Attachments to Letter to C. Auer dated May 25, 2000
Toxicology Studies and Other Information on PFOA

Genotoxicity

1) An Assay of Cell Transformation and Cytotoxicity in the C3H 10T 1/2 Clonal Cell Line for the Test Chemical T-2942 CoC, Environmental Pathology Laboratory, Stone Research Laboratories, University of Minnesota, 3M Ref. No. FC-143 Lot 340, L138679, March 5, 1981

2) Final Report, Mutagenic Evaluation of T-2015 CoC in the Ames Salmonella/Microsome Plate Test, Litton Bionetics, Inc., Study No. 20838, 3M Ref. No. FC-143, February 1978

3) Final Report, Mutagenicity Test on T-6564 in an *In Vivo* Mouse Micronucleus Assay, Corning Hazelton, Inc., Study No. 17750-0-455, 3M Ref. No. FC-1015, L13167, straight-chain APFO, November 1, 1996

4) Final Report, Mutagenicity Test on T-6564 Measuring Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells: with a Confirmatory Assay with Multiple Harvests, Corning Hazelton Inc., Study No. 17750-0-437CO, 3M Ref. No. FC-1015, L13167, straight-chain APFO, September 16, 1996

5) Final Report, Mutagenicity Test with T-6564 in the *Salmonella - Escherichia Coli*/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay, Corning Hazelton Inc., Study No. 17750-0-409R, 3M Ref. No. FC-1015, L13167, straight-chain APFO, September 13, 1996

6) Final Report, Mutagenicity Test on T-6342 Measuring Chromosome Aberrations in Human Whole Blood Lymphocytes with a Confirmatory Assay with Multiple Harvests, Corning Hazelton, Inc., Study No. 17073-0-449CO, 3M Ref. No. FC-1090, L13364, F11426, Lot 1, sodium perfluorooctanoate, November 1, 1996

7) Final Report, Mutagenicity Test on T-6342 Measuring Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells: with a Confirmatory Assay with Multiple Harvests, Corning Hazelton, Inc., Study No. 17073-0-437CO, 3M Ref. No. FC-1090, L13364, F11426, Lot 1, sodium perfluorooctanoate, September 16, 1996

8) Final Report, Mutagenicity Test on T-6342 in an *In Vivo* Mouse Micronucleus Assay, Corning Hazelton, Inc., Study No. 17073-0-455, 3M Ref. No. FC-1090, L13364, F11426, Lot 1, sodium perfluorooctanoate, December 14, 1995

9) Final Report, Mutagenicity Test with T-6342 in the *Salmonella - Escherichia Coli*/Mammalian-Microsome Reverse Mutation Assay, Corning Hazelton, Inc., Study No. 1703073-0-409, 3M Ref. No. FC-1090, L13364, F11426, Lot 1, sodium perfluorooctanoate, December 14, 1995

AN ASSAY OF CELL TRANSFORMATION AND CYTOTOXICITY IN C3H 10T 1/2 CLONAL CELL LINE FOR THE TEST CHEMICAL T-2942 CoC

Submitted to the 3M Corporation

3M Center
St. Paul, Minnesota 55133

By the Environmental Pathology Laboratory
Department of Laboratory Medicine and Pathology
Stone Research Laboratories
Room 110
421 S.E. 29th Avenue
Minneapolis, Minnesota 55414

March 4, 1981

Report Submitted By:

Vincent F. Garry, M.D. — Date 3/5/81
Study Director

Richard L. Nelson — Date 3/5/81
Associate Scientist

TEST ARTICLE SPECIFICATIONS

STUDY NUMBER: 1

TEST ARTICLE I.D.: T-2942 CoC

TEST ARTICLE LOT NUMBER: -

DATE RECEIVED: 8/20/80

DATE STUDY BEGINS: 8/27/80

DATE STUDY COMPLETED: 10/5/80

TEST ARTICLE STORAGE CONDITIONS: Room Temperature

DILUENT(S)USED: DMSO

TEST ARTICLE FORM: GAS ____ LIQUID ____ SOLID X

TEST ARTICLE QUANTITY RECIEVED: 10 grams

SPECIAL HANDLING PRECAUTIONS: None

HAZARD RATING:

