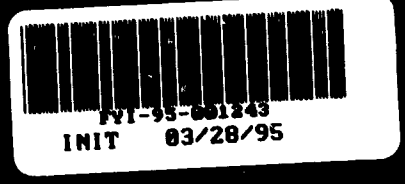
IR-424



PHARMAKON RESEARCH INTERNATIONAL, INC.

Waverly, Pennsylvania 18471

0 0 0 3

PHARMAKON RESEARCH INTERNATIONAL, INC.
WAVERLY, PENNSYLVANIA 18471

PHONE
(717) 586-2411

82-37 NC.

RECEIVED
JUL 06 1982
F. J. KOSCHIER

Genetic Toxicology
Micronucleus Test (MNT)

PH 309A-AC-002-82

Glyoxal 40 LF

301

Submitted to

American Cyanamid Company
Wayne, New Jersey

Ruth M. Sorg
Ruth M. Sorg
Study Director

Robert W. Naismith
Robert W. Naismith, Ph.D.
Director of Toxicology

Richard J. Matthews
Richard J. Matthews, Ph.D.
Director of Research

June 25, 1982

PHARMAKON RESEARCH INTERNATIONAL, INC.
WAVERLY, PENNSYLVANIA 18471

PHONE
(717) 586-2411

Micronucleus Test - Glyoxal 40 LF

SUMMARY DATA

Study Number: PH 309A-AC-002-82

Purpose

To determine the chromosome breaking effects measured by the increase in micronuclei in mice pretreated intraperitoneally.

Methods and Materials

Glyoxal 40 LF was initially supplied as a clear liquid material. The test article, positive control and negative control were prepared fresh daily utilizing distilled water as a vehicle. The test article, positive control and negative control were administered at 20 ml/kg of body weight by the intraperitoneal route.

In a preliminary dose-range study, Glyoxal 40 LF was administered intraperitoneally once daily for two consecutive days to groups of four mice (2 males, 2 females) at concentrations of 50, 166.6, 500, 1666.6, and 3000 mg/kg of body weight. Mortality and signs were observed up to 48 hours following the second dose, at which time the remaining animals were sacrificed.

Signs observed at all dose levels included: piloerection, writhing, hypersensitivity and decreased tone and activity. Additional signs at 500 mg/kg, 1666.6 mg/kg and 3000 mg/kg included abnormal gait and ptosis. All four animals died at the 3000 mg/kg level. The necropsy findings showed one male had a hemorrhage on the lung with red intestines and one female had red intestines. No other visible lesions were found in these two animals or the remaining two animals. All four animals died at the 1666.6 mg/kg level. The necropsy finding showed all animals with red intestines and no other visible lesions. No deaths occurred at the three lower levels.

Based on the results of the dose-range finding study and in discussion with the sponsor, the dose selected for the Micronucleus Assay was 400 mg/kg.

All test animals were dosed by the intraperitoneal injection at a constant volume of 20 ml/kg. The multiple dose groups received the test article in a split dose regimen with two doses of 400 mg/kg each, separated by 24 hours. The time of sacrifice and cell harvest were determined from the initial treatment. The experimental design for the Micronucleus test was as follows:

Group	No. of Mice	Cell Harvest (hrs.)	Total Dose
Positive control	8	30 hours	0.5 mg/kg TEM
Negative control	8	48 hours	2 x distilled H_2O
Single Dose Group I	8	30 hours	400 mg/kg
Single Dose Group II	8	48 hours	400 mg/kg
Multiple Dose Group I	8	48 hours	2 x 400 mg/kg
Multiple Dose Group II	8	72 hours	2 x 400 mg/kg

Immediately following the first dose, writhing, decreased activity and hypersensitivity were observed at all levels. At 4 hours post dose, writhing, piloerection, decreased activity and tone and abnormal gait were observed. At 24 hours piloerection, decreased tone and body drop were exhibited at all levels. Immediately following the second dose and at 4 and 24 hours, writhing, piloerection, decreased tone and activity, body drop, abnormal gait and ptosis were observed in Multiple Dose Group I and II. One male in Multiple Dose Group I and another male in Multiple Dose Group II died prior to sacrifice. Both necropsy findings showed subcutaneous edema and ascites.

All mice were sacrificed by cervical dislocation at the appropriate time interval after dosing and slides were prepared. Slides were scored for numbers of polychromatic erythrocytes (PCE) with micronuclei, in 1000 PCE.

Stability and Purity

There was no apparent change in the physical state of the test article and control article during administration. The purity of the test article is the responsibility of the sponsor.

Results and Conclusions

The results for test article, Glyoxal 40 LF, were negative in the Micronucleus Test (MNT), at a dose of 400 mg/kg in the single dose groups and with a second dose of 400 mg/kg in the multiple dose groups administered in a split dose regimen. These findings are based upon the inability of the chemical to produce a statistically significant increase in the number of micronuclei per 1000 polychromatic erythrocytes in the treated versus the control group.

PHARMAKON RESEARCH INTERNATIONAL, INC.
WAVERLY, PENNSYLVANIA 18471

PHONE
(717) 586-2411

Genetic Toxicology

Micronucleus Test

SUMMARY DATA

Sponsor: American Cyanamid Company

Material: Glyoxal 40 LF

Description: Clear liquid

Date Initiated: June 2, 1982

Study No.: PH 309A-AC-002-82

S.O.P. No.: PH-309A

Pharmakon Ref.: #310, pgs: 74-78
#310, pgs. 79-88

Date Terminated: June 23, 1982

MICRONUCLEI/1000 POLYCHROMATIC ERYTHROCYTES

	Controls		Test Compounds			
			Single Dose		Multiple Dose	
Animal Number	Distilled H_2O	TEM	400 mg/kg			
Male	20 ml/kg	0.5 mg/kg	I	II	I	II
1	0	34	0	1	2	0
2	0	19	2	2	-	3
3	0	39	1	1	0	0
4	0	22	4	0	0	-
Female						
5	2	19	2	0	1	0
6	1	19	0	1	1	2
7	1	25	2	0	1	0
8	0	23	2	0	0	0
Mean	0.500	25.000	1.625	0.625	0.714	0.714
"t" value	-	9.145*	2.113	0.334	0.548	0.394

*,** Denotes statistical significance at the 0.05, 0.01 level, respectively.

