Commercial
Chemicals Division 3M

3M Center
St. Paul, Minnesota 55144
612/733-1110

page 5
186
(41)

4/1/82
Tobkin

3M

March 22, 1982

8EHQ-0380-0394 Supplement
88-8100365

Mr. Terry O'Bryan
Chemical Hazard Identification Branch
U.S. Environmental Protection Agency

Dear Mr. O'Bryan:

At our meeting on Friday, March 12, I left for your evaluation four studies from our laboratory numbered as follows:

0680TR0020	Rat oral alcohol
0681TR0212	Rabbit oral alcohol
0681TR0398	Rabbit oral FC-143
0681TR0110	Rat oral FC-143

Since my return to St. Paul, I have been requested to state that those copies left with you contain confidential business information, since the name and names of several individuals were included. Additionally, one of the documents, contained information concerning the specific chemical composition of the commercial product. Sanitized copies of the reports are being submitted herewith.

For your convenience, the relationship of the several numbers in the reports is:

EPA	REPORT	SAMPLE	CHEMICAL
1180-0373	R0020	2999	
1180-0373	R0212	2999	
0381-0394	R0398	3141	FC143 - Ammonium Carboxylate
0381-0394	R0110	2998	FC143 - Ammonium Carboxylate
0381-0394		2942	FC143 - Ammonium Carboxylate

The original submission of 0373 was based on alcohol. The two substances are generically:

$$C_nF_{2n+1}SO_2N(C_2H_5)C_2H_4OH$$

The relationship of the two is:

A question was raised whether data other than the teratogenicity studies were available to aid in evaluating the safety of the materials. We are accordingly including a number of reports of earlier studies on the three subject materials.

Cell Transformation and Cytotoxicity	143	T(C) 399
90 Day Oral Monkey - 090	143	
90 Day Oral Rat - 089	143	
90 Day Oral Monkey - 088	3422	T(C) [redacted]
90 Day Oral Rat - 086	3423	
90 Day Oral Monkey - 092	95	
90 Day Oral Rat - 085	95	

An epidemiology study of employees in a plant in which these materials have been made for many years is also presented.

These studies are part of an ongoing program to establish the hazards connected with the material we manufacture and process. As such, they present more questions than answers, but we believe they evidence no unreasonable risk to man or the environment.

I apologize again for not providing these studies at an earlier date. It just never occurred to me that they had not been sent to your group.

Since certain of the information presented herein is considered to be confidential business information, this cover letter is confidential. A sanitized copy is also provide for your use.

Yours very truly,

W. H. Pearlson
Corporate Scientist/Regulatory Affairs
Commercial Chemicals/Chemical Resources Division

WHP:rmb

Enclosure

88HQ-0382-0394 Supp
88-8100365

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.
Study Director
Date

Richard L. Nelson
Associate Scientist
Date

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. E. 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 (Rohels 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 702395) 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 I - 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 logarithmic 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 6 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.

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. Maber, 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 CHO TOT 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

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

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

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)	Combined Type II and Type II Colonies: 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}	5.39
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	-	-

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

Dose	Number of Days	Combined Type II and Type III Foci: Total No. Foci	Combined Type II and Type III Foci: Av. No. Foci per plate	Transformation Per Cell Plated Percent
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.

I. 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 (LD50), 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.

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

Selected Type III foci will be retained from living cultures and sub-cultured 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.

4. Special C3H 10T 1/2 Early Reporting

Although the classic methodology for the C3H 10T 1/2 prescribes 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.

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 retrelval 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 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, 17:91, 1976.

5. Bertram, J.S. Cancer Research, 37:2, 514-23, 1977.

6. Heber, 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.

Reference

1. Coulter Hemoglobinometer. Coulter Electronics, 590 W. 20th Street, Hialeah, Florida.

2. Microhematocrit, John H. Miale, 3rd Ed., 1967, The C. V. Mosby Company, p. 1154.

3. Coulter Particle Size Counter, Model Z$_\beta$, Coulter Electronics, 590 W. 20th Street, Hialeah, Florida.

4. Gradwhol's Clinical Laboratory Methods and Diagnosis, Frankel and Reitman, Editors 6th Ed., 1963, The C. V. Mosby Company, p. 1132.