____ CHEMICAL 1 4

____ EXPLOSIVE 1 4

____ BIOLOGICAL 1 4

LD_{50} ORAL ACUTE: 540 mg/kg

SOLUBILITY OF TEST MATERIAL: (H_2O; CH_3OH; Lipid, etc.) H_2O; CH_3OH; Acetone

Quality Assurance Statement

The C3H 10T-1/2 cell transformation assay is a highly reproducible method requiring several internal controls. Cells, media and serum are routinely screened for growth potential and contaminants prior to incorporation into the test system. This function has been carried out by Mr. Nelson and reviewed by Dr. Garry in this initial study. The raw data and the final report has been reviewed by our Quality Control Officer, Mr. Robert Kreiger.

Vincent F. Garry, M.D.
Study Director

Robert Kreiger, M.S.
Quality Control Officer

Executive Summary

Assays for both cytotoxicity and cell transformation have been completed for test article T-2942 CoC, using C3H 10T-1/2 cell cultures *in vitro*. Cytotoxicity was measured by a reduction of cloning efficiency for treated cells. The data showed an LD_{50} of approximately 50 ug/ml. A dose range extending from 0 to 200 ug/ml was tested for cytotoxic effect. Comparison to a wide range of chemicals reported in the literature indicates that the chemical studied (T-2942 CoC) exhibits a low order of cytotoxicity, utilizing C3H 10T-1/2 cells.

Transformation assays were conducted using the previously derived LD_{50} as the median dose, with a dose range spread of four log units. Both the 14 day colony assay for transformation and a longer term foci transformation regimen (38 day) were completed for the test article T-2942 CoC. No evidence of transformation was observed for either the colony or foci assay methods using C3H 10T-1/2 cell cultures.

Introduction

This study was conducted for 3M by V. F. Garry, M.D., M.S., R. Nelson, B.A., R. Krieger, M.S. and M. Sinn, B.A., from 8/27/80 to 10/5/80 at the Environmental Pathology Laboratory of the University of Minnesota, Minneapolis, Minnesota. The experimental procedures employed are the test methods described by Reznikoff, Brankow and Heidelberger for the determination of transformation and cytotoxicity in the C3H 10T-1/2 cell line. Aside from this classical methodology, we have provided 3M an early reporting feature to give a preliminary evaluation of results. All procedures are described in detail in the protocol included in the appendix of the report.

Materials and Methods

Cell Source

Cryopreserved cells of the C3H 10T-1/2 clone 8 (Lot Number 3C1-1-4) utilized in these studies were derived from passage 11. The original cell stock was obtained from Dr. C. Heidelberger, Director for Basic Research of the University of Southern California. The cryopreserved lots were prepared from subcultures of the original cell line obtained at the 5th passage.

Cell Culture Conditions

Stock cultures for these studies were grown in Eagle Basal Medium (BME) containing 10% heat inactivated fetal bovine serum (Reheis Chem. Co., Lot Number T-48609). Prior to the study, the serum lot was tested for cloning efficiency and transformation efficiency in the presence of direct and indirect acting carcinogens. In addition, the entire cell system was tested for possible bacterial or mycoplasma contamination to insure the adequacy of the procedures.

Test Chemicals

The test chemical, T-2942 CoC was received on 8/20/80 from W. McCormick, an employee of 3M Corporation. Contained in a coded glass vial was approximately 10gm of white powder. The material was accompanied by a letter of authorization and information regarding *in vivo* toxicity. The test chemical was stored at room temperature. Immediately before use, the test material was dissolved in spectral grade DMSO (Fisher Chemical Co. Lot Number 702398) and diluted to appropriate concentrations. Within 20 minutes the diluted chemical was added to the cultures under subdued yellow light to avoid photo-inactivation. No more than 20 microliters of the diluted chemical was added to the cultures to avoid solvent effects. The chemicals, Benzo(a)pyrene (Eastman Kodak Lot Number 4941) and di-epoxybutane (Aldrich Chem. Co. Lot Number 082297) were used as positive controls.