Study Director Rita M. Sorg

Date 6/25/82

EXPERIMENTAL PROCEDURE

CHROMOSOMAL ABERRATION ASSAY

Micronucleus Test

Species:	Mouse
Strain:	CD-1
Source:	Charles River Breeding Laboratories Wilmington, Massachusetts
Sex:	Male and female
Age at Initiation:	Seven (7) to twelve (12) weeks
No. on Study:	Forty-eight (48)
Acclimation Period:	Five (5) days
Identification:	Cage number and ear tags.

HUSBANDRY

Research Facility Registration:	U.S.D.A. Registration No. 23-107 under the Animal Welfare Act: SC 2131 *et seq*.
Animal Rooms:	Separate isolation by test system Light cycle - 12 hours light, 12 hours dark Temperature/Humidity - every attempt will be made to maintain a temperature of 22°C ± 3°C and humidity of 30 to 70%.
Housing:	Mice were housed four (4) per box, according to sex, in plastic tote boxes measuring 11-1/4" x 6-3/4" x 5-1/4". fitted with 1/4" galvanized mesh lids. Size in accordance with "Guide for the Care and Use of Laboratory Animals" of the Institute of Laboratory Resources, National Research Council.
Bedding:	Beta Chip ® Bedding, North Eastern Products, Warrensburg, New York. Bedding amount used was sufficient to provide

sanitary conditions for three days.

Sanitization: Bedding was changed on alternate study days. Tote boxes were sanitized at time of bedding change. All bottles, stoppers and sipper tubes were sanitized on alternate working days. Sanitization, filling and stopper insertion are performed in designated areas outside the animal rooms.

Food: Wayne Lab Blox®, ad libitum, checked daily and added or replaced as needed. Feeders are designed to prevent soiling, bridging, and scattering.

Food Analysis: Acute doses minimize the effect of contaminants. There are no interfering contaminants that are reasonably expected to be present in the dietary material known to be capable of interfering with the purpose or conduct of the study.

Water: Availability - fresh tap water, fit for human consumption, ad libitum, in sanitized eight (8) ounce glass bottles with rubber stopper and stainless steel sipper tube.

Water Analysis: Conducted by Pennsylvania Gas and Water Company and results provided to Pharmakon Laboratories annually.

Justification of Test System: Mice have historically been used in the Micronucleus Test and have been shown to exhibit micronuclei indicative of chromosome breaking effects of known mutagens administered intraperitoneally.[1]

Compound Preparation: Proper amounts of test material were ground with a pestle or tissue grinder. In case of a liquid material, proper amounts were weighed in a glass vial. Vehicle was then added in proper increments to achieve a designated dose level.

0 0 0 9

Chromosomal Aberration Assay

Vehicle: Distilled Water

Dose Administration: 400 mg/kg

Group Designation:

Group	No. of Mice	Dose
1	8	TEM - 0.5 mg/kg (sacrificed at 30 hours)
2	8	Distilled Water (20 ml/kg) (sacrificed at 48 hours)
3	8	Glyoxal (400 mg/kg) (MI) (sacrificed at 48 hours)
4	8	Glyoxal (400 mg/kg) (MII) (sacrificed at 72 hours)
5	8	Glyoxal (400 mg/kg) (SI) (sacrificed at 30 hours)
6	8	Glyoxal (400 mg/kg) (SII) (sacrificed at 48 hours)

Volume Administration: 20 ml/kg of body weight

Route of Administration: Intraperitoneally (i.p.)

Justification for Route of Administration: Intraperitoneal administration increases the likelihood of bioavailability and enhances the likelihood of submitting a large proportion of the target cell population to the test article.

Frequency of Administration: Once in the positive control and the single dose groups. Two doses separated by 24 hours in the multiple dose groups, and the negative control.

No. of Mice per Dose Group: Eight (8) - four (4) males and four (4) females.

Method of Randomization: Randomization will be by body weight in order to maintain a consistant mean weight $\pm$ 2 grams among all groups of mice.

Chromosomal Aberration Assay

Methods of Study Performance:

Test chemicals were administered i.p. to four (4) male and four (4) female albino mice in each dose group. Concurrently, triethylenemelamine, the positive control compound was given intraperitoneally to mice at a dose of 0.5 mg/kg. Multiple dose groups and the negative control received a second injection 24 hours after the first. Thirty hours after treatment the positive control animals (Group 1), and the single dose group I (Group 5) were sacrificed. The negative control animals (Group 2) were sacrificed 48 hours after the first injection. Single dose group II (Group 6) animals were also sacrificed at 48 hours. The multiple dose group I animals were sacrificed at 48 hours (Groups 3) and multiple dose group II animals were sacrificed at 72 hours (Group 4) after the first dose. All animals were sacrificed by cervical dislocation and their femurs were removed. The femurs were removed by leaving them intact on the knee and pelvis ends. They were freed from muscle using "Kimwipes" and forceps. The bone was opened carefully at the proximal end with a scissors until a small opening to the marrow canal became visible. A 1 ml tuberculin syringe filled with 0.2 ml fetal calf serum was inserted into the bone and the bone marrow was gently flushed (to assure maximum dispersion) into 1.0 ml of fetal calf serum in a 3 ml conical centrifuge tube. The femur was flushed with fetal calf serum until all the marrow was out and the bone appeared almost transparent. If necessary, the distal end was opened and flushed. The suspension was centrifuged at 1000 rpm for five minutes in an HS-4 Sorvall Centrifuge Head. The supernatant was removed with a pasteur pipette,

leaving a small amount of fetal calf serum with the remaining cell button. The cells remained in fetal calf serum for no longer than 15 minutes. The button was mixed with a pasteur pipette to assure a homogenous mixture. A small drop of the mixture was immediately placed near the frosted end of a glass microscope slide previously cleaned in absolute ethanol and pulled behind a clean slide at a 45° angle. The slides were quick dried on a slide warmer set at 56°C. Following preparation of the smears, they were dipped in absolute methanol and allowed to [illegible] overnight.

