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CYANAMID

American Cyanamid Company
One Cyanamid Plaza
Wayne, NJ 07470

91 DEC 26 PM 1:33

Michael D. Utidjian, M.D.
Corporate Medical Director

8EHQ-1291-1884 Ind.

December 18, 1991

CERTIFIED MAIL
RETURN RECEIPT REQUESTED

88920000530

Document Processing Center (TS-790)
Office of Toxic Substances
Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460

Att'n: Section 8(e) Coordinator (CAP Agreement)

RE: Study or Report Submitted Pursuant to the TSCA Section 8(e) Compliance Audit Program

Identification Number: 8ECAP-0041

Dear Sir/Madam:

American Cyanamid Company is submitting the attached study to the Environmental Protection Agency (EPA) pursuant to the Toxic Substances Control Act (TSCA) Section 8(e) Compliance Audit Program (CAP Agreement) executed by American Cyanamid and EPA. This study does not involve effects observed in humans. There is no confidential business information included.

The enclosed study provides information on the chemical substance ethanedial [CAS 107-22-2].

The title of the enclosed report is "CHO/HGPRT Mammalian Cell Forward Gene Mutation Assay - Glyoxal 40 LF".

Glyoxal was shown to produce an increased mutation frequency in the CHO/HGPRT Mammalian Cell Forward Gene Mutation Assay at 600 ug/ml of media in the presence of a metabolic activation system under the conditions of this assay and should be considered a suspect mutagen.

In total, American Cyanamid is submitting three (3) copies of the enclosed report and this cover letter: an original and two (2) copies.

Further questions regarding this submission may be directed to Dr. Richard A. Davis, Manager, Toxicology Operations at the address above or 201-831-4004.

Sincerely,

H.Michael D. Utidjian, M.D.
Corporate Medical Director

PHARMAKON RESEARCH INTERNATIONAL, INC.
[illegible]

PHONE
[illegible]

RECEIVED
OCT 5 1982
F. J. KOSCHIER

CHO/HGPRT Mammalian Cell Forward
Gene Mutation Assay

Glyoxal 40 LF

PR 114-AC-007-82

CT-046

Submitted to

American Cyanamid Company
Wayne, New Jersey

Edmund G. Godek
Study Director

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

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

September 28, 1982

PHARMAKON RESEARCH INTERNATIONAL, INC.
WAVERLY, PENNSYLVANIA 18471
PHONE
(717) 586-2411

SUMMARY DATA

Study Number: PH 314-AC-002-82

Study Description: CHO/HGPRT Mammalian Cell Forward Gene Mutation Assay with and without metabolic activation preparation according to Standard Operating Procedure PH-314 on:

Glyoxal 40 LF

Sponsor: American Cyanamid Company
Berdan Avenue
Wayne, New Jersey 07470

Purpose: To measure the ability of test article, Glyoxal 40 LF to induce mutations at the hypoxanthine-guanine phosphoribosyl transferase (hgprt) locus in Chinese Hamster Ovary (CHO) cells on the basis that presumptive mutants, by virtue of the loss of the HGPRT activity are unable to convert purine analogues such as 6-thioguanine (6-TG) to toxic metabolites and hence escape their lethal effect which is, however, encountered by the wild-type cells.

Cell Designation: CHO-K1-BH4-7182

Mycoplasma Verification: Cell line CHO-K1-BH4-7182 verified as mycoplasma free by Pharmakon Research International, Inc., August 5, 1982.

Date Cytotoxicity Assay Initiated: June 3, 1982

Date Mutagenicity Assay Initiated: August 26, 1982

Date Completed: September 20, 1982

Pharmakon Reference: Notebook #354; 66-78 and
Notebook #343; 82-85

Test Article Description: Clear liquid

Test Article Preparation: Test article Glyoxal 40 LF was miscible in dH_2O and administered in a volume of 50 ul.

Cytotoxicity: Test article Glyoxal 40 LF was assayed for cytotoxicity at doses of 1000, 333.33, 100, 33.33, 10, 3.3, 1.0, 0.33, 0.1 and 0.033 ug/ml of media both with and without metabolic activation preparation for a 5 hour treatment time. Following a 19 hour recovery period, 200 cells/60 mm dish (6 dishes) were plated

and incubated for 7 days, fixed and stained. The cytotoxicity assay resulted in no cell survival at 1000 ug/ml of media with and without metabolic activation. The 333.33 ug/ml of media level resulted in 25.92 percent relative survival with metabolic activation and 68.95 percent relative survival without metabolic activation preparation. All remaining doses showed greater than 80 percent relative survival. Data from the Cytotoxicity Assay may be found in Table 1. The S-9 fraction used in the Cytotoxicity Assay contained 42 mg protein per ml, as determined by the Lowery Protein Determination Assay.

Dose Selection:

The highest concentration of test article generally used in the CHO/HGPRT Mammalian Cell Forward Gene Mutation Assay should result in 10 percent cell survival (cloning efficiency) after treatment. For test article Glyoxal 40 LF this dose was estimated to be 600 ug/ml of media. Based on the results of the Preliminary Cytotoxicity Assay, additional doses in the Mutation Assay were 300, 150, 75 and 37.5 ug/ml of media.

Mutagenicity Assay:

Cells in logarithmic growth were detached with 0.05% trypsin solution and plated at a density of 5×10^5 cells/25 cm^2 flask in 5 ml of medium F12 containing 5% fetal bovine serum. After a 24 hour growth period, the cultures were washed twice with 5 ml of Saline-G-Complete, and 4-5 ml of serum-free medium F12 were added. In cultures requiring S-9 activation, the S-9 mix was added to bring the final volume to 5 ml. The test article Glyoxal 40 LF was added in 50 ul quantities to give doses of 600, 300, 150, 75 and 37.5 ug/ml of medium. The flasks were gassed with 5 percent CO_2 in air, the caps tightened and the cells incubated at 37°C in 5% CO_2 at 90% + humidity for 5 hours. After the 5 hour treatment time, the cultures were washed three times with 5 ml of Saline-G, 5 ml of medium F12FCM5 were added to each flask, and the cultures incubated for 19 hours. On Day 1, the cells were trypinsized and plated for cloning efficiency determinations (200 cells/plate in 5 ml F12FCM5), and 1×10^6 cells/100 mm dish were plated for phenotypic expression in medium F12FCM5. Approximately 1×10^6 cells were subcultured on Days 3 and 6 to allow for phenotypic expression.
On Day 8, the cells were trypsinized and the cell number determined. The cells were plated at a density of 2×10^5 cells/100 mm dish in 10 ml of hypoxanthine free medium F12FCM5 containing 10 uM 6-TG (5 dishes=1×10^6 cells) and 200 cells/60 mm dish in hypoxanthine free medium F12FCM5 (6 dishes). The cultures were incubated for 7 days, the colonies fixed with 3.7% formalin and stained with dilute giemsa stain.

Stability and Purity:

There was no apparent change in the physical state of the test or control articles during the assay. The purity of the test article was the responsibility of the sponsor.

Results and Conclusions:

The results for test article Glyoxal 40 LF are as summarized in Table II. Glyoxal 40 LF was shown to produce an increased mutation frequency in the CHO/HGPRT Mammalian Cell Forward Gene Mutation Assay at 600 ug/ml of media in the presence of a metabolic activation system under the conditions of this assay and should be considered a suspect mutagen.