Initial Cytotoxicity Determination

Prior to performance of the transformation assay, dose range data was obtained in the form of cytotoxicity measurements as expressed by plating (cloning) efficiency. The test chemical T-2942 CoC was added to cultures seeded the day before at 300 cells per 60mm culture dish. After 24-hour exposure to the test chemicals in 5ml BME media supplemented with 10% fetal calf serum; T-2942 CoC was removed with a complete change of media. The test chemical was applied through a 4 log scale dilutions ranging from 200 to 0.1 ug/ml. Six replicate cultures per dose point were employed and a total of six dose points were studied. The cultures were refed with fresh media every three days. After 7 days in culture, the plates were washed in PBS, fixed with absolute methanol and stained with giemsa stain. Positive controls, solvent controls and serum baseline controls were included in this study. The number of colonies per plate were counted and the plating efficiency was determined by the formula:

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

Transformation Assay

The approximate *in vitro* LD_{50} cytotoxicity dose was chosen as the median dose for the study of the transformation potential of the test chemical (T-2942 CoC). The test was conducted in two phases. In the first phase, cell transformation in the colony mode was assessed. In the second part, foci transformation potential was determined.

Phase 1 - Colony Transformation Potential

Six replicate plates seeded with 300 cells per plate were treated with 200, 100, 50, 10, 1.0 and 0.1 ug/ml to give appropriate two-fold and logarrithmic dose relationships for the test chemical T-2942 CoC. The positive control, benzo(a)pyrene (BP) was tested at 10, 5, 2.5, 1.0, and 0.1 ug/ml. A solvent control (DMSO) was included (10ug/ml). After removal of the chemical after 24 hours exposure, the cultures were refed fresh media and thereafter every 3 days for 14 to 17 days. At 14 days after removal of the test chemical, the plates were washed, fixed and stained as described before. Each colony was

carefully examined macroscopically and microscopically and scored for transformation according to criteria of Reznikoff, et al.

Phase 2 - Foci Transformation Potential

Remaining replicates at 5 replicates per dose were allowed to continue in culture for 38 days. Three dose increments were studied; 100, 10 and 1.0 ug/ml respectively. A positive control (butadiene epoxide) in dose increments of 0.01, 0.001, 0.005 and 0.0001 ug/ml was included along with a DMSO solvent control. The cultures were refed according to the schedule mentioned above for the first fourteen days. For the remainder of time in culture, the cells were refed once weekly with BME supplemented with 10% fetal calf serum. At 38 days after removal of the test chemical, the culture plates were processed as mentioned above.

Scoring for Transformation

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

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, multilayerd, criss-crossed arrays of densely stained cells. Eighty-five percent of Type III foci have been shown to be malignantly transformed.

Selected Type III foci will be retained from living cultures and subcultured twice, after which the cells will be cryopreserved for one year. If, during that time, confirmatory in vivo tumorogenesis studies are requested, these cells will be made available for injection into a suitable host.

Materials and Methods, Continued

Special C3H 10T-1/2 Early Reporting (Phase I)

Although the classic methodology for the C3H 10T-1/2 prescribed a 6-week course of study, recent data obtained by our Laboratory shows that the assay time may be foreshortened to 15 to 20 days. Detailed microscopic study of the colonies allows for quantitation in this somewhat shorter frame using the classic morphologic criteria previously described. This early reporting capability is available as preliminary information and is included in our report.

Recording of Data and Reports

All data from the cytotoxicity screening, transformation assay and early reporting information are recorded in tabular form (see enclosure). A coded access number will be provided to allow retrieval of cryopreserved material, and the fixed and stained cultures. All reports will remain confidential, subject to disclosure by the Corporation. Although there are no specific FDA guidelines for *in vitro* carcinogenesis testing, we will in future, adhere more closely to the Good Laboratory Practices protocol by formal establishment of a quality control unit in our laboratory.

References

1. Reznikoff, C., Brankow, D., Heidelberger, C. Cancer Research, 33:3231-3238, Dec. 1973.
2. Renzikoff, C., Brankow, D., Heidelberger, C. Cancer Research, 33:3239-3249, Dec. 1973.
3. Modal, S., Brankow, D., Heidelberger, C. Cancer Research, 36:7, Part I, 2254-2260, July, 1976.
4. Bertram, J. S. Cancer Research, 17:51, 1976.
5. Bertram, J. S. Cancer Research, 37:2, 514-523, 1977.
6. Haber, D. A., Fox, D. A., Dynan, W. S., Thilly, W. G., Cancer Research, 37:6, 1644-1648, June 1977.
7. Bertram, J. S., Cancer Letters 7:5, 288-298, September, 1979.