Staining: The staining procedure took place in staining jars in the following order:

a) Fixed in absolute methanol - 5 minutes and air dried.
b) Remove metallic film from surface of Giemsa working solution using a paper towel.
c) Stain 20 minutes in a 5% Giemsa stain.
d) Rinse twice. The first rinse was in distilled water adjusted to pH 4.0 to 4.5 and the second rinse in distilled water adjusted to pH 7.0.
e) Clean back of slide with absolute methanol.
f) Dry on 56°C slide warmer.
g) Clear in xylene, 5 minutes.
h) Mount in permount with cover glass.

Evaluation: The slides were screened for good preparation, i.e. well spread, undamaged perfectly stained. One thousand (1000) polychromatic erythrocytes were counted for the presence of micronuclei. The frequency of micronucleated cells was expressed as number polychromatic micronucleated

0 0 1 2

erythrocytes versus total polychromatic erythrocytes. The slides were examined using the oil immersion objective of an American Optical Microstar or Nikon Optiphot Binocular Microscope. Results were analyzed according to the Student's "t" test.

Coding of Slides:

Slides were coded randomly by study number and letter designation. The code was kept on a separate sheet in the sponsor's file until the slides were evaluated. Following evaluation, the slides were decoded and the code sheet was placed in the notebook. These procedures were carried out by personnel not involved in the actual performance of the study.

Interpretation of Results:

If there was no statistically significant increase in the number of micronuclei in polychromatic erythrocytes in both the multiple and single treatment groups as compared to the negative control, the test article was considered negative. If an increase in the frequency of micronuclei was detected in the treatment groups, the test article was considered a suspect mutagen.

Criteria for a Valid Test:

If the spontaneous rate of micronuclei in the polychromatic erythrocytes was less than 0.4% and the positive control was at least three fold (3x's) greater than the spontaneous and at least six animals per group survived the treatment, the results were deemed acceptable.

Records Maintained:

All correspondence pertinent to the study between the sponsor and Pharmakon Research International, Inc., protocol, amendments to the protocol, raw data, test chemical weight or volume, dispensation reports, quality assurance reports and

0 0 1 3

Cytogenetic Assay - MNT Book No. 310

Page No. 24

Sponsor: American Cyanamid Company
Test Article: CYANA CHEM® Glyoxal 40 LF Date Initiated: 6-2-82
Description: Clear Liquid Date Terminated: 6/23/82

Compound Preparation: Positive Control: TEM 0.5 mg/kg
3.5 mg of TEM + 14 ml Distilled Water ⇒ Stock Solution (5 mg/kg)

Dose: 1 ml Stock Solution + 9 ml Distilled Water ⇒ 0.5 mg/kg

Positive Control - TEM at 0.5 mg/kg Sacrifice at 30 hrs. after Single dose.

Dose: 0.5 mg/kg

Mouse No.	Sex	Initial wt. (gms)	Ml. admin	Time of admin	Final wt. (gms)	Date of Sacrifice	No. of P.C.E.	No. of P.C.E. with Microsuclei
2041	♂	36	.72	7:53	35		966	34
2042	♂	34	.68		32		981	19
2043	♂	31	.62		31		961	39
2044	♂	31	.62		30	6/3/82	978	22
2045	♀	30	.60		29		981	19
2046	♀	28	.56		27		981	19
2047	♀	28	.56		27		975	25
2048	♀	29	.58	8:00	29		977	23
		[illegible] 6-2-82	[illegible] 6-2-82	[illegible] 6-2-82	[illegible] 6-3-82	[illegible] 6/3/82	[illegible] 6/23/82	[illegible] 6/23/82

Observations immediate: No signs [illegible] 6-2-82
Hrs pd #1: No signs [illegible] 6-2-82
Hrs pd #2: No signs [illegible] 6-3-82

* Dosed by [illegible] 6/2/82, 6/3/82 [illegible] 6-2-82

To Page No. 86

Witnessed & Understood by me.	Date	Invented by	Date
Ruth M. Sorg	6/23/82	Recorded by [illegible]	6/23/82

Cytogenetic [illegible] — Book No [illegible]

From Page No. 85

Sponsor: American Cyanamid Company

Values obtained from coded slide (and subsequent decoding of test article) CYANA [illegible] (R) Physical [illegible]

Code	Polychromatic Erythrocytes	Polychromatic Erythrocytes with Micronuclei	Dilution
A	966	34	TEM 1♂
B	981	19	TEM 2♂
C	961	39	TEM 3♂
D	978	22	TEM 4♂
E	981	19	TEM 5♀
F	981	19	TEM 6♀
G	975	25	TEM 7♀
H	977	23	TEM 8♀
I	1000	0	dH_2O 1♂
J	1000	0	dH_2O 2♂
K	1000	0	dH_2O 3♂
L	1000	0	dH_2O 4♂
M	998	2	dH_2O 5♀
N	999	1	dH_2O 6♀
O	999	1	dH_2O 7♀
P	1000	0	dH_2O 8♀
Q	1000	0	SI 1♂
R	998	2	SI 2♂
S	999	1	SI 3♂
T	996	4	SI 4♂
U	998	2	SI 5♀
V	1000	0	SI 6♀
W	998	2	SI 7♀
X	998	2	SI 8♀
Y	999	1	SII 1♂
Z	998	2	SII 2♂
AA	999	1	SII 3♂
BB	1000	0	SII 4♀
CC	1000	0	SII 5♀
DD	999	1	SII 6♀
EE	1000	0	SII 7♀

To Page No. [illegible]

Witnessed & Understood by me.	Date	Invented by	Date
Ruth M. Sorg	6/23/82	Recorded by [illegible]	6/22/82

Cytogenetic Assay - MNT

Page No. 86

Sponsor: American Cyanamid Company
Values obtained from coded slides and subsequent decoding of test article CYANA CHEM® Glyoxal 40 LF.

Code [illegible]	Polychromatic Erythrocytes	Polychromatic Erythrocytes with Micronuclei	Decode
FF	1000	0	SII 8♀
GG	998	2	MI 1♂
HH	1000	0	MI 3♂
II	1000	0	MI 4♂
JJ	999	1	MI 5♀
KK	999	1	MI 6♀
LL	999	1	MI 7♀
MM	1000	0	MI 8♀
NN	1000	0	MII 1♂
OO	997	3	MII 2♂
PP	1000	0	MII 3♂
QQ	1000	0	MII 5♀
RR	998	2	MII 6♀
SS	1000	0	MII 7♀
TT	1000	0	MII 8♀

Coded by Margaret H. Kevish 6/9/82
Decoded by Margaret H. Kevish 6/23/82

Less than five micronucleated mature erythrocytes remain the field scanned to find 1000 PCEs.