5. Coulter Particle Size Counter, Model A, Coulter Electronics, 590 W. 20th Street, Hialeah, Florida.

6. General Diagnostics - Warner Chilcott Laboratories Revised April 1965.

7. General Diagnostics - Warner Chilcott Laboratories Revised January 1967.

8. Technicon Auto Analyzer, 6/60 Micro Methodology.

9. Micro Auto Analyzer II, 6/60 Micro Methodology.

10. Atomic Absorption II, Model 353

11. Sigma SGTP Procedure Bulletin #545. Sigma Chem. Co., St. Louis, Mo.

12. Bililabstix (Ames Reagent Strips).

13. Steel, R. G. D. and Torrie, J. H. (1960), Principles and Procedures of Statistics, McGraw-Hill, New York, N. Y.

14. Dunnett, C. W., New Tables for Multiple Comparisons With a Control, Biometrics, McGraw-Hill, New York, N. Y.

137-090

International Research and Development Corporation

SPONSOR: 3M Company

COMPOUND: Fluorad® Fluorochemical FC-143

SUBJECT: Ninety Day Subacute Rhesus Monkey Toxicity Study.

Edwin I. Goldenthal, Ph.D.
Vice President and
Director of Research

Collaborators:

D. C. Jessup, Ph.D., Associate
Director of Research
R. G. Geil, D.V.M., Vice
President and Director of Pathology
J. S. Mehring, Ph.D., Director of
Large Animal Toxicology

Date: November 10, 1978

137-090

000024

TABLE OF CONTENTS
(Continued)

Table No.		Page
5- 7.	Individual Hematological Values	18-20
8.	Means and Significance of Biochemical Values	21
9-11.	Individual Biochemical Values	22-24
12.	Means and Significance of Urinalysis Values	25
13-15.	Individual Urinalysis Values	26-28
16.	Summary of Gross Necropsy Observations	29-30
17.	Absolute and Relative Organ Weights	31-32
18.	Microscopic Observations	33-39

I. SYNOPSIS

In a ninety day oral study in rhesus monkeys, Fluorad® Fluorochemical FC-143 was administered at dosage levels of 0 (control, treated only with 0.5% Methocel®), 3, 10, 30 and 100 mg/kg/day. Two male and two female monkeys were initiated at each dosage level and also in a control group. The monkeys were observed twice daily for general physical appearance and behavior and pharmacotoxic signs. Body weights were recorded weekly. Hematological, biochemical and urinalysis studies were conducted once in the control period, at the end of the first and third months of study.

The monkeys treated with the higher dose, (100 mg/kg/day) all died during weeks 2 through 5 of the study. At the 30 mg/kg/day dosage level, three monkeys died during weeks 7-12. They all showed signs of toxicity in the gastrointestinal tract (anorexia, emesis, sometimes brown in color, black stools), pale face and gums, swollen face and eyes, slight to severe decreased activity and prostration. The monkeys of the 30 and 100 mg/kg/day dosage level showed body weight losses from the first week of the study.

Because of the early deaths of the monkeys at the 100 mg/kg/day dosage level, the clinical laboratory tests were not conducted.

The monkeys at the 30 mg/kg/day dosage level showed, in the first month of the study, slight increase in prothrombin time and in activated partial thromboplastin time (A.P.T.T.) values, as well as decreased alkaline phosphatase activity in the serum (statistically significant). Only one monkey from this dosage level in this period showed a low albumin value. At the end of the study, the only remaining monkey from the 30 mg/kg/day dosage level showed apparent anemia, low blood glucose, alkaline phosphatase, total protein and albumin values.

There was no mortality at the 10 mg/kg/day dosage level. One monkey had black stool on several days in week 12 and occasionally

anorexia and one monkey exhibited pale face and gums. At this dosage level there was a very slight increase in the activated P.T.T. values in the female monkeys during the first month of the study (not statistically significant). There were no changes in the other indices and no changes in the body weight. In single monkey. from the 3 and 10 mg/kg/day dosage levels, there were trends toward decreased alkaline phosphatase in the serum.

In the control and the 3 mg/kg/day dosage level there was no mortality, no changes in the body weights and no signs of toxicity. Soft stool, diarrhea or emesis were observed occasionally.