Results

In the dose ranging cytotoxicity test, the chemical T-2942 CoC showed a plating efficiency of 21.9% of control at 100 ug/ml (Table 1). To provide an efficient description of the transformation potential of the chemical, both colony and foci were studied based on the initial cytotoxicity screening. In the colony mode, eight dose points enclosing the in vitro LD_{50} were assessed in log scale and two-fold dilutions. There was no morphologic evidence of transformation in the fourteen day colonies treated with the test chemical (Table 2)

Longer term foci (38 days in culture) were studied in dose increments of 100, 10, and 1.0 ug/ml. No evidence of morphologic transformation were observed at any of the concentrations studied (Table 3).

The positive control, benzo(a)pyrene showed a dose related transformation frequency in the colony forming mode. In the foci mode, the positive control (butadiene epoxide) transformants were again observed in the dose increments studied. The combined number of type two and type three foci varied from 43.0 to 21.3/plate. No abnormalities were detected in the solvent controls.

Conclusions

The test chemical T-2942 CoC was studied in C3H 10T-1/2 cells for morphologic evidence of transformation in vitro. At the dose levels employed, no evidence of transformation was observed. The positive controls, benzo(a)pyrene and diepoxybutane showed development of either Type II and Type III colonies or Type II or Type III foci. The solvent control showed normal morphology.

Table 1

TOXICITY AND PLATING EFFICIENCY
IN CLONED C3H 10T 1/2 CL 8 CELLS
AFTER EXPOSURE TO CHEMICAL T-2942 CoC

3M Corporation	V. F. Garry, M.D.	T-2942 CoC
Client	Investigator	Test Compound I.D. No.
1	DMSO	200 ug/ml - 0.1 ug/ml
Study Number	Solvent	Dose Range

7 DAY COLONIES

DOSE ug/ml	Av. No. Col 300 Cells/Dish 6 Dishes/Dose	P.E.**** Percent of Controls	Transformed Colonies/ Cell***	Transformed Colonies (Total Colonies Percent)**
T-2942 CoC				
200	0	0		
100	9.0	21.96		
50	22.0	53.66		
10	33.66	81.99		
1.0	37.16	90.04	NONE	NONE
0.1	40.00	97.58		
CONTROL	41.0	-		
S. CONTROL*	40.8	99.27		

*10 ug/ml Solvent Control (DMSO)

**Percent Transformation (T/Col.%) = $\frac{\text{Average No. T/Plate}}{\text{Average No. Col./Plate}}$

***Transformation = $\frac{\text{Average No. T/Plate}}{\text{No. Cells Plated/Dish}}$

****P.E. Percent of Control = $\frac{\text{P.E. of Dose}}{\text{P.E. (Control)}} \times 100$

Table II

COLONY TRANSFORMATION AND CLONING EFFICIENCY
IN CLONED C3H 10T-1/2 CL 8 CELLS
AFTER EXPOSURE TO CHEMICAL T-2942 CoC

3M Corporation	V. F. Garry, M.D.	T-2942 CoC
Client	Investigator	Test Compound I.D. Number
1	DMSO	200 ug/ml - 1 ug/ml
Study Number	Solvent	Dose Range

14 day colonies

Dose ug/ml	Av. No. Col. 300 Cells/Dish 5 Dishes/ Dose	P.E.[d] Percent	P.E.[e] Percent of Controls	Combined Type II and Type II Colonies	
				Transformed Colonies/Cell[c]	Transformed Colonies[b] (Total Colonies Percent)
T-2942 CoC					
200	0	0	0		
100	27.61	9.05	63.15		
50	32.67	10.89	79.99		
20	35.0	11.67	81.44		
10	37.5	12.5	87.23		
5	38.67	12.89	89.95	NONE	NONE
2.5	40.5	13.5	94.21		
1.0	41.0	13.67	95.39		
CONTROL[a]	43.0	14.33	-		
S. CONTROL	41.33	13.77	96.09		
10	26.0	8.67	56.78	11.1×10^{-3}	12.81
5	29.3	9.7	63.52	8.86×10^{-3}	9.08
2.5	37.83	12.6	82.51	7.7×10^{-3}	6.16
1.0	40.5	13.5	88.41	7.2×10^{-3}	5.33
0.1	44.33	14.78	96.79	4.43×10^{-3}	3.0
CONTROL	45.8	15.27	-	-	-
S. CONTROL	43.0	14.3	93.65	-	-