To Page No. 88

Witnessed & Understood by me,	Date	Invented by	Date
Ruth M. Sorg	6/23/82	Recorded by [illegible]	6/22/82

Project No. [illegible]

Book No. 310

88

LE Cytogenetic Array - MNT
[illegible] Test Slide Code

30 A-AC-002-82.

Date: June 9, 1982

[illegible]: CYANA CHEM® Glyoxal 40LF

Coded by: Margaret A. Kivish

[illegible] 400 mg/kg

Decoded by: Margaret A. Kivish

TEM	1♂		X.	SI	8♀
TEM	2♂		Y.	SII	1♂
TEM	3♂		Z.	SII	2♂
TEM	4♂		AA.	SII	3♂
TEM	5♀		BB.	SII	4♂
TEM	6♀		CC.	SII	5♀
TEM	7♀		DD.	SII	6♀
TEM	8♀		EE.	SII	7♀
dH_2O	1♂		FF	SII	8♀
dH_2O	2♂		GG.	MI	1♂
dH_2O	3♂		HH.	MI	3♂
dH_2O	4♂		II.	MI	4♂
dH_2O	5♀		JJ.	MI	5♀
dH_2O	6♀		KK.	MI	6♀
dH_2O	7♀		LL.	MI	7♀
dH_2O	8♀		MM.	MI	8♀
400 mg/kg	1♂	SI	NN.	MII	1♂
400	2♂	SI	OO.	MII	2♂
400	3♂	SI	PP.	MII	3♂
400	4♂	SI	QQ.	MII	5♀
400	5♀	SI	RR.	MII	6♀
400	6♀	SI	SS.	MII	7♀
400	7♀	SI	TT.	MII	8♀

Witnessed & Understood by me.	Date	Invented by	Date
Ruth M. Sorg	6/23/82	Recorded by Susan Mucho Fucenti	6/23/82

PHARMAKON RESEARCH INTERNATIONAL, INC.
WAVERLY, PENNSYLVANIA 18471

PHONE
(717) 586-2411

QUALITY ASSURANCE UNIT STATEMENT

This study was performed in accordance with the Good Laboratory Practices Regulation for non-clinical laboratory studies as developed by the U.S. Food and Drug Administration, as indicated in the Federal Register, Part II of December 22, 1978; Part 58, Title 21.

Study No. PH 309A-AC-002-82

The following inspections were performed:

Interval	Date
Preliminary Phase	6/2/82
Cell + Slide Preparation Phase	6/3/82
Reporting Phase	6/30/82

Results of the above inspections were submitted to the Study Director and Management during the course of the study.

6/30/82
Date

Quality Assurance Unit

	final report, microscope slides prepared during the study and a sample of the test chemical are maintained in the Pharmakon Archives.
Reserve Sample Retention:	The in vivo phases of the study were less than four weeks duration. Studies of less than four weeks duration do not require retention of reserve samples.
Raw Data:	Standard Pharmakon Notebook
Technical Performance:	Ruth Sorg, Frederika Nicholl, Nira Madison, Edmund Godek, Susan Lucenti and Margaret Kevish

NEGATIVE CONTROL ARTICLE

Vehicle:	Distilled Water
Lot No.:	Not applicable
Supplier (Source):	Not applicable
Preparation:	Milli-Q
Description:	Clear liquid
How Supplied:	Not applicable
Special Handling Instructions:	None
Analysis of Purity:	Responsibility of the manufacturer.
Rationale for Control Article:	Suspendability and/or solubility of the test article.

POSITIVE CONTROL ARTICLE

Positive Control:	Triethylenemelamine (TEM)
Lot No.:	6-246
Catalog No.:	1250
Supplier (Source):	Polysciences, Inc. Paul Valley Industrial Park Warrington, Pennsylvania 18976
Preparation:	Stock solution will be maintained in dark container at 5°C until used.

Micronucleus Test
Chromosomal Aberration Assay

Description:	White crystal
Special Handling Instructions:	Standard precautions. Mask and gloves were worn while dosing.
Analysis of Purity:	The purity is the responsibility of the manufacturer.
How Supplied:	One, one gram bottle
Rationale for Positive Control:	Used as a comparison because of its known effects.
Good Laboratory Practices Statement:	This study was conducted in compliance with the Good Laboratory Practices Regulations except if noted to the contrary. There were no significant deviations from the Good Laboratory Practices Regulations which affected the quality or integrity of the study. Q.A.U. findings derived from the inspection(s) during the conduct of this study and from the audit of the final report are documented and have been provided to the study director and to the test facility management.
Reference[1]:	Schmid, W., The Micronucleus Test, Mutation Research, 31, (1975) 9-15. Salamone, Michael, J. Heddle, E. Stuart and M. Katz. Towards An Improved Micronucleus Test, Mutation Research, 74, (1980) 347-356.

0 0 2 0

Project No. [illegible]

Book No. 310

LE Dose Range Finding - MNT

m Page No. —

Purpose: To determine the optimum dose of a test article administered intraperitoneally to mice, for use in the micronucleus test (2 dose)

Sponsor: American Cyanamid Company

Test Article: CYANA CHEM® Glyoxal 40 LF — Date initiated: 5/25/82

Description: Clear liquid — Date Terminated: 5/28/82

Solubility: Soluble in distilled H_2O at all levels tested.

Vehicle: Distilled H_2O — ♂ & ♀ PO# 051082 DTOX Rec'd 5/13/82

Volume Administered: 20 ml/kg

Mouse identification: 1♂ No mark — 3♀ Purple back mark

(magic marker) 2♂ Black head mark — 4♀ Purple tail mark

S.O.P.# PH 344A

Pennsylvania Scale #2 — calibrated: the week of the study

Mettler #2 #35 AR — calibrated: the week of the study

Compound Preparation: 166.6 mg/kg at 20 ml/kg = 8.33 mg/ml

SMZ 5-25-82 *Specific Gravity = 1.04 gm/ml

Dose #1: 48.6 mg CYANA CHEM® Glyoxal 40 LF + 5.79 ml dH_2O

Glyoxal was weighed out in bottle covered with parafilm. The dH_2O was added in entirety. Prior to each group, the dose was mixed with a plastic syringe.