The mortality and the above mentioned signs of toxicity in the 30 and 100 mg/kg/day dosage levels were compound-related. There was a trend toward the same signs of toxicity in single monkeys at the 10 mg/kg/day dosage level. The 3 mg/kg/day dosage level seems to be free of signs of toxicity. There is an evident relationship between the administered doses and the degree of the toxicity.

No gross or microscopic lesions which were considered compound-related were seen in tissues other than the adrenals, bone marrow, spleen and lymph nodes for male and female monkeys at the 30 and 100 mg/kg/day dosage levels. Microscopically, the adrenals from male and female monkeys at the 30 and 100 mg/kg/day dosage levels had compound-related marked diffuse lipid depletion; the bone marrow from male and female monkeys at the 30 and 100 mg/kg/day dosage levels had compound-related slight to moderate hypocellularity; the spleen and lymph nodes from male and female monkeys at the 30 and 100 mg/kg/day dosage levels had compound related moderate atrophy of lymphoid follicles.

Statistically significant variations in sex group mean weights of a few organs occurred between the control and experimental groups. These variations were of unknown biological significance and were not accompanied by morphologic alterations.

II. COMPOUND

The compound was received from 3M Company, Saint Paul, Minnesota on October 24, 1977 as shown below:

Label	Description
Fluorad® Fluorochemical FC-143 3M Stock No. 98-0211-0008-0 Lot 340	white powder

III. CLINICAL STUDIES

A. METHODS:

1. General Procedure:

Ten male rhesus monkeys (weighing from 2.60 to 3.90 kilograms) and 10 females (weighing from 2.95 to 3.80 kilograms) were initiated on this study. The monkeys were purchased from Primate Imports Corporation, Port Washington, N. Y. 11050. The monkeys were housed individually in hanging wire mesh, "squeeze type" cages and maintained in a temperature, humidity and light controlled environment. Purina® Monkey Chow® was fed twice each day and fresh apples were fed 3 times a week. Water was available *ad libitum*.

During the conditioning period, the monkeys were tattooed on the inner surface of the thigh and intrapalpebral tuberculin tests were conducted. Tuberculin tests were conducted at bimonthly intervals during the treatment period. Also a complete physical examination was conducted by the staff veterinarian prior to initiation of compound administration. Only monkeys in good health were selected for the study.

This study was initiated on January 11, 1978. Terminal sacrifices were conducted on April 12, 1978.

2. Compound Administration:

At the end of the conditioning period the monkeys were divided into five groups on a random basis, so that the initial average body weights were similar:

Number of Monkeys		
Male	Female	Dosage Level
2	2	Control
2	2	3 mg/kg/day
2	2	10 mg/kg/day
2	2	30 mg/kg/day
2	2	100 mg/kg/day

The test compound, suspended in 0.5% Methocel®, was administered by gavage, 7 days each week. All doses were given in a constant volume. Also the same volume of 0.5% Methocel® was given to the vehicle control group. Individual daily doses were based upon the body weights obtained weekly.

3. Observations:

The monkeys were observed twice daily for general physical appearance and behavior and pharmacotoxic signs. Individual body weights were recorded weekly. General physical examinations were conducted in the control period and monthly during the study.

4. Clinical Laboratory Tests:

Blood and urine samples were obtained for analysis from all monkeys once during the control period and at 1 and 3 months of study. The monkeys were fasted overnight prior to the collection of blood and urine samples.

a. Hematology:

Hematological studies included: hemoglobin[1], hematocrit[2], erythrocyte count[3], total[3] and differential leucocyte counts, reticulocyte count[4], platelet count[5], prothrombin time[6], activated partial thromboplastin time[7] (A.P.T.T.). Mean corpuscular hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin concentration were calculated.

b. Biochemistry:

Biochemical studies included: fasting blood glucose[8], blood urea nitrogen[8], serum alkaline phosphatase[8], serum glutamic oxalacetic and pyruvic transaminase activities[8], cholesterol[9], total protein[9], albumin[8], sodium[10], potassium[10], chloride[9], inorganic phosphate[9], γ-glutamyl transpeptidase[11] (γ-G.T.P.) and creatinine phosphokinase[9].

c. Urinalysis:

Urinalysis included: measurement of volume, pH[12] and specific gravity; description of color and appearance; qualitative tests for protein[12], glucose[12], ketones[12], occult blood[12] and microscopic examination of the sediment.

d. Statistical Analysis:

Analysis of body weights and clinical laboratory tests were performed. All statistical analyses compared the treatment groups with the control group, by sex. The tests were compared by analysis of variance (one-way classification) Bartlett's test for homogeneity and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie[13] using Dunnett's[14] multiple comparison tables to judge significance of differences.

