

FYI-0794-1246

TITLE PAGE

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IR-424

TITLE OF REPORT: C3H/10T¹/2 Cell Transformation Assay

TEST SUBSTANCE: AEROTEX® Glyoxal 40

TEST SPECIES: C3H/10T¹/2 CL8 cells

ROUTE OF ADMINISTRATION: In vitro Assay

Contains No CBI

DURATION OF STUDY: Five months

TESTING FACILITY: EG&G Mason Research Institute
1530 East Jefferson Street
Rockville, MD 20852

Contains No CBI

TESTING FACILITY'S REPORT NO.: 029-626-292-8

SPONSOR: Organic Chemicals Division
American Cyanamid Company
Wayne, NJ 07470



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SPONSOR'S REPORT NO.: 80-02

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EG&G MASON RESEARCH INSTITUTE

1530 EAST JEFFERSON STREET, ROCKVILLE, MARYLAND 20852

Tel. (301) 770-4400

AEROTEX GLYOXAL 40; C3H/10T $\frac{1}{2}$ CELL TRANSFORMATION ASSAY

Sponsor: American Cyanamid Company
Wayne, New Jersey

Study No.: 029-626-292-8

Test Article I.D.: Aerotex Glyoxal 40

Test Article No.: (R-9195-160) lot 9-745

Test Article Description: Non-Viscous Liquid

Storage Conditions: 4°C

Date Received: 7/3/79

Date Study Started: 7/13/79

Date Study Completed: 12/13/79

Report Date: 1/9/80

Study Coordinator: David R. Brown
American Cyanamid Company

Study Director: A. Thilagar, Ph.D.
EG&G Mason Research Institute

A. Thilagar 12/31/79
A. Thilagar, Ph.D. Date
Study Director

H Sasak 12-31-1979
Halina Sasak Date
Technician

John Cameron 12-31-79
John Cameron Date
Technician

Regina Knight 12-31-79
Regina Knight Date
Technician

Gerry Reichard 1/9/80
Gerry Reichard Date
Data Technician

Summary

Test article Aerotex Glyoxal 40 (MRI #292) was submitted by American Cyanamid Company and was tested at EG&G Mason Research Institute in the C3H/10T $\frac{1}{2}$ Cell Transformation Assay at four dose levels ranging from 0.0098 μ l/ml to 0.0013 μ l/ml.

The results of the assay indicate that the test article did not cause morphological transformation of cells in the C3H/10T $\frac{1}{2}$ Cell Transformation Assay.

Introduction

This study was conducted by A. Thilagar, Ph.D., Regina Knight, Malina Sasak, and John Cameron from July 13, 1979 to December 13, 1979 at EG&G Mason Research Institute, Rockville, Maryland. The experimental procedure employed was essentially that described by Bertram (Cancer Research 37:514-523, 1977) and is described in detail in the appendix to this report.

Materials and Methods

Stock Cultures

The C3H/10T $\frac{1}{2}$ CL8 cells used in this study were derived from the cryopreserved Lot No. 3-2-1978, Passage No. 8. The C3H/10T $\frac{1}{2}$ CL8 cell line was originally obtained at its sixth passage from Dr. C. Heidelberger of the McArdle Laboratory for Cancer Research, University of Wisconsin. The cryopreserved lots were prepared from the subculture of this line.

Test Article

The test article Aerotex Glyoxal 40 was received on July 3, 1979 and stored at room temperature. Compound 7,12-dimethylbenz(a)anthracen , Eastman Kodak Lot #A8X was used as the positive compound.

Test article Aerotex Glyoxal 40 was measured, dissolved and diluted in acetone to appropriate concentrations, immediately before use. Approximately 10 to 20 minutes were taken between the time the test article was drawn from the stock bottle and the final treatment of cells. All test article preparation and culture treatments were conducted under subdued yellow lights to avoid possible problems of photo-inactivation.

Initial Toxicity Determination

Dose range study to determine the toxicity of the test article Aerotex Glyoxal 40 was conducted prior to testing for transformation potential. The test article was tested in duplicate using 60 mm culture plates seeded with 200 cells per plate and grown in 5 ml of BME medium supplemented with 10% fetal bovine serum. Test article Aerotex Glyoxal 40 was tested using 2-fold dilutions over a concentration range of 10 μ l/ml to 0.0013 μ l/ml.