TABLE I

PH 314-AC-002-82

Mammalian Cell Forward Gene Mutation Assay
Cytocoxicity Data (5 hour Treatment)

Test Article	Dose ug/ml	S-9 (±)	Number of Surviving Colonies	Avg. No. of Surviving Colonies/6 plates	Percent Relative* Survival
untreated	-	-	183, 174, 166, 224, 178, 186	185.17	100.00
untreated	-	+	183, 192, 193, 224, 213, 185	198.33	100.00
dH_2O	-	-	206, 172, 173, 173, 196, 193	185.50	100.00
dH_2O	-	+	194, 172, 179, 183, 153, 162	173.83	93.88
Glyoxal 40 LF	1000	-	Cytotoxic	-	-
Glyoxal 40 LF	1000	+	Cytotoxic	-	-
Glyoxal 40 LF	333.33	-	117, 118, 148, 146, 109, 128	127.67	68.95
Glyoxal 40 LF	333.33	+	49, 53, 29, 39, 50, 68	48.00	25.92
Glyoxal 40 LF	100	-	131, 159, 149, 162, 182, 164	157.83	85.24
Glyoxal 40 LF	100	+	168, 158, 174, 187, 191, 143	170.17	91.90
Glyoxal 40 LF	33.33	-	158, 151, 149, 124, 154, 160	149.33	80.65
Glyoxal 40 LF	33.33	+	148, 168, 154, 136, 131, 148	147.50	79.65

* Relative to untreated control

TABLE I (Continued)

PH 314-AC-002-82

Mammalian Cell Forward Gene Mutation Assay
Cytotoxicity Data (5 hour Treatment)

Test Article	Dose ug/ml	S-9 (±)	Number of Surviving Colonies	Avg. No. of Surviving Colonies/6 plates	Percent Relative* Survival
Glyoxal 40 LF	10	-	228, 221, 191, 176, 194, 197	201.17	100.00
Glyoxal 40 LF	10	+	192, 191, 172, 181, 191, 179	184.33	99.55
Glyoxal 40 LF	3.3	-	213, 226, 199, 193, 171, 189	198.50	100.00
Glyoxal 40 LF	3.3	+	202, 190, 210, 177, 176, 188	190.50	100.00
Glyoxal 40 LF	1.0	-	181, 193, 177, 188, 168, 164	178.50	96.39
Glyoxal 40 LF	1.0	+	195, 172, 186, 192, 162, 181	181.33	97.93
Glyoxal 40 LF	0.33	-	197, 196, 168, 168, 191, 192	185.33	100.00
Glyoxal 40 LF	0.33	+	160, 192, 184, 185, 192, 174	181.17	97.82
Glyoxal 40 LF	0.1	-	171, 183, 188, 152, 189, 176	176.50	95.31
Glyoxal 40 LF	0.1	+	174, 156, 173, 181, 208, 200	182.00	98.28
Glyoxal 40 LF	0.033	-	191, 147, 180, 181, 179, 163	173.50	93.69
Glyoxal 40 LF	0.033	+	182, 180, 191, 178, 180, 186	182.83	98.74

* relative to untreated controls

TABLE II

PH 314-AC-002-82

Mammalian Cell Forward Gene Mutation Assay

Mutagenicity Data

			Mutant Scoring		Post-"Expression Survival"		
Test Article	ug/ml	S-9 (±)	Total No. of Mutants (5 plates)	No. of cells seeded/plate	Avg. No. of surviving cells x/6 plates	Percent "expression survival"	x Mutation Frequency (Mutants/10^6 "expression survivors")
untreated	-	-	6	2 x 1	205.83	102.92	
untreated	-	-	4	2 x 10^5	218.67	109.33	4.75/10^6
untreated	-	+	4	2 x 10^5	192.17	96.08	
untreated	-	+	6	2 x 10^5	191.33	95.67	5.22/10^6
dH_2O	-	-	11	2 x 10^5	217.67	108.83	
dH_2O	-	-	9	2 x 10^5	235.83	117.92	8.19/10^6
dH_2O	-	+	7	2 x 10^5	217.50	108.75	
dH_2O	-	+	9	2 x 10^5	220.83	110.42	7.30/10^6
Glyoxal 40 LF	600	-	22	2 x 10^5	166.33	83.17	
Glyoxal 40 LF	600	-	18	2 x 10^5	180.00	90.00	23.23/10^6
Glyoxal 40 LF	600	+	23	2 x 10^5	168.67	84.33	
Glyoxal 40 LF	600	+	52	2 x 10^5	148.83	74.42	48.57/10^6

TABLE II (Continued)

PH 314-AC-002-82

Mammalian Cell Forward Gene Mutation Assay

Mutagenicity Data

			Mutant Scoring		Post-"Expression Survival"		
Test Article	ug/ml	S-9 (±)	Total No. of Mutants (5 plates)	No. of cells seeded/plate	Avg. No. of surviving cells $\bar{x}$/6 plates	Percent "expression survival"	x Mutation Frequency (Mutants/10^6 "expression survivors")
Glyoxal 40 LF	300	-	16	2×10^5	193.50	96.75	
Glyoxal 40 LF	300	-	17	2×10^5	181.67	90.83	$17.63/10^6$
Glyoxal 40 LF	300	+	13	2×10^5	157.17	78.83	
Glyoxal 40 LF	300	+	12	2×10^5	164.50	82.25	$15.54/10^6$
Glyoxal 40 LF	150	-	28	2×10^5	184.67	92.33	
Glyoxal 40 LF	150	-	22	2×10^5	186.33	93.17	$26.97/10^6$
Glyoxal 40 LF	150	+	11	2×10^5	191.83	95.92	
Glyoxal 40 LF	150	+	27	2×10^5	200.67	100.33	$19.19/10^6$
Glyoxal 40 LF	75	-	26	2×10^5	200.67	100.33	
Glyoxal 40 LF	75	-	12	2×10^5	198.33	99.17	$19.01/10^6$
Glyoxal 40 LF	75	+	17	2×10^5	178.67	89.33	
Glyoxal 40 LF	75	+	11	2×10^5	179.67	89.83	$15.64/10^6$

TABLE II (Continued)