(14 Days)

(a) 10 ug/ml Solvent Control (DMSO)

(b) Percent Transformation (T/Col.Percent) = $\frac{\text{Average No. T/Plate}}{\text{Average No. Col./Plate}}$

(c) Transformation (T/Cell) = $\frac{\text{Average No. T/Plate}}{\text{No. Cells Plated/Dish}}$

(d) P. E. Percent = $\frac{\text{Average No. of Col./Dish}}{\text{No. Cells Plated/Dish}} \times 100$

(e) P. E. Percent of Control = $\frac{\text{P. E. of Dose}}{\text{P. E. (Control)}} \times 100$

Table III

C3H 10T-1/2 CELLS
TRANSFORMED FOCI FORMATION ASSAY
AFTER EXPOSURE TO CHEMICAL T-2942 CoC

3M Corporation Client	V. F. Garry M.D. Investigator	T-2942 CoC Test Compound I.D. No.
1 Study Number	DMSO Solvent	100 ug/ml - 1.0 ug/ml Dose Range

Dose	Number of Days	Combined Type II and Type III Foci		Transformation Per Cell Plated Percent
		Total No. Foci	Av. No. Foci per plate	
T-2942 CoC				
100	38	0/6		
10	38	0/6		
1.0	38	0/6	NONE	NONE
CONTROL	38	0/6		
S. CONTROL	38	0/3		
+ Control Butadiene Diepoxide				
0.01	36	0	0	0
0.001	36	129/3	43.0	14.33
0.0005	36	84/3	28.0	9.33
0.0001	36	64/3	21.3	7.1

$$\frac{\text{Average No. of Type II and Type III Foci per Dish}}{\text{No. of Cells Plated per Dish}} \times 100$$

C3H 10T 1/2 CLONE 8 TRANSFORMATION ASSAY

The C3H 10T 1/2 cell line is derived from mouse embryonic cells. In the early 1970's Reznikoff, Bankow and Heidelberger[1,2] utilized clonal cells from the initial isolate to establish an *in vitro* measure of carcinogenesis, the transformation assay. In brief, the assay consists of exposure of mammalian cells to a potential carcinogen *in vitro* for a precise period of time; followed by a cultivation of newly seeded cells for a period of 30 to 35 days. During this time, cytotoxicity and morphologic evidence of neoplastic transformation is obtained. Because the cells are morphologically homogenous and sensitive to post confluence inhibition, abnormal cellular foci can easily be detected. The number of foci detected are proportional to the dose of the carcinogen applied. Similarly, the number of colonies of cells detected within the first 7-10 days of culture provide a quantitative basis for the determination of cytotoxicity. To confirm the carcinogenic potential of the transformed foci, the abnormal cells are injected into a susceptible host. The development of macroscopic tumors within 90 days constitutes confirmation of the neoplastic character of injected, morphologically abnormal cells.

The assay has good reproducibility in our hands as well as in those of our colleagues in the field. Each assay to be performed includes a positive control, solvent control and five dose points for the test chemical. Six separate cultures are used to establish each dose point.

1. Procedures

   A. Cell Source

      Low passage cryopreserved cells of the C3H 10T 1/2 clone 8 were obtained from Dr. Heidelberger. From the initial culture, a low passage stock of cells has been established. For long-term storage, the cells are first suspended in 8 percent DMSO plus media and are preserved in liquid nitrogen. For short-term storage, we routinely maintain cells in a -80° centigrade freezer. Periodically, sample cultures are tested for mycoplasma contamination, cloning efficiency and postconfluence inhibition. In the past sixteen months, cloning efficiency has varied from 12 to 16 percent. There is no evidence of "spontaneous transformation" in any of our cultures tested to date.