After about 18 hours, the cells were refed with fresh medium and incubated for 8 days. The plates were washed with PBS, fixed with absolute methanol and stained with Giemsa stain. The number of colonies per plate was counted and the cloning efficiency (CE) and the relative cloning efficiency (RCE) were determined by the following formula:

$$CE = \frac{\text{Average No. Colonies/plate}}{\text{No. of Cells Seeded/plate}} \times 100$$

$$RCE = \frac{\text{Test Culture Cloning Efficiency}}{\text{Solvent Control Cloning Efficiency}} \times 100$$

Transformation Assay

The transformation potential of test Aerotex Glyoxal 40 was tested at four dose levels in decreasing 2-fold dilutions from the concentration which caused over 50% reduction in cloning efficiency. Twelve replicate plates seeded with 1000 cells/plate were treated by 0.0098 µl/ml, 0.0049 µl/ml, 0.0025 µl/ml and 0.0013 µl/ml. The positive compound 7,12-dimethylbenz(a)anthracene was tested at 0.5 µg/ml and 0.25 µg/ml. In parallel with the test plates, four toxicity plates per dilution, each containing 200 cells were treated with the same compound dilutions.

Approximately 18 hours after treatment, the test article was removed from all assay and toxicity plates which were refed with growth medium and reincubated. The toxicity plates were incubated for 8 days, stained and the relative cloning efficiency was determined as previously described. This was to assure that the assay was being conducted at compound concentrations approaching the LD₅₀.

The remaining culture plates were refed weekly with BME medium supplemented with 5% fetal bovine serum.

At 35 days after removal of the test article, all plate cultures were washed, fixed, stained, examined microscopically and macroscopically and scored for transformation.

Scoring for Transformation

Focal areas of transformation were classified according to the criteria of Reznikoff et al. as follows:¹

Type I. Foci composed of monolayer cells are more densely packed than the background cells. This type is not considered malignant and was not scored.

Type II. Foci show massive piling up into virtually opaque multilayers. The cells are only moderately polar, thus criss-crossing is not pronounced.

Type III. Foci are composed of highly polar, fibroblastic, multilayered, criss-crossed arrays of densely stained cells.

Recording of Data

The data generated by each assay was recorded on Forms No. WL-88, WL-89, and WL-108. This includes data from the initial toxicity assay and the transformation assay with its parallel toxicity test.

The number of Type II and III foci per plate were recorded.

All raw data and stained plates of this test will be maintained at E&G Mason Research Institute Archives located at 1530 East Jefferson Street, Rockville, Maryland.

¹Reznikoff et al., Cancer Research 33:3239-3249, 1973.

Results

In the initial toxicity test, the test article Aerotex Glyoxal 40 showed a relative cloning efficiency of 11% at 0.0098, which falls in the desired toxic level of over 50% reduction in the relative cloning efficiency (Table 1). The transformation assay was therefore conducted at four 2-fold dilutions starting at 0.0098 $\mu\text{l/ml}$.

The toxicity study conducted in parallel to the assay showed the attainment of desired toxic level at 0.0098 $\mu\text{l/ml}$ (Table 2).

No indications of transformation were observed in any of the test article plates or in the solvent control, (Table 3). The 7,12-dimethylbenz(a)anthracene control plates at 0.5 $\mu\text{g/ml}$ and 0.25 $\mu\text{l/ml}$ showed development of both Type II and Type III foci.

Table 1

ED&B MASON RESEARCH INSTITUTE
C3H 10T½ TRANSFORMATION ASSAY
INITIAL COMPOUND TOXICITY TEST

<u>American Cyanamid</u> Client	<u>A. Thilagar, Ph.D.</u> Investigator	<u>10 µl/ml-0.0013 µl/ml</u> Dose Range
<u>Aerotex Glyoxal 40</u> Test Compound Identity	<u>029-626-292-8</u> Study Number	<u>Acetone</u> Solvent