SMZ 5-26-82

Dose #2: 53.5 mg CYANA CHEM® Glyoxal 40 LF + 6.37 ml distilled H_2O

Prepared as above.

Dose: 166.6 mg/Kg

Mouse No.	Sex	Initial wt. (gms)	Ml. admin	Time of Dose	Day 2 wt. (gms)	ml admin	Time of Dose	Final wt. (gms)	Total Deaths
1	♂	36	5 of 5/25/82 RMS .72	9:24	36	.72	9:39	33	
2	♂	35	.70	↓	35	.70	↓	32	0/4
3	♀	26	.52	↓	26	.52	↓	27	
4	♀	25	.50	9:27	25	.50	9:42	23	
		SMZ 5/25/82	RMS 5/25/82	RMS 5/25/82	RMS 5/26/82	RMS 5/26/82	RMS 5/26/82	SMZ 5/28/82	SMZ 5/28/82

Observations: Immediate - writhing hypersensitive, decreased activity RMS 5/25/82

4 hrs pd #1: Pilorection SMZ 5-26-82

24 hrs pd #1: Pilorection, body drop (in ♂♂) decreased tone. RMS 5/26/82

Immediate pd #2 - Writhing, decreased tone and activity, pilorection, body drop in ♂♂. RMS 5/26/82

4 hrs pd #2: Pilorection; ♂♂ body drop; decreased tone. SMZ 5-26-82

8 hrs pd #2: Pilorection; ♂♂ body drop; decreased body tone (♂ & ♀) SMZ 5-27-82

8 hrs pd #2: ♂ & ♀ Pilorection; decreased tone; body drop SMZ 5-28-82 SMZ 5-27-82

To Page No. 75

Witnessed & Understood by me,	Date	Invented by	Date
Ruth M. Sorg	5/28/82	Recorded by Susan Mueller Lucenti	5/28/82

0 0 2 1

Project No. [illegible]

Book No. 310

From Page No. 94

Sponsor: American Cyanamid Company

Test Article: CYANA CHEM® Glyoxal 40 LF

Description: Clear liquid

Date Initiated: 5/25/82

Date Terminated: 5/28/82

Compound Preparation: 500 mg/kg at 20 ml/kg = [illegible] mg/ml

Smt 5-25-82 Dose #1: 127.8 mg of CYANA CHEM® Glyoxal 40 LF + 4.99 ml [illegible]

Article was prepared as on pg. 71.

Smt 5-26-82 Dose #2: 135.5 mg of CYANA CHEM® Glyoxal 40 LF + 5.29 ml distilled [illegible]

Dose prepared as on pg. 71

Dose 500 mg/kg

Mouse No.	Sex	Initial wt. (gms)	ml admin	Time of Dose	Day 2 wt. (gms)	ml admin	Time of Dose	Final wt. (gms)	Total Deaths
1	♂	36	.72	9:30	35	.70	9:44	35	
2	♂	34	.68	↓	33	.66	↓	35	0/4
3	♀	27	.54	↓	26	.52	↓	24	
4	♀	26	.52	9:35	26	.52	9:47	26	
		Smt 5/25/82	RMS 5/25/82	RMS 5/25/82	RMS 5/26/82	RMS 5/26/82	RMS 5/26/82	Smt 5/28/82	Smt 5/28/82

Observations: Immediate: Writhing, hypersensitive, decreased activity. RMS 5/25/82

4 hrs pd #1: abnormal gait, writhing; decrease tone & activity; piloerection Smt 5-26

24 hrs pd #1: decreased tone, piloerection, body drop Smt 5/26

Immediate pd #2: Decreased tone and activity, writhing, piloerection, ptosis, ♀♀ hypersensitive. RMS 5/26/82

4 hrs pd #2: decreased tone & activity, ptosis (1♂) piloerection; hypersensitive (♀♀) Smt 5/26/82

24 hrs pd #2: decrease body tone & activity; 2♀ 1♂ abnormal gait; ♂, ♀ have body drop

48 hrs pd #2: piloerection; decreased tone; body drop; decreased activity; 2♀♀; 1♂ abnormal gait Smt 5-28-82 1♂ & 1♀ slight ptosis Smt 5-28-82

To Page No.

Witnessed & Understood by me, Ruth M. Sorg

Date 5/28/82

Invented by

Recorded by Susan Murch & Lucenti

Date 5/28/82

Project No. PR-2444-AC Test #2

Title: Acute Range Finding - MRT Book No. 310

From Page No. 75

Sponsor: American Cyanamid Company
Test Article: CYANA CHEM® Glyphosate 10 LF
Description: Clear liquid

Date Initiated: 5/25/82
Date Terminated: 5/28/82

Compound Preparation: 50 mg/kg at 20 ml/kg = 2.5 mg/ml

Dose #1: 44.6 mg CYANA CHEM® glyphosate 10LF + 17.8 ml dist H2O (5-25-82 Smk)
Dose prepared as on pg. 74.

Dose #2: 13.5 mg CYANA CHEM® glyphosate 10LF + 4.9 ml distilled H2O (5-26-82 Smk)
Dose prepared as on pg. 74.