B. RESULTS:

1. General Behavior, Appearance and Survival:

There was no mortality in monkeys at 0, 3 and 10 mg/kg/day dosage levels.

The monkeys from the control and 3 mg/kg/day dosage levels did not show any unusual behavior or signs of toxicity. Soft stool or moderate to marked diarrhea were noted occasionally. Frothy emesis was also noted occasionally.

At the 10 mg/kg/day dosage level the monkeys did not show any unusual signs of toxicity, except Monkey 7363. In week 7 its face appeared swollen and pale. It had been occasionally anorexic in week 4 and black stools appeared for several days in week 12 of the study.

At the 30 mg/kg/day dosage level, three monkeys died during weeks 7, 12 and 13 of the study. From week 4, the monkeys were anorexic. Slight to moderate and sometimes severe decreased activity was noted occasionally to frequently for the four monkeys. Emesis and ataxia were very rarely noted, for one monkey.

Swollen face, eyes and vulva, as well as pallor of the face and gums were noted. From week 6, for two monkeys, black stools were noted. Monkey 7387 showed slight to moderate dehydration and ptosis of the eyelids.

All monkeys from the 100 mg/kg/day dosage level died during weeks 2 through 5 of study. They showed the same symptoms of toxicity as the previous group, but they appeared sooner in the study (from week 1) and were more marked: anorexia, frothy emesis (sometimes brown in color) pale face and gums, swollen face and eyes, decreased activity from slight to severe, prostration and body trembling.

2. Body Weights (Tables 1-3):

Changes in body weight were similar for monkeys from the control and the 3 and 10 mg/kg/day dosage levels. Monkeys at the 30 and 100 mg/kg/day dosage levels lost body weight after the first week of study. There was statistically significant decreases in the body weight for the male monkeys at the 30 mg/kg/day dosage level in week 13 of the study. The female monkeys of the same dosage level and the monkeys from the 100 mg/kg/day dosage level were dead in this period.

3. Laboratory Test (Tables 4-15):

a. Hematology:

There were no noteworthy changes in monkeys from the 3 and 10 mg/kg/day dosage levels. In the first month of the study there was a slight increase (not statistically significant) of the A.P.T.T. values in the females at the 10 mg/kg/day dosage level and a statistically significant increase of the A.P.T.T. and prothrombin time values in monkeys at the 30 mg/kg/day dosage level. In the third month of the study there was a high increase in the above mentioned indices for the one surviving monkey from the 30 mg/kg/day dosage level. The same monkey (07455) had pronounced anemia as well.

The statistically significant increase in the hematocrit in monkeys at the 10 mg/kg/day dosage level and in the platelet count in monkeys at the 3 mg/kg/day dosage level at 3 months of study, were within the normal physiological limits.

b. Biochemistry:

There were no noteworthy changes in monkeys from the control, 3 and 10 mg/kg/day dosage level. Only one monkey from the 3 mg/kg/day dosage level and one monkey from the 10 mg/kg/day dosage level showed trends toward decreases of alkaline phosphatase (432 and 474 units/l, respectively), without statistical significance.

In the first month of the study, decrease in serum alkaline phosphatase was noted in monkeys at the 30 mg/kg/day dosage level (statistically significant) and in one monkey in the same dosage level, the albumin in the serum was lower (3.22 g/100ml). The one surviving monkey (7455) from the 30 mg/kg/day dosage level showed decreasing of: blood sugar (66 mg/100ml), total protein (5.52 g/100ml) with albumin (2 g/100ml) and alkaline phosphatase (360 units/l) and slightly elevated cholesterol (240 mg/100ml).

c. Urinalysis:

No changes considered to be related to compound were seen in the urinalysis studies.

IV. PATHOLOGICAL STUDIES

A. METHODS:

1. Gross Pathology:

After completion of the compound administration period all surviving monkeys were anesthetized with Sernylan®*, exsanguinated and necropsied. At necropsy, the heart, liver, adrenals, spleen, pituitary, kidneys, testes/ovaries and brain were weighed and representative tissues were collected in buffered neutral 10% formalin. Eyes were fixed in Russell's fixative. The thyroid/parathyroid was weighed after fixation.