Compound Concentration	No. Colonies No. Plates	Ave. No. Colonies per Plate	Cloning Efficiency	Relative* Cloning Efficiency
10 µl/ml	0+0/2	0	0	0
5 µl/ml	0+0/2	0	0	0
2.5 µl/ml	0+0/2	0	0	0
1.25 µl/ml	0+0/2	0	0	0
0.625 µl/ml	0+0/2	0	0	0
0.312 µl/ml	0+0/2	0	0	0
0.156 µl/ml	0+0/2	0	0	0
0.078 µl/ml	0+0/2	0	0	0
0.039 µl/ml	0+0/2	0	0	0
0.0195 µl/ml	0+0/2	0	0	0
0.0098 µl/ml	6+7/2	6.50	3.25	11%
0.0049 µl/ml	22+26/2	24.00	12.00	40%
0.0025 µl/ml	32+40/2	36.00	18.00	61%
0.0013 µl/ml	35+38/2	36.50	18.25	61%
Solvent Control	59+60/2	59.50	29.75	100%

* Relative Cloning Efficiency = $\frac{\text{Test Culture Cloning Efficiency}}{\text{Solvent Control Cloning Efficiency}} \times 100$

Table 2

ED:0 MASON RESEARCH INSTITUTE
C3H 10T_{1/2} TRANSFORMATION ASSAY

ASSAY RESULTS

American Cyanamid Client	A. Thilagar, Ph.D. Investigator	0.0098 µl/ml-0.0013 µl/ml Dose Range
Aerotex Glyoxal 40 Test Compound Identity	029-626-292-8 Study No.	Acetone Solvent

PARALLEL TOXICITY

Compound Concentration	No. Colonies No. Plates	Ave. No. Colonies per plate	Cloning Efficiency	Relative Cloning Efficiency
0.0098 µl/ml	17+16+18+10/4	17.25	8.62	32.71%
0.0049 µl/ml	23+26+31+28/4	27.00	13.50	51.18%
0.0025 µl/ml	40+42+31/3	37.67	18.84	71.42%
0.0013 µl/ml	44+40+45+32/4	40.25	20.13	76.31%
Test Compound Solvent Acetone	49+50+58+54/4	52.75	26.38	100.00%
7,12-DMBA 0.5 µg/ml	12+11+11+14/4	12.00	6.00	22.74%
7,12-DMBA 0.25 µg/ml	20+25+15+23/4	20.75	10.38	39.35%
7,12-DMBA Solvent Acetone				

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AT&T MASON RESEARCH INSTITUTE

CM 1074 TRANSFORMATION ASSAY

ASSAY RESULTS

American Cyanamid Client A. Thilagar, Ph.D. Investigator 0.0098 µl/ml-0.0013 µl/ml Dose Range

Aerotex Glyoxal 40 Test Compound Identity 029-626-292-8 Study No. Acetone Solvent

TRANSFORMATION

Compound Concentration	Type II Foci/Plate												Totals
	1	2	3	4	5	6	7	8	9	10	11	12	
0.0098 µl/ml	0	0	0	0	0	0	0	0	0	0	0	0	0
0.0049 µl/ml	0	0	0	0	0	0	0	0	0	0	0	0	0
0.0025 µl/ml	0	0	0	0	0	0	0	0	0	0	0	0	0
0.0013 µl/ml	0	0	0	0	0	0	0	0	0	0	0	0	0
Test Compound Solvent Acet	0	0	0	0	0	0	0	0	0	0	0	0	0
7,12-DNA 0.5 µg/ml	0	1	2	1	0	1	1	2	0	1	1	1	11
7,12-DNA 0.25 µg/ml	1	0	1	0	0	0	0	3	1	1	0	1	8
7,12-DNA Solvent Acet													

Compound Concentration	Type III Foci/Plate												Totals
	1	2	3	4	5	6	7	8	9	10	11	12	
0.0098 µl/ml	0	0	0	0	0	0	0	0	0	0	0	0	0
0.0049 µl/ml	0	0	0	0	0	0	0	0	0	0	0	0	0
0.0025 µl/ml	0	0	0	0	0	0	0	0	0	0	0	0	0
0.0013 µl/ml	0	0	0	0	0	0	0	0	0	0	0	0	0
Test Compound Solvent Acet	0	0	0	0	0	0	0	0	0	0	0	0	0
7,12-DNA 0.5 µg/ml	0	0	2	0	1	0	0	1	0	0	0	0	4
7,12-DNA 0.25 µg/ml	1	0	1	0	1	0	1	0	0	0	1	1	6
7,12-DNA Solvent Acet													

0019

Conclusions

Test article Aerotec Glyoxal 40 was submitted by American Cyanamid Company and was tested in the C3H/10T $\frac{1}{2}$ Cell Transformation Assay at four dose levels. The results of the assay indicate that the test article did not cause morphological transformation of cells in the C3H/10T $\frac{1}{2}$ Cell Transformation Assay.