Dose: 50 mg/kg

Animal No.	Sex	Initial wt. (gms)	ml admin	Time of Dose	Day 2 wt. (gms)	ml admin	Time of Dose	Final wt. (gms)	Total Deaths
1	♂	36	.72	10:15	35	.70	10:31	34	0/4
2	♂	35	.70	↓	35	.70	↓	34	
3	♀	28	.56	↓	27	.54	↓	25	
4	♀	24	.48	10:18	24	.48	10:35	24	
		Smk 5/25/82	RMS 5/26/82	RMS 5/25/82	RMS 5/26/82	RMS 5/26/82	RMS 5/26/82	Smk 5/28/82	Smk 5/28/82

Observations: Immediate Writhing, hypersensitive RMS 5/25/82

Thurs pd #1: piloerection (Smk 5-27-82)
24hrs pd #1: Hypersensitive, piloerection, RMS 5/26/82
Immediate pd #2 - Writhing decreased tone and activity, hypersensitive RMS 5/26/82

Thurs pd #2: piloerection, hypersensitive (Smk 5/26/82)
48hrs pd #2: piloerection; slight decrease in body tone (Smk 5/27/82)
72hrs pd #2: piloerection; decrease in body tone (Smk 5-28-82)

To Page No. 77

Witnessed & Understood by me,	Date	Invented by	Date
Ruth M. Sorg	5/28/82	Recorded by Susan Thurkettle	5/28/82

0023

Title: Dose Range Finding - MNT Book No. 310

Page No. 77

Sponsor: American Cyanamid Company
Test Article: CYANA CHEM® Glyoxal to LF Date Initiated: 5/25/82
Description: Clear liquid Date Terminated: 5/28/82

Compound Preparation: 1666.6 mg/kg at 20 ml/kg = 83.33 mg/ml
Smt 5-25-82 Dose #1: 543.6 mg CYANA CHEM® Glyoxal to LF + 6 ml of H_2O
Dose prepared as on pg. 74.

Smt 5-26-82 Dose #2: 542.0 mg CYANACHEM® Glyoxal to LF + 5.98 ml distilled H_2O
Dose prepared as on pg. 74.

Dose: 1666.6 mg/kg

Mouse No.	Sex	Initial wt. (gms)	ml admin	Time of Dose	Day 2 wt. (gms)	ml admin	Time of Dose	Final wt. (gms)	Total Deaths
1	♂	36	.72	10:20	dead	5/26/82	→	35	
2	♂	34	.68	↓	33	.66	10:37	32	4/4
3	♀	28	.56	↓	dead	5/26/82	→	28	
4	♀	26	.52	10:24	dead	5/26/82	→	25	
		Smt 5/26/82	Smt 5/26/82	RMS 5/26/82	RMS 5/26/82	RMS 5/26/82	RMS 5/26/82	Smt 5/27/82	Smt 5/28/82

Observations, Immediate: Writhing, hypersensitive, piloerection, 1♀ ptosis, decreased activity RMS 5/26/82
4hrs pd #1: Piloerection, decreased activity; hypersensitive; 1♂ ptosis; dyspnea; 2♀ moribund; ptosis Smt 5-25

24hrs pd #1 - Piloerection, hypersensitivity in remaining ♂ RMS 5/26/82
1♂, 2♀♀ dead. Necropsy findings - Red intestines with no other visible lesions
Immediate pd #2 - Piloerection, writhing, decreased tone and activity, ptosis RMS 5/26/82
4hrs pd #2: decreased tone, activity, prostrate, dyspnea, ptosis Smt 5/26/82
24hrs pd #2 ♂ dead Necropsy Findings: Red intestines with no other visible lesions Smt 5/27/82

To Page No. 78

Witnessed & Understood by me, Ruth M. Sorg Date 5/28/82 Invented by Date
Recorded by Susan Murphy Date 5/28/82

0024

Project No. [illegible]

Dose Range Finding - MNT Book No. 310

Page No. 77

Sponsor: American Cyanamid Company
Test Article: CYANA CHEM® Glyoxal 40 LF
Description: Clear liquid

Date Initiated: 5/25/82
Date Terminated: 5/28/82

Compound Preparation: 3000 mg/kg at 20 ml/kg = 150 mg/ml
SmL 5-25-82 Dose #1: 942.3 mg CYANA CHEM® Glyoxal 40 LF + 5.37 ml dH_2O
Dose prepared as on pg. 74.

Dose: 3000 mg/kg

Mouse No.	Sex	Initial wt. (gms)	ml admin	Time of Dose	Day 2 wt (gms)	ml admin	Time of Dose	Final wt. (gms)	Total Deaths
1	♂	34	.68	10:26	33 dead 5/25/82			33	
2	♂	35	.70	↓	dead 5/25/82			35	4/4
3	♀	28	.56	↓	dead 5/25/82			28	
4	♀	26	.52	10:29	dead 5-26-82			26	
		SmL 5/25/82	RMS 5/25/82	RMS 5/25/82				SmL 5/25/82	SmL 5/28/82

RMS 5/25/82

Observations: Immediate: Writhing, hypersensitive, piloerection, abnormal gait, decreased activity, ptosis, ocular discharge.
4hrs pd #1: ~~4/4 dead~~ 3/4 dead DEMIL 5-26-82;
Necropsy Finding:
18 - hemorrhage on the lung; pink intestines; 2♂; 3♀ - No visible lesion.
4♀ - moribund; dyspnea; prostrate; ptosis SmL 5-25-82
24 hrs pd #1 - 4♀ dead -
Necropsy findings - Red intestines RMS with no visible lesions 5/26/82

To Page No. ____

Witnessed & Understood by me,	Date	Invented by	Date
Ruth M. Sorg	5/28/82	Recorded by Susan Mucha Lucenti	5/28/82

0025

Project No. - [illegible]
Book No. 310 79
Page No. 79

Purpose: To determine the chromosome breaking effects measured by the increase in micronuclei in mice pretreated intraperitoneally with the test article.

S.O.P.#: PH 309A-AC-002-81 Initiation Date of Dose Range: 5/25/82
Sponsor: American Cyanamid Company Initiation Date: 6-2-82
Test Article: Glyoxal 40 LF (CYANACHEM®) Termination Date: 6-23-82
Description: Clear Liquid
Solubility: Good Solution
Vehicle: Distilled H_2O
Volume Administered: 20 ml/kg
Compound Receipt: 5/12/82
Amount submitted: 1 quart

Species: Mouse Strain - CD-1 Sex: 24♂; 24♀
Animal P.O.# ♂ 051082 DTox Rec'd 5/13/82 ♀ 051082 DTox Rec'd 5/13/82
No. of Animals per group: 8 (4♂; 4♀)
Fasting: Not Applicable Animal Randomization: By weight
Animal identification: Cage number and ear tags according to PH 026.