PH 314-AC-002-82

Mammalian Cell Forward Gene Mutation Assay

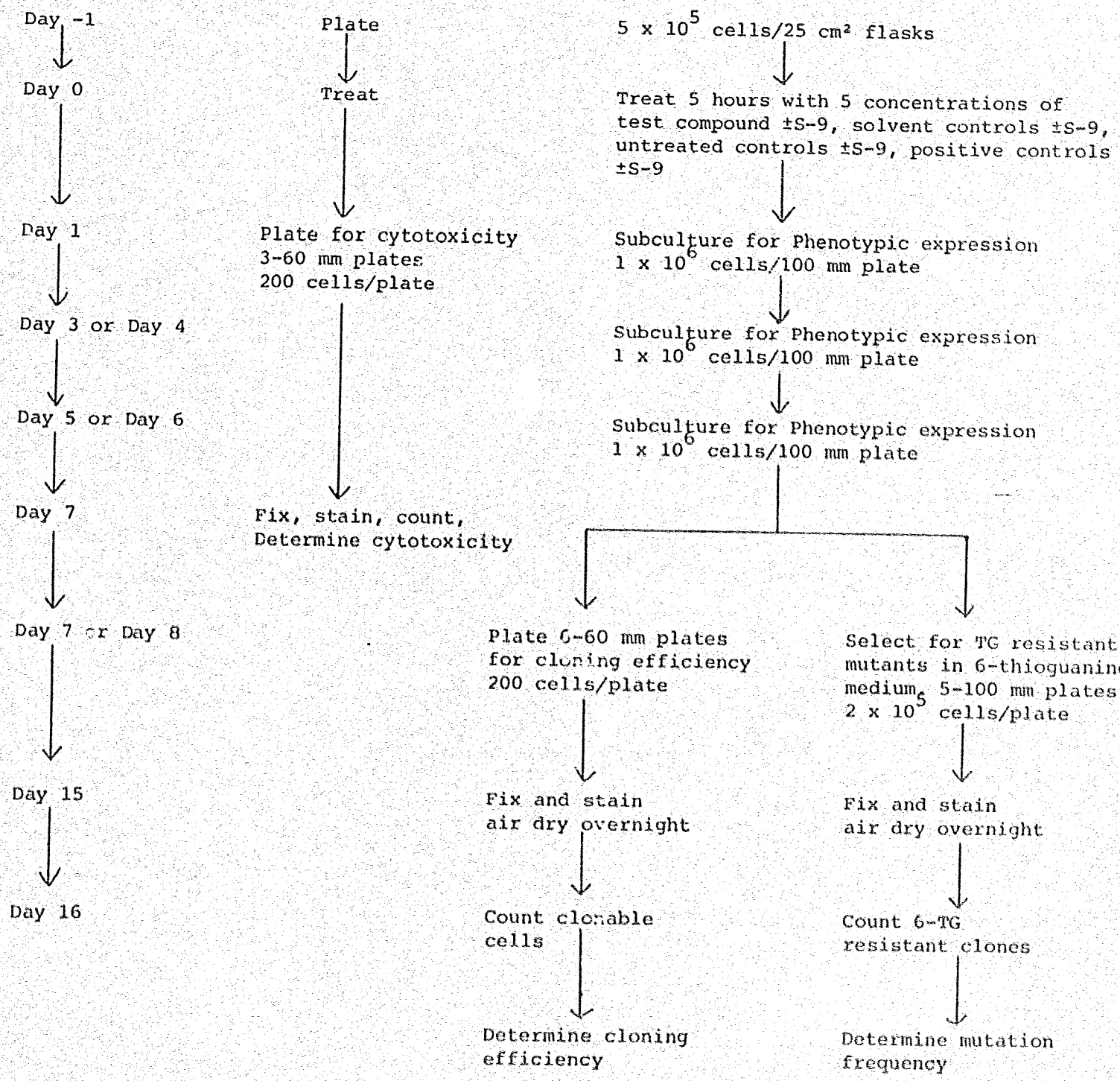
Mutagenicity Data

			Mutant Scoring		Post-"Expression Survival"		
Test Article	ug/ml	S-9 (±)	Total No. of Mutants (5 plates)	No. of cells seeded/plate	Avg. No. of surviving cells $\bar{x}$/6 plates	Percent "expression survival"	x Mutation Frequency (Mutants/10^6 "expression survivors")
Glyoxal 40 LF	37.5	-	13	2×10^5	179.83	89.92	
Glyoxal 40 LF	37.5	-	14	2×10^5	191.33	95.67	$14.55/10^6$
Glyoxal 40 LF	37.5	+	9	2×10^5	180.00	90.00	
Glyoxal 40 LF	37.5	+	8	2×10^5	182.50	91.25	$9.38/10^6$
EMS	200	-	243	2×10^5	180.67	90.33	
EMS	200	-	239	2×10^5	187.83	93.92	$261.74/10^6$***
DMN	100	+	155	2×10^5	127.67	63.83	
DMN	100	+	200	2×10^5	133.50	66.75	$271.23/10^6$***

*** denotes a positive test response

Experimental Procedure
CHO/HGPRT
Mammalian Cell Forward Gene Mutation Assay

Figure I
Summary Flow Sheet
CHO/HGPRT Mammalian Cell Forward Gene Mutation Assay



Experimental Procedure

CHO/HGPRT
Mammalian Cell Forward Gene Mutation Assay

Objective: To measure the ability of a test substance to induce mutations at the hypoxanthine-guanine phosphoribosyl transferase (hgprt) locus in Chinese Hamster Ovary (CHO) cells on the basis that the presumptive mutants, by virtue of the loss of the HGPRT activity are unable to convert purine analogues such as 6-thioguanine (6-TG) to toxic metabolites and hence escape their lethal effect which is, however, encountered by the wild-type cells.

Study Director: Edmund G. Godek, Pharmakon Research

Technical Performance: Edmund G. Godek, Ruth Sorg, and Frederika Nicholl

Cell Culture: Chinese Hamster Ovary cells, clone K1

Cell Line Designation: CHO-K1-BH4

Source: Dr. Abraham W. Hsie
Biology Division
Oak Ridge National Laboratories
P O Box Y
Oak Ridge, Tennessee 37380

Aseptic Technique: All aseptic techniques, where possible, were carried out in a Baker NCB6 Hood.

Storage: The stock cultures of CHO-K1-BH4 cell line are maintained in frozen aliquots in a Revco Ultra-low Freezer.

Experimental Procedure
CHO/HGPRT
Mammalian Cell Forward Gene Mutation Assay

Working Cultures: Fresh cultures of CHO-K1-BH4 cell line were prepared frozen stock cultures known to have a stable spontaneous mutation frequency of 0 - 10 x 10^{-6} mutants per cell, however, values up to 20 x 10^{-6} were deemed acceptable.

Negative Controls: CHO-K1-BH4 cells were assayed untreated, treated with metabolic activation system only, and treated with the appropriate solvent, both with and without metabolic activation.

Positive Controls: Positive controls used in the assay were:
Ethylmethane sulfonate (EMS) at 200 ug/ml, a mutagen not requiring an S-9 activation system.
Dimethylnitrosamine (DMN) at 100 ug/ml, a mutagen requiring an S-9 activation system.

Replication: CHO-K1-BH4 cells were treated with five levels of the test article. All negative and positive controls were treated in duplicate. All treatment groups were tested in duplicate.

Dose Selection: Cytotoxicity of a test substance to CHO cells was determined by a reduction in colony forming ability of the cells following a 5 hour treatment with the test substance in the presence or absence of a metabolic activation system. Generally, for test substances with unknown toxicity, they were run at 10 doses with 1000 ug/ml initially being the highest one. The remaining doses were 300, 100, 30, 10, 3, 1, 0.3, 0.1 and

0.03 ug/ml. The highest concentration of test substance used in the CHO/HGPRT Mammalian Cell Forward Gene Mutation Assay was that which resulted in 10% cell survival (cloning efficiency). Four additional doses which yield 10 - 100% survival were also tested.

Treatment of CHO Cells with Test Substance for Cytotoxicity (non-activated):

The CHO-K1-BH4 cells were removed from the incubator, the medium was aspirated from the flasks and the cultures were washed twice with 5 ml of Saline G. After the final wash, 5 ml of serum free medium F12 was pipetted into each flask. The test substance being assayed for cytotoxicity was diluted in the proper solvent to the concentrations desired for testing and 20 - 50 µl of the test substance was added to each flask and mixed well. The flasks were gassed with 5% CO_2 in air, the caps tightened and the cells incubated for 5 hours at 37°C. After the 5 hour incubation, the medium was aspirated from the flasks, the cultures washed three times with 5 ml of Saline G and 5 ml of F12FCM5 added. The cultures were incubated for an additional 19 hours.

Treatment of CHO Cells with Test Substance for Cytotoxicity (activated):

The CHO-K1-BH4 cells were removed from the incubator, the medium was aspirated from the flasks, and the cultures were washed twice with 5 ml of Saline G.

After the final wash, 4 ml of serum free medium F12 were pipetted into each flask. The S-9 mix contained (per ml) 8 µmol $MgCl_2$, 8 µmol $CaCl_2$, 33 µmol KCl, 5 µmol glucose-6-phosphate, 4 µmol NADP, 50 µmol sodium phosphate buffer (pH 8.0) and 0.1 ml of the microsomal preparation containing 30 mg protein/ml. The test substance being assayed for cytotoxicity was diluted in the proper solvent to the concentrations desired for testing and 20 - 50 µl of the test substance was added to each flask and mixed well. The flasks were gassed with 5% CO_2 in air, the caps tightened and the cells incubated for 5 hours at 37°C. After the 5 hour incubation, the medium was aspirated from the flasks, the cultures washed three times with 5 ml of Saline G and 5 ml of F12FCM5 added. The cultures were incubated for an additional 19 hours.

Cytotoxicity Determination:

Following the 19 hour incubation after test substance treatment, the cultures were removed from the incubator and the medium aspirated from the flasks. The cultures were washed twice with 5 ml of Ca-Mg-free Saline G. The cells were then placed at ambient temperature in 0.5 ml of a 0.05% trypsin solution. The flasks were examined under an inverted microscope to ensure that the cells had rounded and 1.5 ml of

medium F12FCM5 were added. The cells were washed off the surface of the flask using a plugged pasteur pipette. A cell number was determined for each culture. An aliquot of the cells was diluted to a cellular density of 1000 cells/ml in medium F12FCM5 and 0.2 ml (200 cells/plate) was added to each of 6 - 60 mm plates containing 5 ml of medium F12FCM5. The plates were incubated for 7 - 8 days at 37°C in a 5% CO_2 in air incubator. After the incubation period, the medium was removed and the colonies fixed with 3.7% formaldehyde, stained with a 1% Giemsa solution, counted and the colony numbers recorded. These survival frequencies were used to determine which levels of test substance yielded a 10 - 100% survival. Five levels of test substance yielding 10 to 100% survival were chosen to be used in the CHO Mammalian Cell Forward Gene Mutation Assay.

Plating CHO cells for Experiment:

CHO-K1-BH4 cells for mutagenesis testing were obtained from frozen stocks of cultures known to have a stable spontaneous mutation frequency of 0 - 10 x 10^{-6} mutants per cell. Exponentially growing cells were plated in 25 cm² plastic tissue culture flasks at an initial density of 5 x 10^5 cells in 5 ml of medium F12FCM5 and incubated for 16 - 24 hours. Normal cell

growth was inspected using an A.O. Inverted Microscope. Cells in exponential growth state were treated.

Treatment of CHO Cells with Test or Control Substance: (non-activated):

The CHO-K1-BH4 cells were removed from the incubator, the medium was aspirated from the flasks and the cultures were washed twice with 5 ml of Saline G. After the final wash, 5 ml of serum free medium F12 were pipetted into each flask. The test or control substance being assayed was diluted in the proper solvent to the concentration desired for testing and 20 - 50 µl of the test or control substance was added to each flask and mixed well. The flasks were gassed with 5% CO_2 in air, the caps tightened and the cells incubated for 5 hours at 37°C. After the 5 hour incubation, the medium was aspirated from the flasks, the cultures washed three times with 5 ml of Saline G and 5 ml of F12FCM5 added. The cultures were incubated for an additional 19 hours.