In this study, the solvent control did not show any indication of transformed foci. The positive control, 7,12-dimethylbenz(a)anthracene showed development of both Type II and Type III transformed foci.

APPENDIX

0015



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1530 EAST JEFFERSON STREET, ROCKVILLE, MARYLAND 20852

Tel. (301) 770-4400

PROTOCOL NO. 8

AEROTEX GLYOXAL 40; C3H/10T $\frac{1}{2}$ CELL TRANSFORMATION ASSAY

sponsor: American Cyanamid Company
Wayne, New Jersey

Testing Facility: EG&G Mason Research Institute
Rockville, Maryland

Study No.: 029-626-292-8 MRI #: 292

Test Article: Aerotex Glyoxal 40 (ACCO Glyoxal R9195-160)

Proposed Starting Date: 7-9-1979

Proposed Completion Date: 8-29-1979

This study will probably be submitted () , will probably not be submitted (), to the Food and Drug Administration in support of an application.

Study Director: A. Thilagar 6-26-79
A. Thilagar, Ph.D. Date
EG&G Mason Research
Institute

Study Coordinator: David R Brown 6-29-79
Date

Aerotex Glyoxal 40; C3H/10T_{1/2} Cell Transformation Assay

1.0 Purpose of Study

To evaluate the test materials actions relative to cell transformation in a mammalian cell line.

2.0 General Procedures

2.1 Good laboratory practices shall be followed in all experimental and record keeping procedures in this study (43 FR 60013-25).

2.2 The testing facility shall designate responsible personnel for each part of the study and promptly notify Cyanamid of any changes.

2.3 All records will be available for inspection by Cyanamid personnel throughout the study.

2.4 Any modifications in this protocol must, insofar as practical, be recorded and approved by the Cyanamid study director in advance.

3.0 Test Material

3.1 The test material, Aerotex Glyoxal 40, is specified in Annex 1 including chemical name, chemical abstract services registry number, sponsor code numbers, synonyms, and molecular structure.

3.2 Cyanamid will provide data for physical identification of test material.

3.3 Cyanamid will supply the test material and provide batch numbers, source, sample size, and storage conditions information.

3.4 Cyanamid will supply a material safety data sheet and other relevant information necessary for the safe handling of the test material.

4.0 Test Procedure

4.1 The toxic level of the test compound will be determined by measuring the cloning efficiency of cells exposed to two-fold dilutions of the compound for 18 hours.

4.2 Replicate plate cultures of the cells will be exposed to four dose levels of the compound for 18 hours.

4.3 Place cultures will be incubated for 35 days with refeedings at 7-day intervals; at eight days the serum content is lowered to 5%.

4.4 An appropriate solvent control will be included with each assay.

4.5 An appropriate positive control will be included with each assay.

4.6 An appropriate parallel toxicity control will be included with each assay.

4.7 The plates are washed, stained, and examined after 35 days.

4.8 The number of foci of transformed cells is determined and each is classified according to the criteria of Reznikoff.

5.0 Methodology

5.1 The test facility will prepare a working protocol which sets forth the methods to be used including measurements, observations, and statistical procedures. See Annex 2.

6.0 Reporting

6.1 An interim report is to be provided Cyanamid at the conclusion of the transformation assay.

6.2 If confirmation of Type II or Type III transformants is elected, a final report will be provided within ninety days after sacrifice of the last animal.

7.0 Retention of Specimen Tissues and Records

7.1 All wet tissues, tissue blocks, and slides shall be identified and stored in a manner consistent with current Good Laboratory Practices regulations.

7.2 One year after the date of the final report, the test facility shall contact Cyanamid to determine the disposition of all raw data and other materials.

8.0 Amendments to Protocol

8.1 This protocol shall include provisions of Appendix 1.

8.2 Subsequent amendments to this protocol shall be drawn up and numbered serially as Annexes. Each shall show:

- (a) Signatures of authorized representatives of Cyanamid and the test facility.
- (b) The date upon which the Annex was signed.
- (c) The date upon which the amendment became effective.