Monkeys which died during the study were necropsied as above.

2. Histopathology:

Microscopic examination of formalin fixed hematoxylin and eosin stained paraffin sections was performed for all monkeys in the control and treatment groups. The following tissues were examined:

adrenals	kidneys	lumbar spinal cord
aorta	liver	pituitary
bone	lung	stomach
brain	skin	testes/ovaries
esophagus	mesenteric lymph node	thyroid
eyes	retropharyngeal lymph node	parathyroid
gallbladder	mammary gland	thymus
heart (with coronary vessels)	nerve (with muscle)	trachea
duodenum	spleen	tonsil
ileum	pancreas	tongue
jejunum	prostate/uterus	urinary bladder
cecum	rib junction (bone marrow)	vagina
colon	salivary gland	tattoo
rectum		

and any other tissue(s) with lesions

*Phencyclidine HCl - Bio-Ceutic Laboratories, Inc., St. Joseph, Missouri.

B. RESULTS:

1. Gross Pathology (Table 16) and Organ Weights (Table 17):

No gross lesions considered compound related were seen in male and female rhesus monkeys which died on study or were sacrificed after 90 days of study.

Statistically significant variations in sex group mean weights of few organs occurred between the control and experimental groups. The following statistically significant organ weight variations occurred:

Organ	Dosage Level mg/kg/day	Sex	Weight	Change	p<
Heart	10	F	absolute,relative	decrease,decrease	0.05,0.01
Brain	10	F	absolute	decrease	0.01
Pituitary	3	M	relative	increase	0.05

The biological significance of these variations is unknown. These organ weight variations were not accompanied by morphologic changes which were considered compound related.

2. Histopathology (Table 18):

One male and two female rhesus monkeys at the 30 mg/kg/day dosage level and all male and female rhesus monkeys at the 100 mg/kg/day dosage level had marked diffuse lipid depletion in the adrenals. All male and female rhesus monkeys at the 30 and 100 mg/kg/day dosage levels had slight to moderate hypocellularity of the bone marrow. All male and female rhesus monkeys at the 30 and 100 mg/kg/day dosage levels had moderate atrophy of lymphoid follicles in the spleen. One female at the 30 mg/kg/day dosage level and all male and female rhesus monkeys at the 100 mg/kg/day dosage level had moderate atrophy of the lymphoid follicles in the lymph nodes.

No microscopic changes considered compound related were seen in the adrenals, bone marrow, spleen and lymph nodes of male and female rhesus monkeys at the 3 and 10 mg/kg/day dosage levels. No microscopic

lesions in tissues other than the adrenals, bone marrow, spleen and lymph nodes at the 30 and 100 mg/kg/day dosage levels were considered compound-related.

FC-143: Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE I. Mean Body Weights of Monkeys Week 13 of Study.

Sex	Group I (Control)	Group II (3 mg/kg/day)	Group III (10 mg/kg/day)	Group IV (30 mg/kg/day)	Group V (100 mg/kg/day)
M	3.78	3.50	3.68	2.32*	dead
F	3.55	3.68	3.78	dead	dead

*Statistical significance.

FC-143: Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 2. Individual Body Weights, Kilograms.