Treatment of CHO with Test or Control Substance (activated):

The CHO-K1-BH4 cells were removed from the incubator, the medium was aspirated from the flasks, and the cultures were washed twice with 5 ml of Saline G. After the final wash, 4 ml of serum free medium F12 were pipetted into each flask. The S-9 mix contained (per ml) 8 µmol $MgCl_2$, 8 µmol $CaCl_2$, 33 µmol KCl, 5 µmol glucose-6-phosphate, 4 µmol NADP, 50 µmol sodium phosphate buffer (pH 8.0) and 0.1 ml of the

microsomal preparation containing 30 mg protein/ml. The test or control substance being assayed was diluted in the proper solvent at the concentrations desired for testing and 20 - 50 ul of the test or control substance was added to each flask and mixed well. The flasks were gassed with 5% CO_2 in air, the caps tightened and the cells incubated for 5 hours at 37°C. After the 5 hour incubation, the medium was aspirated from the flasks, the cultures washed three times with 5 ml of Saline G and 5 ml of F12FCM5 added. The cultures were incubated for an additional 19 hours.

Subculturing for Expression of Mutation:

Following the 19 hour incubation after test substance treatment, the cultures were removed from the incubator and the medium aspirated from the flasks. The cultures were washed twice with 5 ml of Ca-Mg-free Saline G. The cells were then placed at ambient temperature in 0.5 ml of a 0.05% trypsin solution. The flasks were examined under an inverted microscope to ensure that the cells had rounded and 1.5 ml of medium F12FCM5 was added. The cells were washed off the surface of the flask using a plugged pasteur pipette. A cell number was determined for each culture. An aliquot yielding 1×10^6 cells were subcultured into a 100 mm dish containing 10 ml of medium F12FCM5. These cultures were incubated at 37°C

in a 5% CO_2 in air for phenotypic expression. A subculture was taken on Day 3 and Day 5. On Day 3 the subculture was performed by washing the cells twice with 5 ml of Ca-Mg-free Saline G and placing them with 0.5 ml of 0.05% trypsin solution at ambient temperature. The cells were allowed to round and 1.5 ml of F12FCM5 was added to each plate. The cells were washed off the surface of the plate using a plugged pasteur pipette. A cellular density was determined and an aliquot of the suspension containing 1×10^6 cells was added to a plate containing 10 ml of medium F12FCM5. The subculture was repeated on Day 5.

Selection of Mutant CHO Cells (6-TG-Resistant):

On Day 7 the cells were washed once with 5 ml of Ca-Mg-free Saline G and then placed in 0.5 ml of a 0.05% trypsin solution at ambient temperature. When the cells rounded up, 1.5 ml of hypoxanthine-free medium F12FCM5 was added and the cells washed from the 100 mm dish with a pasteur pipette. A cell density was determined and the cells diluted to 1×10^5 cells/ml in hypoxanthine free medium F12FCM5. This aliquot of cells was used for selection and an additional aliquot was reserved for a determination of cloning efficiency. For mutant selection, 6.25 ml of a 10^{-3}M 6-thioguanine solution was added to 494 ml of hypoxanthine free medium F12FCM5. To each of 5 100 mm plates, 8 ml of the TG medium were added and 2 ml of

the 1 x 10^5 cells/ml aliquot, for a total of 2 x 10^5 cells/plate. The plates were incubated for 7 days at 37°C in 5% CO_2 in air to allow for colony formation. The colonies were then fixed in 3.7% formaldehyde, stained with a 1% Giemsa solution, counted, and the clone numbers recorded. The total number of mutant clones on the five plates was determined.

Cloning Efficiency: From the aliquot reserved before mutant selection, a 1:100 dilution was performed in Saline G to a cellular density of 1 x 10^3 cells/ml. This aliquot was used to determine cloning efficiency. From this dilution, 0.2 ml was aliquoted into each of six 60 mm plates containing 5 ml of hypoxanthine free medium F12FCM5 (200 cells/plate). These plates served as viable counts (cloning efficiency) plates and were incubated for 7 days at 37°C in 5% CO_2 in air, fixed, stained and counted for a measure of cloning efficiency (average of the six 60 mm plates).

Calculation of Mutation Frequency: The mutation frequency was calculated by dividing the total number of mutant clones by the number of cells plated, corrected for the cloning efficiency of the cells prior to mutant selection.

Interpretation of Results: After treatment with the test substance, cells which had undergone mutation to hgprt$^-$ form colonies in the presence of 6-thioguanine (resistant to 6-thioguanine). The total number of mutant colonies

(per 10^6 surviving cells) observed at each concentration of test substance was compared to the total number of mutant colonies (per 10^6 surviving cells) observed in the negative control. A test substance showing a dose-dependent increase of mutation induction with at least one dose exhibiting a mutation frequency that was greater than or equal to 50×10^{-6} per cell was considered a suspect mutagenic response, and the sponsor will be advised to repeat the assay bracketing the positive response dose. A test substance showing a true positive response in this assay should also exhibit a dose-response relationship. The spontaneous background mutation frequency (forward mutation frequency) is usually $0 - 10 \times 10^{-6}$, however, values up to 20×10^{-6} are deemed acceptable. Data is presented in this final study report in tabular form as the number of mutant colonies per 10^6 surviving cells at each concentration of test substance. In those cases where the test substance was positive, a dose-response curve was also provided showing the increase in mutant frequency vs. increasing concentration. All assays were performed in duplicate and data presented as the average number of mutants per 10^6 surviving cells.

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 the final report are maintained in the Pharmakon Archives.

Raw Data: Pharmakon Research Notebook

Good Laboratory Practices Statement: This study was conducted in compliance with the Good Laboratory Practice Regulations except if noted. There were no significant deviations from the GLP 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 the test facility management.

Test Article: The identity, purity, quality, and strength of the test article are the responsibility of the sponsor.

Bibliography[1]:

O'Neill, J. P., P. A. Brimer, R. Machanoff, G. P. Hirsch, and A. W. Hsie. 1977a. A quantitative assay of mutation induction at the hypoxanthine-guanine phosphoribosyl transferase locus in Chinese hamster ovary cells (CHO/HGPRT system): Development and definition of the system. Mutation Research 45: 91-101.

O'Neill, J. P., D. B. Couch, R. Machanoff, J. R. San Sebastian, P. A. Brimer, and A. W. Hsie. 1977b. A quantitative assay of mutation induction at the hypoxanthine-guanine phosphoribosyl transferase locus in Chinese hamster ovary cells (CHO/HGPRT system): Utilization with a variety of mutagenic agents. Mutation Research 45: 103-109.

.E Preliminary Cytotoxicity Screen - Glyoxal 40LF Project No. [illegible] Book No. 343

n Page No. [illegible]

'bjectives: To determine cytotoxicity of a test article towards the CHO cell line. Cytotoxicity of a test article to CHO cells is determined by a reduction in colony forming ability of the cells following 5 hour treatment with the test article in the presence and absence of a metabolic activating system.

Methods and Materials: Refer to Standard Operating Procedure PH314.

Sponsor: American Cyanamid Company
Test Article: Glyoxal 40LF
Description: clear liquid

5/27/82 - Frozen aliquot of cell line CHO-K1-BH4, Lot # 11882, inoculated into 15 ml of media F12FCS10 in a T75 flask (1
Aminopterin treated: 1/11/82. Frozen: 1/18/82.
Lot # 11882 certified mycoplasm free 2/23/82.
Media F12FCS10 prepared - 3/25/82 Fetal Bovine Serum Lot # KC3

E Jodek 5/27/82.

5/28/82 - CHO-K1-BH4 cells subcultured into 2-T75 flasks (2×10^5 cells/flask) in 15 ml media F12FCS10 (3/25/82).
E Jodek 5/28/82

5/31/82 - CHO-K1-BH4 cells subcultured into 8-T75 flasks (2.5×10^5 cells/flask) in 15 ml media F12FCS10 (3/25/82).
E Jodek 5/31

6/2/82 - CHO-K1-BH4 cells subcultured into 26-T25 flasks (5×10^5 cells/flask) in 5 ml media F12FCM5.
Media F12FCM5 prepared: 3/25/82 Fetal Bovine Serum Lot # KC3 free

To Page [illegible]

sed & Understood by me,	Date	Invented by	Date
Ruth R. Sorg	6/2/82	Witnessed by Edward J. Jodek	6/2/82

Preliminary Cytotoxicity Screen - Alyoxalyl F Book No. 343

Page No. 82

6/3/82 - Test article Preparation:

8.7890g.
8.2260g. 563 mg / 5.6 ml total volume. (100mg/ml).
0.5630g. 563mg contained in 0.45ml ⇒ 563mg / 0.45eq vol. + 5.15ml d.