ANNEX 2

EG&G MASON RESEARCH INSTITUTE

Aerotex Glyoxal 40;

C3H/10T $\frac{1}{2}$ Cell Transformation Assay

Study No.: 029-626-292-8

MRI #: 292

Test Article: Aerotex Glyoxal 40 (ACCO Glyoxal R9195-160)

Effective date:

Study Director:

A. Thilagar
A. Thilagar, Ph.D.
EG&G Mason Research
Institute

6-26-79

Date

Study Coordinator:

David R Brown

7-9-79

Date

0022

A number of in vitro cell transformation systems are available today for the detection of potentially carcinogenic compounds. EG&G Mason offers the C3H/10T $\frac{1}{2}$ Mouse Embryo Cell Transformation assay. This cell line is malignantly transformed by a variety of agents and is particularly appealing for transformation studies. Spontaneous transformations are extremely rare with the cell line, and transformed foci are readily detected against a very uniform background of normal cells. EG&G Mason's standard protocol yields final results in approximately 50 days.

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PAGE 2 OF 4

C3H/10T $\frac{1}{2}$ CELL TRANSFORMATION ASSAY

A. INTRODUCTION

The cell line designated C3H/10T $\frac{1}{2}$ CL8 was derived from a primary C3H mouse embryo culture and established by Reznikoff, Brankow, and Heidelberger.¹ This cell line is highly sensitive to postconfluence inhibition of cell division and has been used extensively in studies of chemical transformation. Thus far, C3H/10T $\frac{1}{2}$ CL8 has been shown to be sensitive to transformation by certain polycyclic aromatic hydrocarbons, MNNG, 4NQO, smoke condensate, and X-rays. Recently, it was shown that maintenance of carcinogen treated C3H/10T $\frac{1}{2}$ CL8 cells on reduced serum levels (5%), 8 days post treatment, leads to a 2- to 6-fold enhancement in transformation frequency in comparison with cultures maintained in 10% serum throughout the transformation assay.² This cell line is particularly appealing for transformation studies because, in general, no spontaneous transformations are observed.

Briefly, the assay is conducted as follows:

The toxic level of the test compound is determined by establishing the cloning efficiency of cells exposed to 2-fold dilutions of the compound for 18 hours. Replicate plate cultures of the cells are exposed to four dose levels of the compound for 18 hours. The plate cultures are incubated for 35 days with refeedings at 7-day intervals; at 8 days the serum content is lowered to 5%. Appropriate solvent and positive control plates are included in each assay.

After 35 days, the plates are washed, stained, and examined. Foci of transformed cells stand out clearly against the uniform background of untransformed cells. The total number of foci is determined and each is classified according to the criteria of Reznikoff.

B. PROCEDURES

1. Stock Cultures

The culture of the C3H/10T $\frac{1}{2}$ CL8 cell line was obtained at its sixth passage directly from Dr. C. Heidelberger of the McArdle Laboratory for Cancer Research, University of Wisconsin. Subcultures of this line were propagated to levels sufficient for preparation and cryopreservation of 50 to 100 ampules. These ampules serve as stock for initiation of new subcultures for each assay, assuring a uniformity of inoculum in replicate assays. Prior to using an inoculum stock in the assay, a representative culture is examined for typical growth characteristics and freedom from spontaneous transformation.

2. Culture Conditions

Stock cultures are grown in antibiotic-free Eagle's basal medium (BME) containing 10% heat-inactivated fetal bovine serum (FIFBS). Cultures are subpassaged weekly at a 1:5 split ratio and maintained in a humidified incubator at 37°C with an atmosphere of 5% CO₂ in air.

¹ Reznikoff, Brankow, and Heidelberger. *Cancer Research* 33:3231-3238, 1973.

² Bertram, J. S. *Cancer Research* 37:514-523, 1977.

3. Test Compounds

All test compounds are weighed, dissolved, and diluted to desired concentrations immediately before use. Unless otherwise specified, sterile deionized water, ethanol, dimethylsulfoxide (DMSO), or acetone is used as a solvent. All compound preparation and culture treatments are conducted under subdued or yellow lights to avoid possible problems of photo-inactivation. Also, all test compounds are considered to be carcinogenic and are handled by our standard biohazard procedures.