Group, Monkey Number	Sex	Control 1	Control 2	Week of Study 1	2	3	4	5	6	7	8	9	10	11	12	13
Control:																
7362	M	3.15	3.30	3.15	3.30	3.35	3.10	3.20	3.20	3.00	3.15	3.20	3.05	3.20	3.40	3.50
7365	M	3.50	3.50	3.50	3.50	3.50	3.40	3.55	3.60	3.60	3.85	3.75	3.75	3.80	4.00	4.05
7336	F	3.05	3.20	3.25	3.25	3.15	3.15	3.00	3.15	3.20	3.30	3.45	3.30	3.35	3.35	3.60
7386	F	3.90	3.70	3.70	3.65	3.55	3.45	3.40	3.55	3.40	3.40	3.55	3.40	3.50	3.50	3.50
Mean		3.40	3.43	3.40	3.43	3.44	3.28	3.29	3.38	3.30	3.41	3.49	3.38	3.46	3.56	3.66
3 mg/kg/day:																
7364	M	3.70	3.90	3.85	3.95	3.85	3.85	3.80	3.80	3.85	4.10	4.10	4.05	4.05	4.20	4.30
7366	M	2.60	2.60	2.70	2.60	2.65	2.65	2.70	2.70	2.55	2.70	2.70	2.45	2.55	2.50	2.70
7384	F	3.55	3.60	3.70	3.80	3.80	3.80	3.70	3.70	3.60	3.55	3.80	3.55	3.70	3.90	3.75
7385	F	3.50	3.55	3.45	3.45	3.45	3.45	3.40	3.40	3.50	3.55	3.60	3.40	3.30	3.40	3.60
Mean		3.34	3.41	3.43	3.45	3.44	3.44	3.40	3.40	3.38	3.48	3.55	3.36	3.40	3.50	3.59
10 mg/kg/day:																
7363	M	3.55	3.70	3.70	3.65	3.65	3.65	3.65	3.60	3.60	3.70	3.65	3.75	3.85	3.90	3.90
7458	M	3.10	3.10	3.25	3.20	3.10	3.05	2.95	3.20	3.00	3.15	3.10	3.10	3.25	3.25	3.45
7328	F	3.30	3.30	3.45	3.40	3.40	3.30	3.20	3.30	3.25	3.45	3.60	3.50	3.40	3.60	3.75
7383	F	3.60	3.60	3.50	3.80	3.60	3.55	3.50	3.60	3.60	3.65	3.80	3.65	3.75	3.75	3.80
Mean		3.39	3.43	3.48	3.51	3.44	3.39	3.33	3.43	3.36	3.49	3.54	3.50	3.56	3.63	3.73

PC-143: Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 2. Cont. Individual Body Weights, Kilograms.

Group, Monkey Number	Sex	Control 1	Control 2	Week of Study 1	2	3	4	5	6	7	8	9	10	11	12	13
30 mg/kg/day:																
7367	M	3.40	3.40	3.25	3.25	3.10	2.95	2.45	2.30	2.10*	Died					
7455	M	3.50	3.30	3.20	3.05	2.85	2.65	2.45	2.50	2.55	2.60	2.70	2.70	2.65	2.50	2.30
7382	F	3.25	3.30	3.20	3.20	3.05	3.00	2.85	2.80	2.80	2.80	2.80	2.80	2.80	2.60	2.25* Died
7387	F	3.70	3.75	3.50	3.55	3.50	3.45	3.10	2.95	2.85	2.85	2.70	2.65	2.50	2.25*	Died
Mean		3.46	3.44	3.29	3.26	3.13	3.01	2.76	2.64	2.73	2.75	2.73	2.72	2.65	2.55	2.30
100 mg/kg/day:																
7361	M	3.50	3.85	3.50	3.30	3.00	2.55	2.40*	Died							
7456	M	3.10	3.10	2.60	2.70*	Died										
7335	F	2.80	2.95	2.70	2.45	2.05*	Died									
7381	F	3.85	3.80	3.55	3.20	2.80	2.60*	Died								
Mean		3.31	3.43	3.09	2.98	2.90	2.55									

*Terminal weight not included in mean.

0000339

FC-1431 N ety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 3. T-Test Comparison of Body Weights.

Study Week	Sex	Control	3 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
13	M	3.78	3.50	3.68	2.30[a]	-
	F	3.55	3.68	3.78	-	-

*p<0.05
**p<0.01
[a]Not included in statistical analysis due to only one surviving animal.
- Line indicates animals had died prior to week 13.

137-090

FC-1431 Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 4. Means and Significance of Hematological Values.