.100 mg/ml —1:3→ 33.33 mg/ml —1:10→ 3.33 mg/ml —1:10→ 0.33 mg/ml —1:10→ 0.033 mg/ml —1:10→

↓ 1000 ↓ 333.33 ↓ 33.33 ↓ 3.3 ↓ 0.33

10 mg/ml —1:10→ 1.0 mg/ml —1:10→ 0.1 mg/ml —1:10→ 0.01 mg/ml ug/ml of media

↓ 100 ↓ 10 ↓ 1 ↓ 0.1

- Mixture:

Sterile Milli Q H_2O	-	10.9ml
0.2M $NaPO_4$ (pH 8.0)	-	5.0ml
0.1M NADP	-	0.8ml
1.0M Glucose-6-Phosphate	-	0.1ml
1.5M KCl	-	0.4ml
0.5M $MgCl_2$	-	0.4ml
0.5M $CaCl_2$	-	0.4ml
S-9 Fraction	-	2.0ml
		20ml total

S-9 prepared 4/27/82, 2.5mg protein/ml

Treatment time 5hrs. 10:00 A.M. to 3:00 P.M.

All test article and control articles delivered to test system at dose volume of 50ul.

2:00 P.M. - All flasks washed 3X with Saline-G-Complete. 5ml Media F12Fcm5 (5/26/82) added to each flask. All flasks incubated at 37°C, 5% CO_2 in air at 90+% humidity.

To Page No.

Witnessed & Understood by me,	Date	Invented by	Date
Ruth M. Sorg	6/3/82	Recorded by Edmund Y. Arak	6/3/82

LE Preliminary Cytotoxicity Screen - Glyoxal LF Book No. 343

m Page No. 54

;1 /82 - Plates removed from incubator, media aspirated off, plates washed with phosphate buffered saline, fixed in 3.7% formalin solution, stained with 1% Giemsa stain, rinsed in running tap water and allowed to air dry.

E Godek 6/11/82.

6/14/82 - Scoring for Initial Survival.

Plate#	Compound	±S9	ug/ml	Plate Counts	# cells seeded/plate	Avg. # clones	% survival	% relative survival
2AC-1	untreated	−	—	183 174 166 224 178 186	200	185.17	92.59	100
2AC-2	untreated	+	—	183 192 193 224 213 185	200	198.33	99.17	100
2AC-3	H_2O	−	—	206 172 173 173 156 193	200	185.50	92.75	100
2AC-4	H_2O	+	—	194 172 179 183 153 162	200	173.83	86.92	93.8
2AC-5	Glyoxal 40% LF	−	1000	0 0 0 0 0 0	200	0.00	0	0
2AC-6	Glyoxal 40% LF	+	1000	0 0 0 0 0 0	200	0.00	0	0
2AC-7	Glyoxal 40% LF	−	333.33	117 118 148 146 109 128	200	127.67	63.84	68.9
2AC-8	Glyoxal 40% LF	+	333.33	49 53 29 39 50 68	200	48.00	24.00	25.9
2AC-9	Glyoxal 40% LF	−	100	131 159 149 162 182 164	200	157.83	78.92	85.2
2AC-10	Glyoxal 40% LF	+	100	168 158 174 187 191 143	200	170.17	85.09	91.9
2AC-11	Glyoxal 40% LF	−	33.33	158 151 149 124 154 160	200	149.33	74.67	80.65
2AC-12	Glyoxal 40% LF	+	33.33	148 165 154 136 131 148	200	147.50	73.75	79.65
2AC-13	Glyoxal 40% LF	−	10	223 221 191 176 194 197	200	201.17	100.59	100
2AC-14	Glyoxal 40% LF	+	10	192 191 172 181 191 194	200	184.33	92.17	99.5
2AC-15	Glyoxal 40% LF	−	3.33	213 216 199 193 171 189	200	198.50	99.25	100
2AC-16	Glyoxal 40% LF	+	3.33	202 190 210 172 176 188	200	190.50	95.25	100
2AC-17	Glyoxal 40% LF	−	1.0	181 193 172 158 165 164	200	178.50	89.25	96.39
2AC-18	Glyoxal 40% LF	+	1.0	195 172 186 192 162 181	200	181.33	90.67	97.93
2AC-19	Glyoxal 40% LF	−	0.33	197 196 168 168 191 192	200	185.33	92.67	100
2AC-20	Glyoxal 40% LF	+	0.33	160 192 194 185 192 174	200	181.17	90.59	97.8
2AC-21	Glyoxal 40% LF	−	0.1	171 183 158 152 189 176	200	176.50	88.25	95.31
2AC-22	Glyoxal 40% LF	+	0.1	174 156 173 181 205 200	200	182.00	91.00	98.2
2AC-23	Glyoxal 40% LF	−	0.033	191 142 180 181 179 163	200	173.50	86.75	93.69
2AC-24	Glyoxal 40% LF	+	0.033	182 180 191 178 180 186	200	182.83	91.42	98.7

To Page No. 6

Witnessed & Understood by me,	Date	Invented by	Date
Ruth M. Sorg	6/14/82	Recorded by Edmund J. Godek	6/14/82

E. CHO/HGPRT Mutation Assay - Glyoxal 40LF Book No. 354

Page No. 85, 88. 343

l ectives: To measure the ability of a test substance to induce muta
at the hypoxanthine guanine phosphoribosyl transferase (hgprt) l
in Chinese Hamster Ovary (CHO) cells on the basis that the presump
mutants, by virtue of the loss of the HGPRT activity are un
to convert purine analogs, such as 6-thioguanine (6-TG) to
toxic metabolites, and hence escape their lethal effects, whi
is however, encountered by the wild type cells.

aterials and Methods: Refer to Standard Operating Procedure PH314.
s. sor: American Cyanamid Company
est Article: Glyoxal 40 LF
e cription: clear liquid

Date Preliminary Cytotoxicity Initiated: 6/3/82
Date CHO/HGPRT Forward Gene Mutation Assay Initiated: 8/26/82

0-K1-BH4 Lot # 7182 received from Oak Ridge National Laboratories 7
Routine subcultures were done every Friday (a.m.) and Monday (p.m.)
where 1×10^5 cells were subcultured into each of 3-75 cm^2 flasks
containing 15 ml of media F12FCS10. CHO-K1-BH4 Lot # 7182 Amung
treated 7/23/82. Routine subculture regime carried out.

8/23/82 - CHO-K1-BH4 cells (Lot # 7182) subcultured into 10-T75cm
flasks (3×10^5 cells/flask) in 15 ml of media F12FCS10.

E Jodek 8/23/82.

25/82 - CHO-K1-BH4 cells (Lot # 7182) subcultured into 36-T25cm
flasks (5×10^5 cells/flask) in 5 ml of media F12 FCM5, in
preparation for treatment. (7/29/82). Fetal Bovine Serum Lot # KC-331005

To Page

nessed & Understood by me,	Date	Invented by	Date
J. Mecca	8/25/82	Recorded by Edmund A. Jodek	8/25/82

8/26/82 - Test Article Preparation:

7.7250g.
7.4110g.
0.3140g.

314 mg / 5.23 ml dH2O ⇒ 60 mg/ml.

60 mg/ml $\xrightarrow{1:2}$ 30 mg/ml $\xrightarrow{1:2}$ 15 mg/ml $\xrightarrow{1:2}$ 7.5 mg/ml $\xrightarrow{1:2}$ 3.75 mg/ml

g/ml 600 300 150 75 37.5

media delivered in a volume of 50 ul.

Positive Control Preparation:

EMS - 200 ug/ml of media.

7.5440g.
7.4950g.
0.0490g.

49 mg / 2.45 ml total volume density EMS: 1.
eq. vol. = 0.034 ml.
49 mg / 0.034 ml eq. vol + 2.416 ml DMSO ⇒ 20
delivered in 50 ul ⇒ 200 ug/ml of media.

DMN - 100 ug/ml of media.

7.5120g.
7.4890g.
0.0230g.

23 mg / 2.3 ml DMSO ⇒ 10 ug/ml
delivered in 50 ul ⇒ 100 ug/ml of media.