Dose Levels: Single dose (low): [illegible] mg/kg in a single dose
Multiple dose (high): 2 doses of 400 mg/kg separated by 24 hours
Route of administration: Intraperitoneal Injection
Length of Study: 4 days (in vivo)
Food Lot # PO 4132 Rec'd 4/22/82
Pennsylvania Scale # 2 calibrated: the week of the study
Mettler Balance: H35 AR # 2 calibrated: the week of the study
Light cycle checked on: 4/2/82 12 hrs light; 12 hrs dark

To Page No. 80

Witnessed & Understood by me,	Date	Invented by	Date
Ruth N. Sorg	6/23/82	Recorded by Susan Mucho Lucente	6/23/82

[illegible] Assay [illegible]

Page No. 79

Sponsor: American Cyanamid Company
Test Article: CYANA CHEM® Glyoxal 40 LF — Date Initiated: 6-2-82
Description: Clear Liquid — Date Terminated: 6/23/82

Compound Preparation: Single Dose Group I: 400 mg/kg at 20 ml/kg = 20 mg/ml
Dose #1: 618.5 mg CYANA CHEM® Glyoxal 40 LF + 30.34 ml Distilled Water
Compound was weighed out in a bottle covered with parafilm. All of the distilled water was added and the dose was mixed with a plastic syringe prior to each group. SmL 6-2-82

Single Dose Group I - Sacrifice 30 hrs after single dose.

Dose: 400 mg/kg

Mouse No.	Sex	Initial wt. (gms)	ml admin	Time of admin	Final wt. (gms)	Date of Sacrifice	No. of P.C.E.*	No. of P.C.E. with Micronuclei	Observations, immediate:
2001	♂	36	.72	8:45	35		1000	0	Writhing; decreased activity, hypersensitive SmL 6-2-82
2002	♂	37	.74		35		998	2	4 hrs pd. #1: Pilorrection;
2003	♂	33	.66		32		999	1	abnormal gait; decreased
2004	♂	32	.64		31	6/3/82	996	4	tone & activity; body drop;
2005	♀	28	.56		28		998	2	writhing SmL 6-2-82
2006	♀	29	.58		29		1000	0	24 hrs pd #1: Pilorrection; SmL 6-3-82
2007	♀	28	.56		28		998	2	decreased tone, body drop
2008	♀	27	.54	8:55	25		998	2	
		SmL 6-2-82	SmL 6-2-82	SmL 6-2-82	SmL 6/3/82	SmL 6/3/82	SmL 6/23/82	SmL 6/23/82	

*P.C.E. - Polychromatic Erythrocyte.
Recd by SmL 6/3/82, 6/2/82 IDF SmL 6-2-82

To Page No. 81

Issued & Understood by me,	Date	Invented by	Date
Ruth M. Sorg	6/23/82	Recorded by Susan Mushakhicenti	6/23/82

0029

Cytogenetic Assay - MWT

Project No. [illegible]

Book No. 310

Page No. 81

Sponsor: American Cyanamid Company

Test Article: CYANA CHEM® Glyoxal 40 LF

Date Initiated: 6-2-82

Description: Clear Liquid

Date Terminated: 6/23/82

Compound Preparation: Single Dose Group II: 400 mg/kg at 20 ml/kg = 20 mg/ml

Dose: 1 Refer to pg. 80 SmL 6-2-82

- Single Dose Group II - Sacrifice: 48 hrs after single injection.

Dose: 400 mg/kg

Mouse No.	Sex	Initial wt (gms)	ml. admin	Time of admin	Final wt. (gms)	Date of Sacrifice	No. of P.C.E.	No. of P.C.E. with Micronuclei	Observations, immediate:
2009	♂	37	.74	9:41	38		999	1	Decreased activity; SmL 6-2-82
2010	♂	36	.72		37		998	2	writhing; hypersensitive
2011	♂	35	.70		37	6/4/82	999	1	thro pd #1: Pilorection;
2012	♂	32	.64		33		1000	0	writhing; decreased
2013	♀	28	.56		30		1000	0	activity & body tone;
2014	♀	30	.60		31		999	1	abnormal gait SmL 6-2-82
2015	♀	27	.54		29		1000	0	thro pd #1: Pilorection;
2016	♀	27	.54	9:52	28		1000	0	decreased body tone;
		SmL 6-2-82	SmL 6-2-82	SmL 6-2-82	SmL 6/4/82	SmL 6/4/82	SmL 6/23/82	SmL 6/23/82	body drop; 40° hypersensitive SmL 6-3-82

Dosed by SmL 6/2/82

To Page No. 82

Attended & Understood by me. Ruth M. Sorg

Date 6/23/82

Invented by

Date

Recorded by Susan Kusha Thesenti 6/23/82

0028

Cytogenetic Assay - MIT

Page No. 81

Sponsor: American Cyanamid Company

Test Article: CYANA CHEM® Glyoxal 40% LF — Date Initiated: 6-2-82

Description: Clear Liquid — Date Terminated: 6/23/82

Proposed Preparation: Multiple Dose Group I: 4x 40 mg/kg at 20 ml/kg = 80 mg/ml

Dose #1: Refer to pg. 80 (Smk 6-2-82)

Dose #2: 296 mg CYANA CHEM® Glyoxal 40% LF + 14.52 ml distilled H_2O (Smk 6-3-82)

Prepared same as Dose #1 on p. 80.

Multiple Dose Group I - Sacrificed 48 hours after first dose

given 2 doses of 40 mg/kg separated by 24 hours.

