dosed with 25 microliters of distilled water. The mice were observed daily for general health, particularly lesions of the skin, and were weighed after 0, 1, 3, 4, 6, 8, 9, and 10 doses on the semi-automatic weighing system. After the tenth dose, the mice were sacrificed and a complete necropsy was performed on each.

The results of this preliminary skin painting study are summarized in Table 1. The mice dosed with the 1:4 dilution of U.S. process glyoxal in water showed significantly higher body weight changes after 4 doses ($0.01 > P > 0.001$), and again after 10 doses ($0.05 > P > 0.01$) when compared with the negative control group. Two of the 5 mice had open sores on the skin at necropsy. The mice dosed with the 1:8 dilution of U.S. process glyoxal in water, had significantly higher body weight changes after 1 dose ($0.05 > P > 0.01$) and also after 4, 6, and 10 doses ($0.01 > P > 0.001$) when compared to the negative control. When the group dosed with the 1:4 dilution of European process glyoxal in water was compared to the negative control group, it showed significantly higher body weight changes after all doses (dose 1, $P < 0.001$; dose 3, $0.01 > P > 0.001$; doses 4 and 6, $P < 0.001$; dose 8, $0.05 > P > 0.01$; dose 9, $0.01 > P > 0.001$; and dose 10, $P < 0.001$). Three of the 5 mice had open sores of the skin at necropsy. The group dosed with the 1:8 dilution of European process glyoxal in water showed significantly greater body weight changes after dose 1 ($0.01 > P > 0.001$), dose 4 ($0.05 > P > 0.01$), and dose 10 ($0.01 > P > 0.001$) when compared to the negative control group.

In conclusion, none of the groups showed any ill effect on body weights. However, the 1:4 dilutions of both the U.S. and European glyoxal produced sores on the skin while the 1:8 dilutions did not. Therefore, due to the absence of any adverse effects on skin or body weights both the U.S. process glyoxal and European process glyoxal will be dosed at a 1:8 dilution in water for the lifespan studies.

JHC/MJC:acc
Attachment: 1

Table 1

## Summary of Ten-Day Preliminary Mouse Skin Painting Studies

### C3H/HeJ Male Mice

| Material | Glyoxal (U.S.) | Glyoxal (U.S.) | Glyoxal (Eur.) | Glyoxal (Eur.) | Distilled Water Negative Control |
|---|---|---|---|---|---|
| Concentration, % in Distilled Water | 1:4 | 1:8 | 1:4 | 1:8 | - |
| Dosage, in microliters | 25 | 25 | 25 | 25 | 25 |
| **Weight Change (gms):** | | | | | |
| 1 Dose | -0.7 | -0.4(a) | 0.4(c) | 0.0(b) | -1.6 |
| 3 Doses | -0.6 | 0.3 | 0.7(b) | 0.0 | -0.8 |
| 4 Doses | -0.1(b) | -0.1(b) | 0.2(c) | -0.5(a) | -1.2 |
| 6 Doses | -0.1 | 0.5(b) | 0.9(c) | -0.3 | -0.8 |
| 8 Doses | -1.0 | -0.3 | 0.0(a) | -1.1 | -1.2 |
| 9 Doses | -0.4 | 0.2 | 0.8(b) | -0.5 | -0.7 |
| 10 Doses | -0.3(a) | 0.3(b) | 1.3(c) | 0.2(b) | -1.6 |
| **Gross Lesions:** | | | | | |
| Internal | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| Open Sores on Skin | 2/5 | 0/5 | 3/5 | 0/5 | 0/5 |
| Flaky and/or Peeling Skin | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |
| Mortality | 0/5 | 0/5 | 0/5 | 0/5 | 0/5 |

(a) = $0.05 > P > 0.01$ (b) = $0.01 > P > 0.001$ (c) = $0.001 > P$

AMTEK CORP.

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