S-9 Mixture:

Sterile Milli Q H2O	-	10.9 ml
0.2M Na2HPO4·7H2O - (pH 7.4)	-	5.0
0.1M NADP	-	0.8
1.0M Glucose-6-Phosphate	-	0.1
1.5M KCl	-	0.4
0.5M MgCl2	-	0.4
0.5M CaCl2	-	0.4
S-9 Fraction	-	2.0
		20.0 ml total

S-9 prepared: 4/2
4/2 mg protein/

Treatment time: 10:00 A.M. to 3:00 P.M.

To Pa

Witnessed & Understood by me,	Date	Invented by	Date
[illegible] J. Meca	8/26/82	Recorded by [illegible]	8/26/82

Project No. [illegible] NC-00[illegible]-[illegible]

LI CHO/HGPRT Mutation Assay - Glycol 40LF Book No. 354

om Page No. 67 8/27/82 - Subculture for expression of Mutation and Sur
survival.

Expression of Mutation - Subculture 1x10^6 cells / 100 mm plate containing
of media F12FCM5.

Initial Survival - Subculture an aliquot of cells containing 1x10^3 c
0.2 ml of aliquot (200 cells) in each of 3-60 mm dishes containing 5 ml

Plate #	Compound	±S9	ug/ml	cell count	# cells per ml	ant. trans.	# cells in suspension
GLV - 1-IS	untreated	-	-	10500	1.05×10^6	0.95	2.10×10^6
2-IS	untreated	-	-	10500	1.05×10^6	0.95	2.10×10^6
3-IS	untreated	+	-	10400	1.04×10^6	0.96	2.08×10^6
4-IS	untreated	+	-	10100	1.01×10^6	0.99	2.02×10^6
5-IS	H_2O	-	-	9700	9.7×10^5	1.03	1.94×10^6
6-IS	H_2O	-	-	10300	1.03×10^6	0.97	2.06×10^6
7-IS	H_2O	+	-	11100	1.11×10^6	0.90	2.22×10^6
8-IS	H_2O	+	-	9800	9.8×10^5	1.02	1.96×10^6
9-IS	Glycol 40LF	-	600	4600	4.6×10^5	1.7	9.2×10^5
10-IS	↓	-	600	5100	5.1×10^5	1.7	1.02×10^6
11-IS	↓	+	600	4400	4.4×10^5	1.7	8.8×10^5
12-IS	↓	+	600	4500	4.5×10^5	1.7	9.0×10^5
13-IS	↓	-	300	7700	7.7×10^5	1.30	1.54×10^6
14-IS	↓	-	300	8500	8.5×10^5	1.18	1.70×10^6
15-IS	↓	+	300	5900	5.9×10^5	1.69	1.18×10^6
16-IS	↓	+	300	5700	5.7×10^5	1.7	1.14×10^6
17-IS	↓	-	150	11200	1.12×10^6	0.89	2.24×10^6
18-IS	↓	-	150	12600	1.26×10^6	0.79	2.52×10^6
19-IS	↓	+	150	9000	9.0×10^5	1.11	1.80×10^6
20-IS	↓	+	150	8600	8.6×10^5	1.16	1.72×10^6
21-IS	↓	-	75	11900	1.19×10^6	0.84	2.38×10^6
22-IS	↓	-	75	13900	1.39×10^6	0.72	2.78×10^6
23-IS	↓	+	75	10000	1.00×10^6	1.00	2.00×10^6
24-IS	↓	+	75	10400	1.04×10^6	0.96	2.08×10^6
25-IS	↓	-	37.5	12400	1.24×10^6	0.81	2.48×10^6
26-IS	↓	-	37.5	13400	1.34×10^6	0.75	2.68×10^6
27-IS	↓	+	37.5	12100	1.21×10^6	0.83	2.42×10^6
28-IS	Glycol 40LF	+	37.5	11600	1.16×10^6	0.86	2.32×10^6

To Fo

...essed & Understood by me, ...no J Mecca	Date 8/27/82	Invented by	Date
		Witnessed by	8/27/82

E CHO/HGPRT Mutat. Assay - Glycol 40LF Project No. Book No. 354

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Plate #	Compound	±S9	ug/ml	cell count	# cells per ml	ant. trans.	# cells in suspension
Gev-29-IS	EMS	−	200	9200	9.2×10^5	1.09	1.84×10^6
30-IS	EMS	−	200	11900	1.19×10^6	0.84	2.38×10^6
31-IS	DMN	+	100	10700	1.07×10^6	0.93	2.14×10^6
32-IS	DMN	+	100	10800	1.08×10^6	0.93	2.16×10^6

Hodik

3/82 - Subculture for expression of mutation. Subculture 1×10^6 c into 100 mm plates containing 5 ml media f/12 FCMS.

#	Compound	±S9	ug/ml	cell count	# cells per ml	ant. trans.	# cells in suspension
1	untreated	−	−	29100	2.91×10^6	0.34	5.82×10^6
2	untreated	−	−	27300	2.73×10^6	0.37	5.46×10^6
3	untreated	+	−	19700	1.97×10^6	0.51	3.94×10^6
4	untreated	+	−	23700	2.37×10^6	0.42	4.74×10^6
5	H_2O	−	−	26900	2.69×10^6	0.37	5.38×10^6
6	H_2O	−	−	24800	2.48×10^6	0.40	4.96×10^6
7	H_2O	+	−	25300	2.53×10^6	0.40	5.06×10^6
8	H_2O	+	−	24100	2.41×10^6	0.41	4.82×10^6
9	Glycol 40LF	−	600	10600	1.06×10^6	0.94	2.12×10^6
10		−	600	10700	1.07×10^6	0.93	2.14×10^6
11		+	600	1300	1.3×10^5	1.7	2.6×10^5
12		+	600	3100	3.1×10^5	1.7	6.2×10^5
13		−	300	23000	2.30×10^6	0.43	4.60×10^6
14		−	300	25500	2.55×10^6	0.39	5.10×10^6
15		+	300	19800	1.98×10^6	0.51	3.96×10^6
16		+	300	20900	2.09×10^6	0.48	4.18×10^6
17		−	150	21600	2.16×10^6	0.46	4.32×10^6
18		−	150	23800	2.38×10^6	0.42	4.76×10^6
19		+	150	18300	1.83×10^6	0.55	3.66×10^6
20		+	150	19000	1.90×10^6	0.53	3.80×10^6
21		−	75	27800	2.78×10^6	0.36	5.56×10^6
22	Glycol 40LF	−	75	22600	2.26×10^6	0.44	4.52×10^6

To Page

essed & Understood by me,	Date	Invented by	Date
J. Meece	8/30/82	Witnessed by	8/30/82

E CHO/HGPRT Mutation Cross - Hycol 40LF

Project No. [illegible] HC-002-42

Book No. 354

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#	Compound	±S9	ug/ml	cell Count	# cells per ml	cont. tens.	# cells in suspension
15	Hycol 40LF	+	300	11500	1.15×10^6	0.87	2.30×10^6
16		+	300	14700	1.47×10^6	0.68	2.94×10^6
17		−	150	16800	1.68×10^6	0.59	3.36×10^6
18		−	150	13300	1.33×10^6	0.75	2.66×10^6
19		+	150	15500	1.55×10^6	0.65	3.10×10^6
20		+	150	14800	1.48×10^6	0.68	2.96×10^6
21		−	75	19700	1.97×10^6	0.51	3.94×10^6
22		−	75	15100	1.51×10^6	0.66	3.02×10^6
23		+	75	17300	1.73×10^6	0.58	3.46×10^6
24		+	75	20500	2.05×10^6	0.49	4.10×10^6
25		−	37.5	19300	1.93×10^6	0.52	3.86×10^6
26		−	37.5	18400	1.84×10^6	0.54	3.68×10^6
27		+	37.5	12900	1.29×10^6	0.78	2.58×10^6
28	Hycol 40LF	+	37.5	14200	1.42×10^6	0.70	2.84×10^6
29	EMS	−	200	11400	1.14×10^6	0.88	2.28×10^6
30	EMS	−	200	7000	7.0×10^5	1.42	1.40×10^6
31	DMN	+	100	5200	5.2×10^5	1.7	1.04×10^6
32	DMN	+	100	7600	7.6×10^5	1.32	1.52×10^6

C. Boelck 9/1/82

9/3/82 - Selection of Mutant CHO clones:

Media F12FCMS - hypoxanthine free 8/26/82

Fetal Bovine Serum Lot # KC-301005

6-TG (6-thioguanine) Media - Add 6.25 ml of a 10^{-3} M solution of 6-TG to 494 ml of media F12FCMS - hypoxanthine free. 5 ml of 6-TG media in each of 5 - 100 mm plates + 3 ml (3×10^5 cells) of cells in hypoxanthine free F12FCMS.