Dose: 40 mg/kg

Animal No.	Sex	Initial wt (grams)	Vol. admin.	Time of admin.	Days wt. (grams)	Vol. admin.	Time of admin.	Final wt. (grams)	Date of Sacrifice	No. of P.C.E.	No. of P.C.E. with micronuclei	Observations; comments
017	♂	36	.72	9:53	34	.68	10:01	36		998	2	Decreased activity, writhing, body tone, hypersensitive
018	♂	34	.74 +3		33	.66		34	*Dead 6/4/82			
019	♂	36	.72		35	.70		37	6/4/82	1000	0	Grp #1: Piloerection, writhing, decreased activity & body tone; abnormal gait (Smk 6-3-82)
020	♂	34	.74**		34	.68		33		1000	0	
021	♀	27	.54		28	.56		30		999	1	
022	♀	28	.56		27	.54		28		999	1	
023	♀	26	.52		26	.52		27		999	1	
024	♀	28	.56	10:04	27	.54	10:14	29		1000	0	Grp #1: Piloerection, decreased body tone, body drop (Smk 6-3-82)
		Smk 6-2-82	Smk 6-2-82	Smk 6-2-82	Smk 6/3/82	RMS 6/3/82	RMS 6/3/82	Smk 6/4/82	Smk 6/4/82	Smk 6/23/82	Smk 6/23/82	

Immediate dose #2: piloerection; (Smk 6-3-82) decreased tone; activity; body drop writhing; abnormal gait; slight ptosis

Grp'd #2: piloerection; slight ptosis; writhing; decreased activity & body tone; body drop; abnormal gait (Smk 6-3-82)

Grp'd #2 piloerection; slight ptosis; decreased activity & body tone; body drop writhing; abnormal gait; (Smk 6-4-82) #18 dead Necropsy findings: subcutaneous edema; ascites

Dose before sacrifice time — mean: 0.714

Animals inadvertently overdosed (due miscalculated) "t" value: 0.548

* used by Smk 6/4/82; 6/5/82

To Page No. 83

Read & Understood by me.	Date	Invented by	Date
Ruth M. Sorg	6/23/82	Recorded by Susan Thrun Schmitt	6/23/82

Cytogenetic Assay - MNT Book No. 316

Page No. 82

Sponsor: American Cyanamid Company
Test Article: CYANA CHEM® Glyoxal 40 LF Date Initiated: 6-2-82
Description: Clear Liquid Date Terminated: 6/23/82

Compound Preparation: Multiple Dose Group II: 400 mg/kg at 20 ml/kg = 20 mg/ml
Dose #1: Refer to pg 80 SmL 6-2-82

Dose #2: Refer to pg 82 SmL 6-3-82

Multiple Dose Group II: Sacrifice 72 hrs after first dose.
Given 2 doses of 400 mg/kg separated by 24 hrs.

Dose: 400 mg/kg

Mouse No.	Sex	Initial wt. (gms)	ml. admin	Time of admin	Day 2 wt. (gms)	ml. admin	Time of admin	Final wt. (gms)	Date of Sacrifice	No. of P.C.E.	No. of P.C.E. with Micronuclei	Observations; Immediate:
2025	♂	38	.76	10:06	35	.70	10:16	34		1000	0	Decreased activity, writhing, hypersensitive SmL 6-2-82
2026	♂	35	.70		33	.66		33		997	3	
2027	♂	34	.68 SEE SmL 6-2-82		34	.68		34		1000	0	4 hrs pd #1: Pilorection; writhing, decreased activity & body tone, abnormal gait SmL 6-2-82
2028	♂	32	.64		32	.64		32	*Dead	6/5/82		
2029	♀	27	.54		25	.50		27	6/5/82	1000	0	
2030	♀	29	.58		29	.58		31		998	2	
2031	♀	27	.54		27	.54		27		1000	0	24 hrs pd #1: Pilorection; decreased tone; body drop SmL 6-3-82
2032	♀	26	.52	10:17	25	.50	10:24	28		1000	0	
		SmL 6-2-82	SmL 6-2-82	SmL 6-2-82	SmL 6/3/82	RMS 6/3/82	RMS 6/3/82	SmL 6/5/82	SmL 6/5/82	SmL 6/23/82	SmL 6/23/82	

Dosed by SmL 6/2/82, 6/3/82
Immediate: Pilorection; decreased tone & activity; body drop, writhing, abnormal gait; slight ptosis SmL 6/3/82
4 hrs pd #2: Pilorection; slight ptosis; decreased activity & body tone; writhing; abnormal gait; body drop SmL 6-3-82 SEE SmL 6-3-82
24 hrs pd #2 Pilorection; slight ptosis; decreased activity & tone; body drop; writhing; abnormal gait SmL 6-4-82
* 4♂-2028 dead before sacrifice time; Necropsy Findings showed subcutaneous edema; ascites SmL 6/5/82 RMS

To Page No. 84

Witnessed & Understood by me, Ruth M. Sorg | Date 6/23/82 | Invented by | Date
Recorded by Susan Trucha Thiscanti 6/23/82

Cytogenetic Assay: MNT Book No. 310

From Page No. 83

Sponsor: American Cyanamid Company
Test Article: CYANA CHEM® Glyoxal 40 LF Date Initiated: 6-2-82
Description: Clear Liquid Date Terminated: 6/23/82

Compound Preparation: Negative Control: Distilled Water

Negative Control: Distilled Water : Sacrifice at 48 hrs after first dose.
Given 2 doses of 20 ml/kg each separated by 24 hours.

Dose: 20 ml/kg

Mouse No.	Sex	Initial wt (gms)	Ml. admin	Time of admin	Dose wt (gms)	Ml. admin	Time of admin	Final wt (gms)	Date of Sacrifice	No. of P.C.E.	No. of P.C.E. with micronuclei
2033	♂	37	.74	10:20	35	.70	10:26	35		1000	0
2034	♂	34	.68		33	.66		34		1000	0
2035	♂	34	.68		32	.64		33		1000	0
2036	♂	31	.62		30	.60		29	6/4/82	1000	0
2037	♀	29	.58		28	.56		27		998	2
2038	♀	30	.60		29	.58		28		999	1
2039	♀	28	.56		27	.54		27		999	1
2040	♀	30	.60	10:26	29	.58	10:30	29		1000	0
		SmL 6-2-82	SmL 6-2-82	SmL 6-2-82	SmL 6/2/82	RMS 6/3/82	RMS 6/3/82	SmL 6/4/82	SmL 6/4/82	SmL 6/23/82	SmL 6/23/82

Dosed by SmL 6/2/82, 6/3/82
Observations, immediate: No signs SmL 6-2-82
24 hrs pd #1: No signs SmL 6-2-82
24 hrs pd #2: No signs SmL 6-3-82
Immediate dose #2: No signs SmL 6/3/82
24 hrs pd #2: No signs SmL 6/3/82
24 hrs pd #2: No signs SmL 6/4/82

To Page No. 85

Witnessed & Understood by me, Ruth M. Sorg | Date 6/23/82 | Invented by | Date
Recorded by Susan Thursby Lucente 6/23/82

0091