4. Initial Toxicity Determinations

Dose range studies are conducted on all test compounds prior to testing for transformation potential. Compounds are tested in duplicate using 60 mm culture plates seeded with 200 cells per plate and grown in 5 ml of BME supplemented with 10% HIFBS. Each compound is tested using 15 2-fold dilutions over a concentration range of 1024 $\mu\text{g/ml}$ to 0.03 $\mu\text{g/ml}$. The diluted compound is delivered in 25 μl volumes, resulting in a maximum solvent concentration level of 0.5% which, in the case of ethanol, acetone, and DMSO, is compatible with the C3H/10T $\frac{1}{2}$ CL8 cell culture.

After 18 hours exposure, the cells are washed, refed with fresh medium, and incubated for 10 days. The plates are washed twice with PBS, fixed for 20 minutes with absolute methanol, air dried, and stained with Giemsa stain for 30 minutes. The number of colonies per plate is counted, and the cloning efficiency (CE) is determined by the following formula:

$$\text{CE} = \frac{\text{Average No. Colonies/Plate}}{\text{No. of Cells/Plates}} \times 100$$

If a desired toxicity level (approximately 50% decrease in cloning efficiency) is not achieved, the toxicity study will be repeated at additional concentrations.

5. Transformation Assay

Cells in late logarithmic phase are trypsinized with 0.1% trypsin for 4 minutes and are plated at a concentration of 1×10^3 cells/60 mm petri dish in 5 ml of BME supplemented with 10% HIFBS, penicillin (50 units/ml), and streptomycin (50 $\mu\text{g/ml}$). A second series of replicate cultures containing 200 cells/plate is set up from the same cell suspension for toxicity determinations.

After 24 hours, the cultures are treated with the appropriate concentrations of test compound delivered in 25 μ l volumes. Solvent control cultures receive an equal amount of solvent only. Each compound is tested at four dose levels in decreasing 2-fold dilutions from the concentration which causes a 50% to 75% reduction in cloning efficiency. In parallel with the test plates, four toxicity plates containing 200 cells each are treated with the same compound dilutions.

Eighteen hours after treatment, the test compound is removed from all assay and toxicity plates which are refed with growth medium and reincubated. The toxicity plates are incubated for 10 days, stained, and the cloning efficiency is determined as previously described. This is to assure that the assay is being conducted at compound concentrations approaching the LD_{50} .

The remaining cultures are refed weekly with BME supplemented with 5% HIFBS.

At 35 days after removal of the compound, all plate cultures are washed, fixed, stained, examined microscopically and macroscopically, and scored for transformation.

6. Scoring of Transformation

Focal areas of transformation are classified according to the criteria of Reznikoff as follows:³

Type I. Foci composed of monolayer cells are more densely packed than the background cells. This type is not considered malignant and is not scored.

Type II. Foci show massive piling up into virtually opaque multilayers. The cells are only moderately polar, thus criss-crossing is not pronounced. Fifty percent of Type II foci have been shown to be malignantly transformed.

Type III. Foci are composed of highly polar, fibroblastic, multilayered, criss-crossed arrays of densely stained cells. Eighty-five percent of Type III foci have been shown to be malignantly transformed.

C. RECORDING OF DATA

The data generated by each assay is recorded on Forms No. WL-88 and WL-89. This includes data from the initial toxicity assay and the transformation assay with its parallel toxicity test.

The number of Type II and III foci per plate is recorded.

³ Reznikoff. Cancer Research 33:3239-3249, 1973.

ANNEX 3
PROTOCOL AMENDMENT

Study No.: 029-626-292-8

Test Article: Aerotex Glyoxal 40 (ACCO Glyoxal R9195-160)

On page 1 of the protocol the proposed date
of initiation was 7-9-79, the actual date of
initiation was 7-13-79.

A. Thilagar 12/5/79
A. Thilagar, Ph.D. Date
Study Director

DATA SHEET FOR TOXICITY STUDIES

THIS SHEET WILL BE PHOTOCOPIED; PLEASE TYPE ALL INFORMATION.