Cloning efficiency Plates - 6 - 60 mm plates containing 5 ml of media F12FCMS - hypoxanthine free + 0.2 ml of a 1×10^3 cells/ml aliquot (300 cells/pl.).

To Page No.

essed & Understood by me,	Date	Invented by	Date
J. Mezza	9/3/82	Received by	9/3/82

E CHO/HGPRT Mutation Assay - Glycol 40LF Book No. 354

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Sel. Plate #	Compound	±S9	ug/ml	cell Count	# cells per ml	# cells in suspension	Cloning Plate
AC-Dy-1-S	untreated	−	−	13200	1.32×10^6	5.28×10^6	AC-Dy-
-2-S	untreated	−	−	12000	1.20×10^6	4.80×10^6	
-3-S	untreated	+	−	13900	1.39×10^6	5.56×10^6	
-4-S	untreated	+	−	13100	1.31×10^6	5.24×10^6	
-5-S	H_2O	−	−	10700	1.07×10^6	4.28×10^6	
-6-S	H_2O	−	−	10000	1.00×10^6	4.00×10^6	
-7-S	H_2O	+	−	11900	1.19×10^6	4.76×10^6	
-8-S	H_2O	+	−	12000	1.20×10^6	4.80×10^6	
-9-S	Glycol 40LF	−	600	9200	9.2×10^5	3.68×10^6	
-10-S		−	600	10000	1.00×10^6	4.00×10^6	
-11-S		+	600	7500	7.5×10^5	3.00×10^6	
-12-S		+	600	10000	1.00×10^6	4.00×10^6	
-13-S		−	300	8400	8.4×10^5	3.36×10^6	
-14-S		−	300	10000	1.00×10^6	4.00×10^6	
-15-S		+	300	10300	1.03×10^6	4.12×10^6	
-16-S		+	300	8800	8.8×10^5	3.52×10^6	
-17-S		−	150	12300	1.23×10^6	4.92×10^6	
-18-S		−	150	13000	1.30×10^6	5.20×10^6	
-19-S		+	150	10600	1.06×10^6	4.24×10^6	
-20-S		+	150	12100	1.21×10^6	4.84×10^6	
-21-S		−	75	10000	1.00×10^6	4.00×10^6	
-22-S		−	75	11600	1.16×10^6	4.64×10^6	
-23-S		+	75	12400	1.24×10^6	4.96×10^6	
-24-S		+	75	11100	1.11×10^6	4.44×10^6	
-25-S		−	37.5	13000	1.30×10^6	4.80×10^6	
-26-S		−	37.5	11000	1.11×10^6	4.44×10^6	
-27-S		+	37.5	13900	1.39×10^6	5.56×10^6	
-28-S	Glycol 40LF	+	37.5	14000	1.40×10^6	5.60×10^6	
-29-S	EMS	−	200	12700	1.27×10^6	5.08×10^6	
-30-S	EMS	−	200	13900	1.39×10^6	5.56×10^6	
31-S	DMN	+	100	12400	1.24×10^6	4.96×10^6	
32-S	DMN	+	100	11100	1.11×10^6	4.44×10^6	

To Page No

essed & Understood by me,	Date	Invented by	Date
Lo J. Mecca	9/3/82	Recorded by Edward A. Dorel	9/3/82

E CHO/HGPRT Mutation Assay - Glyoxal HOLF Project No.: Book No. 354

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9/3/82 - Staining of Initial Survival Plates. Plates removed from incubator, washed, fixed, stained, rinsed in running tap water and allowed to air dry.

9/8/82 - Scoring of Initial Survival Plates:

Plate #	Compound	±S9	ug/ml	Plate Count	avg. no. $\bar{Y}$	# cells seeded plate	% Survival	Avg. % survival	Avg. rel.
GXY-1-IS	untreated	−	−	190 173 165	176.00	200	88.00		
2-IS	untreated	−	−	171 166 170	169.00	200	84.50	86.25	100
3-IS	untreated	+	−	173 158 166	175.67	200	87.83		
4-IS	untreated	+	−	158 170 165	164.33	200	82.17	85.00	98.
5-IS	H_2O	−	−	177 170 155	167.33	200	83.67		
6-IS	H_2O	−	−	177 163 167	169.00	200	84.50	84.09	97.
7-IS	H_2O	+	−	163 179 151	164.33	200	82.17		
8-IS	H_2O	+	−	152 162 182	165.33	200	82.67	82.42	95.
9-IS	Glyoxal HOLF	−	600	58 72 53	61.00	200	30.50		
10-IS		−	600	52 47 42	47.00	200	23.50	27.00	31.
11-IS		+	600	11 16 16	14.33	200	7.17		
12-IS		+	600	18 22 18	19.33	200	9.67	8.42	9.
13-IS		−	300	152 177 181	170.00	200	85.00		
14-IS		−	300	129 150 144	141.00	200	70.50	77.75	90
15-IS		+	300	113 112 130	118.33	200	59.17		
16-IS		+	300	127 146 125	133.67	200	66.83	63.00	73.
17-IS		−	150	135 138 137	136.67	200	68.34		
18-IS		−	150	148 156 114	139.33	200	69.67	69.01	80.
19-IS		+	150	95 143 129	122.33	200	61.17		
20-IS		+	150	133 143 157	144.33	200	72.17	66.67	77
21-IS		−	75	141 130 133	134.67	200	67.34		
22-IS		−	75	155 161 147	154.33	200	77.17	72.76	83.
23-IS		+	75	176 191 169	178.67	200	89.33		
24-IS		+	75	154 167 145	155.33	200	77.67	83.50	96
25-IS		−	37.5	177 170 158	168.33	200	84.17		
26-IS		−	37.5	154 158 186	166.00	200	83.00	83.59	96.
27-IS		+	37.5	152 171 148	157.00	200	78.50		
28-IS	Glyoxal HOLF	+	37.5	152 168 143	154.33	200	77.17	77.84	90.

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Witnessed & Understood by me,	Date	Invented by	Date
D. M. J. Mecca	9/8/82	Recorded by [illegible]	9/8/82

E CHO/HGPRT Mutation Assay - Glycol 40LF Book No. 354

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Plate #	Compound	±S9	ug/ml	Plate Counts	avg. no. $\bar{x}$	# cells seeded/plate	% Survival	Avg. % Survival	% mu
Day -									
29-IS	EMS	—	200	154 155 174	161.00	200	80.50		
30-IS	EMS	—	200	145 145 111	133.67	200	66.83	73.67	85
31-IS	DMN	+	100	61 62 66	61.00	200	30.50		
32-IS	DMN	+	100	47 52 50	49.67	200	24.83	27.67	32

[illegible] 9/8/82.

9/10/82 - Staining of resistant clones and cloning efficiency plates.

Plates removed from incubator, media aspirated off. Plates washed, fixed, and stained. Plates rinsed in running tap water and allowed to air dry.

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Witnessed & Understood by me,	Date	Invented by	Date
[illegible] Mecca	9/10/82	Read & Signed by [illegible]	9/10/82

Continuation of TG 354 Notebook Page 74

	Compound	± S9	ug/ml	# cells seeded/plate	mutant plate counts	total # mutants	surviving expression clones	# cells seeded/plate	$\bar{x}$ # clones	% expression survival
AC-Gly-1 1-S CE	untreated	−	−	2×10^5	2 2 0 1 1	6	21, 187, 183, 224, 237, 193	200	205.83	102.92
Ac-Gly-2 2-S CE	untreated	−	−	2×10^5	0 1 2 1 0	4	228, 218, 201, 199, 219, 247	200	218.67	109.33
AC-Gly-3 3-S CE	untreated	+	−	2×10^5	1 1 0 0 2	4	174, 175, 207, 198, 199, 200	200	192.17	96.08
Ac-Gly 4 S CE	untreated	+	−	2×10^5	1 1 2 1 1	6	210, 186, 181, 188, 205, 178	200	191.33	95.67
Ac-Gly 5 S CE	H_2O	−	−	2×10^5	1 3 3 2 2	11	212, 213, 252, 214, 191, 224	200	217.67	108.83
Ac Gly 6 S CE	H_2O	−	−	2×10^5	1 4 0 2 2	9	214, 213, 260, 232, 247, 219	200	235.83	117.92
Ac Gly 7 S CE	H_2O	+	−	2×10^5	1 1 3 2 0	7	198, 211, 247, 220, 211, 218	200	217.50	108.75
Ac Gly 8 S CE	H_2O	+	−	2×10^5	0 1 2 2 4	9	228, 249, 205, 224, 231, 188	200	220.83	110.42
Ac Gly 9 S CE	Glyoxal 40LF	−	600	2×10^5	0 5 5 6 6	22	201, 170, 173, 159, 149, 140	200	166.33	83.17
Ac Gly 10 S CE	Glyoxal 40LF	−	600	2×10^5	3 3 6 3 3	18	183, 186, 147, 208, 174, 182	200	180.00	90.00
Ac Gly 11 S CE	Glyoxal 40LF	+	600	2×10^5	8 6 3 5 1	23	175, 188, 156, 162, 184, 147	200	168.67	84.33
Ac Gly 12 S CE	Glyoxal 40LF	+	600	2×10^5	9 4 12 16 11	52	151, 158, 145, 151, 153, 135	200	148.83	74.42
Ac Gly 13 S CE	Glyoxal 40LF	−	300	2×10^5	5 4 3 2 2	16	200, 218, 187, 180, 160, 216	200	193.50	96.75
Ac Gly 14 S CE	Glyoxal 40LF	−	300	2×10^5	2 5 2 6 2	17	206, 173, 199, 180, 172, 160	200	181.67	90.83