   B. Cell Culture Conditions

      Routinely, stock cultures are grown in Eagle Basal Medium (BME) containing 10 percent heat-inactivated fetal bovine serum without the use of antibiotics. The media is made from powdered form in high purity water obtained from certified commercial sources. Fetal calf serum lots are periodically tested for their effect on cloning efficiency and selected on this basis. To insure continuity of testing sufficient quantities of serum are held on reserve.

C. Test Chemicals

Chemicals to be tested should be identified as precisely as possible. Information regarding the physical characteristics (eg) solubility, in vivo toxicity (LD_{50}), and source are important considerations. Each chemical received is coded and duplicate records of the test procedure are maintained by the Laboratory.

Chemicals to be studied will be tested on a ug/ml basis in an appropriate solvent (eg) DMSO. Addition of the chemicals to culture is accomplished by media dilutions under photographic safety light. Subsequent media changes are carried out under gold fluorescent light.

2. Cytotoxicity

Prior to performance of the transformation assay, dose range data is obtained in the form of cytotoxicity measurements. In accordance with the protocol established by Reznikoff, Bertram, Brankow, and Heidelberger[1], cytotoxicity is measured in terms of plating efficiency. A fixed number of cells (approximately 300/dish) are placed in culture and allowed to grow for 7 to 10 days. The percentage of surviving colonies divided by the number of cells initially plated is then determined (ie) the cloning efficiency. Routinely, chemicals to be tested are added to the cultures 24 hours after plating. After a standard 24-hour exposure period, the chemicals are removed with a change of media. The cells are refed fresh media every three days. The test chemicals are applied through a 4-log dose scale using 10 dose points. Three replicate cultures per point are employed. Positive control, solvent control and serum baseline controls are included with each study. All chemical or solvent additions to the media are not allowed to exceed 0.5% of the total media volume. At the end of 7 to 10 days, the cultures are fixed, stained and the number of colonies are counted macroscopically and microscopically.

3. Transformation Assay

The transformation assay is performed in accord with the methodology developed by Reznikoff, et al[1,2] and modified by Bertram, et al[4]. In line with this classical approach, approximately 300 cells are plated in each of six separate culture dishes to achieve six replicates per dose point, once the desired concentration range has been determined by measurements of cloning efficiency. Routinely, a minimum of four dose points are studied in increasing dilutions from a dose which causes 50 to 75 percent decrease in cloning efficiency. Dilutions will be made two-fold or more, depending on the slope of the cytotoxicity curve. The procedures for chemical additions to the cultures are identical to that employed for cytotoxicity.

All cultures are refed with Basal Eagles media with 10 percent fetal calf serum. Routinely, re-feeding is done every three days until a confluent monolayer of cells is acheived; usually 15 to 20 days. Thereafter, the cultures are refed weekly.

-----Transformation Assay Continued

At 35 days after removal of the compound, all culture dishes are fixed, stained, and examined macroscopically and microscopically, and scored for transformation. All of the fixed and stained cultures are retained for one year as a semi-permanent record of the study. Scoring of morphological transformation is in accordance with the types described by Reznikoff, et al[1].

A. Scoring of Transformation

Focal areas of transformation are classified according to the criteria of Reznikoff[1] as follows:

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.

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5. Recording of Data and Reports

All data from the cytotoxicity screening, transformation assay and early reporting information are recorded in tabular form (see enclosure). A coded access number will be provided to allow retreival of cryopreserved material, and the fixed and stained cultures. All reports will remain confidential,

-----Recording of Data and Reports, Continued

subject to disclosure by the Corporation. Although there are no specific FDA guidelines for in vitro carcinogenesis testing, we adhere to the Good Laboratory Practices Protocol put forward by this Federal Agency.

6. References

1. Reznikoff, C., Brankow, D., Heidelberger, C. Cancer Research, 33:3231-3238, Dec. 1973.

2. Reznikoff, C., Brankow, D., Heidelberger, C. Cancer Research, 33:3239-3249, Dec. 1973.

3. Mondal, S., Brankow, D., Heidelberger, C. Cancer Research, 36:7, Part I, 2254-2260, July, 1976.

4. Bertram, J.S. Cancer Research, 17:51, 1976.

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

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