TO: DIRECTOR OF TOXICOLOGY
CENTRAL MEDICAL DEPARTMENT
AMERICAN CYANAMID COMPANY
WAYNE, NEW JERSEY 07470

PREPARED BY

Carole M. Dixon

DATE PREPARED

7/12/79

OUR SAMPLE NO.

HOW

OCD

DEPARTMENT

ITC

1. IDENTIFICATION OF PRODUCT

1.1 SALES NAME (GIVE PRODUCT CODE NUMBER, IF ANY)

ABROTEX Glyoxal 40 code number 91274

1.2 CHEMICAL NAME IF THIS PRODUCT IS A DYE OR PIGMENT. GIVE COLOR INDEX NAME AND NUMBER, IF ANY

40% glyoxal

1.3 AMOUNT OF SAMPLE SUPPLIED (WEIGHT OR VOLUME)

1 gallon

1.4 YOUR SAMPLE NO.

(R-9195-160) lot 9-745

2. STRUCTURAL FORMULA AND COMPOSITION

INCLUDE IMPURITIES IN STATEMENT OF COMPOSITION. FOR MIXTURES, GIVE PERCENTAGE COMPOSITION AND STRUCTURAL FORMULAS OF PRINCIPAL COMPONENTS. FOR SOLUTIONS, GIVE PERCENTAGE SOLIDS AND COMPOSITION OF SOLVENT AND SOLIDS, SEPARATELY, EACH ON BASIS OF 100. FOR RESINS, IDENTIFY THE MONOMERS, AND GIVE STRUCTURE OF REPEATING UNIT. GIVE PROPORTION OF MONOMERS IN COPOLYMERS, WHERE APPLICABLE. GIVE PERCENTAGE OF RESIDUAL INTERMEDIATES OR STARTING MATERIALS, AND UNNEUTRALIZED ACID OR ALKALI. DO NOT USE SALES NAMES OF OTHER MANUFACTURERS WITHOUT IDENTIFYING THE MATERIALS BY CHEMICAL NAME.

Solids = 46.9%

Solvent = 53.1%

Glyoxal	85.3%
Formaldehyde	12.6%
Acid **	1.5%
Glycolaldehyde	0.6%

Water	98.3%
ethylene glycol	1.7%
	100.0%

** Acid is expressed as acetic acid. It is really some combination of formic, glycolic, oxalic, and glyoxylic acids which we are unable to distinguish.

IMPORTANT: PLEASE BE SURE TO ENTER YOUR SAMPLE IDENTIFICATION NUMBER IN 1.4 ABOVE. TOXICITY TESTS ARE SOMETIMES INFLUENCED GREATLY BY IMPURITIES IN A SAMPLE. IT IS NOT UNUSUAL FOR A NEED TO ARISE TO GO BACK, OVER A LONG PERIOD OF TIME, TO DETERMINE THE EXACT COMPOSITION OR ORIGIN OF THE SAMPLE THAT WAS USED.

3. PHYSICAL PROPERTIES

3.1 APPEARANCE: DESCRIBE AS POWDER, CRYSTAL, FLAKE, GRANULE, LUMP, PASTE, VOLATILE SOLID, VISCOUS LIQUID, MOBILE LIQUID, VOLATILE LIQUID, COMPRESSED GAS, SELF-PRESSURIZED CONTAINER, ETC. WHERE APPLICABLE, GIVE APPROXIMATE MESH SIZES OF SOLIDS.

Clear, water-white to light yellow liquid

3.2 MELTING POINT

3.3 BOILING POINT

3.4 VAPOR PRESSURE (SPECIFY AMBIENT TEMPERATURE AND PRESSURE)

3.5 FLASH POINT, DEGREES FAHRENHEIT, TAG OPEN CUP

3.6 SOLUBILITY IN WATER

Complete

4. PAYMENT OF CHARGES

WORK ON THIS SAMPLE WILL BE PERFORMED BY AN INDEPENDENT CONTRACTOR WHO WILL SUBMIT A BILL TO THE CENTRAL MEDICAL DEPARTMENT. PLEASE INDICATE BELOW THE OFFICE OR INDIVIDUAL TO WHOM THE BILL SHOULD BE REFERRED FOR PAYMENT. INCLUDE ANY CHARGE NUMBERS THAT ARE APPLICABLE.

R.L.von Glahn charge 022



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