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Book No. 354
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Witnessed & Understood by me, [illegible]

Date 9/20/82

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Date 9/20/82

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Project No. [illegible]

Book No. 354

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Cytg/HGPRT Mutation Assay: Glycol 40LF

			mx	plate	plate counts	mutants	Surviving expression clones	Cells seeded/plate	% cloning	% expression assumed
Ac Gly 15-S 15 CE	Glycol 40LF	+	300	2x10^5	2 5 1 3 2	13	163 170 141 150 175 144	200	157.17	78.83
Ac Gly 16 S 16 CE	Glycol 40LF	+	300	2x10^5	1 2 2 7 0	12	164 157 185 124 164 188	200	164.50	82.25
Ac Gly 17 S 17 CE	Glycol 40LF	−	150	2x10^5	5 9 8 4 2	28	188 189 224 191 183 128	200	184.67	92.33
Ac Gly 18 S 18 CE	Glycol 40LF	−	150	2x10^5	4 7 5 3 3	22	247 169 173 195 157 177	200	186.33	93.17
Ac Gly 19 S 19 CE	Glycol 40LF	+	150	2x10^5	1 4 2 1 3	11	208 210 181 195 216 171	200	191.83	95.92
Ac Gly 20 S 20 CE	Glycol 40LF	+	150	2x10^5	9 5 3 6 4	27	222 182 206 186 241 192	200	200.67	100.33
Ac Gly 21 S 21 CE	Glycol 40LF	−	75	2x10^5	4 4 3 1 0 5	26	221 236 243 147 172 185	200	200.67	100.33
Ac Gly 22 S 22 CE	Glycol 40LF	−	75	2x10^5	3 0 3 3 3	12	221 222 169 167 184 227	200	198.33	99.17
Ac Gly 23 S 23 CE	Glycol 40LF	+	75	2x10^5	4 4 2 6 1	17	173 232 148 180 164 177	200	178.67	89.33
Ac Gly 24 S 24 CE	Glycol 40LF	+	75	2x10^5	4 3 1 2 1	11	182 168 200 204 191 133	200	179.67	89.83
Ac Gly 25 S 25 CE	Glycol 40LF	−	37.5	2x10^5	2 2 1 4 4	13	181 190 191 173 184 160	200	179.83	89.92
Ac Gly 26 S 26 CE	Glycol 40LF	−	37.5	2x10^5	3 2 3 1 5	14	157 195 170 246 189 191	200	191.33	95.67
Ac Gly 27 S 27 CE	Glycol 40LF	+	37.5	2x10^5	2 2 0 1 4	9	218 173 168 149 166 206	200	180.00	90.00
Ac Gly 28 S 28 CE	Glycol 40LF	+	37.5	2x10^5	1 2 4 0 1	8	176 195 196 188 189 151	200	182.50	91.25

Read & Understood by me, J. Mecca

Date 9/30/82

Invented by Recorded by J. Mecca / J. Hook

Date 9/30/82

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CHO/HGPRT Mutation Assay - Hybrid Wolf

Project No. [illegible]
Book No. 354

	Compound	S9	µg/ml	# cells seeded/plate	Colony Counts	# mutants	# Colonies in expansion	# cells seeded/plate	# Colonies	% exp survival
Ac Rly 29 5 22 CE	EMS	−	200	2×10^5	50 51 47 43 52	243	157 157 183 195 188 194	200	186.67	90.33
Ac Rly 30 5 30 CE	EMS	−	200	2×10^5	49 47 48 45 50	239	163 180 195 231 163 165	200	187.83	93.92
Ac Rly 31 5 31 CE	DMN	+	100	2×10^5	26 29 38 32 30	155	97 134 113 144 135 143	200	127.67	63.83
Ac Rly 32 5 32 CE	DMN	+	100	2×10^5	40 44 41 43 32	200	130 161 155 113 124 118	200	133.5	66.75

Cytek Colony Counter calibrated
from sensitivity 6.0 – 8.3
Calibration = 419

Std. Sensitivity = 7.3
Comp. = 15%

Hand Count	Cytek Count
211	179
187	161
183	158

Witnessed & Understood by me,
[signature]

Date 9/20/82

Invented by
Recorded by [signature]

Date 9/20/82

To Page

E Cito/Hoppa Mutation Assay - Glyoxal Golf Project No. [illegible] Book No. 354

n Page No. 77

Compound	±S9	ug/ml	% relative survival (mean x̄)	total # mutants	Cloning efficiency (% survival)	Corrected Mutation Freq. (/10⁶ clonable cells)	Average Mutation Frequency
untreated	−	−		6	102.92	5.83 / 10⁶	
untreated	−	−	100.00	4	109.33	3.66 / 10⁶	4.75 /10⁶
untreated	+	−		4	96.08	4.16 / 10⁶	
untreated	+	−	98.55	6	95.67	6.27 / 10⁶	5.22 /10⁶
H_2O	−	−		11	108.83	10.11 / 10⁶	
H_2O	−	−	97.50	9	117.92	7.63 / 10⁶	8.19 /10⁶
H_2O	+	−		7	108.75	6.44 / 10⁶	
H_2O	+	−	95.56	9	110.42	8.15 / 10⁶	7.30 /10⁶
Glyoxal Golf	−	600		22	83.17	26.45 / 10⁶	
	−	600	31.30	18	90.00	20.00 / 10⁶	23.23 /10⁶
	+	600		23	84.33	27.27 / 10⁶	
	+	600	9.76	52	74.42	69.87 / 10⁶	48.57 /10⁶
	−	300		16	96.75	16.54 / 10⁶	
	−	300	90.14	17	90.83	18.72 / 10⁶	17.63 /10⁶
	+	300		13	78.83	16.49 / 10⁶	
	+	300	73.04	12	82.25	14.59 / 10⁶	15.54 /10⁶
	−	150		28	92.33	30.33 /10⁶	
	−	150	80.01	22	93.17	23.61 /10⁶	26.97 /10⁶
	+	150		11	95.92	11.47 /10⁶	
	+	150	77.30	27	100.33	26.91 /10⁶	19.19 /10⁶
	−	75		26	100.33 ~~[illegible]~~	25.91 /10⁶	
	−	75	83.78	12	99.17	12.10 /10⁶	19.01 /10⁶
	+	75		17	89.33	19.03 /10⁶	
	+	75	96.81	11	89.83	12.25 /10⁶	15.64 /10⁶
	−	37.5		13	89.92	14.46 /10⁶	
	−	37.5	96.92	14	95.67	14.63 /10⁶	14.55 /10⁶
	+	37.5		9	90.00	10.00 /10⁶	
Glyoxal Golf	+	37.5	90.25	8	91.25	8.77 /10⁶	9.38 /10⁶
EMS	−	200		243	90.33	269.01 /10⁶	
EMS	−	200	85.41	239	93.92	254.47 /10⁶	261.74 /10⁶
DMN	+	100		155	63.83	242.83 /10⁶	
DMN	+	100	32.08	200	66.75	299.63 /10⁶	271.23 /10⁶

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Witnessed & Understood by me,	Date	Invented by	Date
[illegible] J. Mecca	9/20/82	Recorded by [illegible]	9/20/82

PHARMAKON RESEARCH INTERNATIONAL, INC.
WAVERLY, PENNSYLVANIA 18471

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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 314-AC-002-82

The following inspections were performed:

Interval	Date
Treatment Phase	8/26/82
Selection for Mutation Phase	9/3/82
Scoring Phase	9/20/82
Reporting Phase	10/1/82

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

10/1/82
Date

Sheila Maas
Quality Assurance Unit

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