Hematology	Month of Study	Control	3 mg/kg/day	10 mg/kg/day	30 mg/kg/day
Erythrocytes, 10^6/cmm	1	4.46	4.26	4.71	4.53
	3	4.90	4.74	5.47	3.84[a]
Hemoglobin, g/100 ml	1	11.7	11.4	12.1	11.7
	3	12.9	12.7	13.3	9.7[a]
Hematocrit, %	1	38	37	39	36
	3	37	37	40**	30[a]
Platelets, 10^3/cmm	1	253	233	210	219
	3	210	285*	216	261[a]
Reticulocytes, %	1	0.2	0.5	0.5	0.2
	3	0.3	0.2	0.2	0.2[a]
Prothrombin Time, sec	1	12	12	13	15**
	3	11	11	11	30[a]
Activated P.T.T., sec	1	28	28	31	35**
	3	26	26	24	65[a]
Leucocytes, 10^3/cmm	1	9.49	9.78	9.93	8.44
	3	9.40	9.83	11.96	10.14[a]
Neutrophils, %	1	24	19	26	15
	3	16	19	25	36[a]
Lymphocytes, %	1	75	76	72	85
	3	80	76	67	54[a]
Eosinophils, %	1	1	5*	2	0
	3	3	3	6	3[a]
Monocytes, %	1	0	0	0	0
	3	1	2	2	7[a]
Basophils, %	1	0	0	0	0
	3	0	0	0	0[a]
MCV, μ^3	1	86	86	82	80
	3	75	78	73	78[a]
MCH, $\mu\mu g$	1	27	27	26	26
	3	26	27	24	25[a]
MCHC, g/100 ml	1	31	31	32	32*
	3	36	35	34	32[a]

*Significantly different from control group, $p<0.05$.
**Significantly different from control group, $p<0.01$.
[a]Value not used in statistical analysis due to only one animal surviving.

137-090

[illegible]: Short-Term Toxicity Study.

TABLE 5: Individual Hematological Values - Control 1.

Group, Animal Number	Sex	Erythrocytes 10^6/mm³	Hemoglobin g/100 ml	Hematocrit %	Platelets 10^3/mm³	Reticulocytes %	Prothrombin Time sec	Activated P.T.T. sec	Leucocytes 10^3/mm³	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %	Neutrophils %	Band %	Myelocytes %	Metamyelocytes %	Blast %
Control:																		
7262	M	5.68	11.9	45	267	0.1	13	25	10.96	3	1	62	1	0	0	28	0	0
7265	M	4.21	11.9	38	325	0.3	13	26	11.79	23	0	72	1	0	0	26	0	0
7266	F	5.37	12.9	38	325	0.6	14	29	7.86	18	0	58	1	0	0	40	0	0
7286	F	4.36	11.1	34	237	0.5	14	21	12.09	58	0	36	1	0	0	42	0	0
Mean		4.82	12.2	38	245	0.4	14	23	11.43	40	0	58	2	0	0	32	0	0
1 mg/kg/day:																		
7264	M	4.50	11.5	37	255	0.4	13	23	8.98	43	0	57	0	1	0	28	0	0
7266	M	4.68	12.0	37	287	0.3	14	24	7.39	41	0	58	0	0	0	28	0	0
7284	F	4.55	11.7	38	260	0.2	13	22	14.22	31	0	64	5	0	0	24	0	0
7285	F	4.29	11.4	35	265	0.6	13	24	8.56	38	0	55	3	0	0	44	0	0
Mean		4.43	11.7	37	232	0.4	13	23	9.81	38	0	60	2	0	0	30	0	0
10 mg/kg/day:																		
7263	M	5.24	13.7	42	236	0.4	13	31	12.47	46	0	46	5	0	0	28	0	0
7458	M	5.29	12.2	36	262	0.2	13	29	17.34	26	0	70	4	0	0	26	0	0
7285	F	5.32	12.5	38	192	0.8	13	31	7.89	35	0	65	0	0	0	28	0	0
7381	F	5.04	12.5	41	312	0.4	13	28	8.22	47	0	48	4	1	0	28	0	0
Mean		5.22	12.9	40	210	0.5	13	30	11.45	36	0	60	4	0	0	30	0	0
30 mg/kg/day:																		
7267	M	4.98	12.4	38	143	0.2	12	28	10.84	41	0	52	3	0	0	28	0	0
7455	M	5.16	13.6	40	331	0.5	12	24	8.45	21	0	74	3	0	0	32	0	0
7282	F	4.96	12.8	36	152	0.6	13	26	5.03	26	0	73	1	0	0	32	0	0
7287	F	4.80	12.2	35	115	0.4	14	27	5.30	25	0	68	1	2	0	32	0	0
Mean		4.94	12.8	38	180	0.5	13	26	7.61	29	0	68	2	0	0	32	0	0
300 mg/kg/day:																		
7268	M	4.75	12.4	36	282	0.1	12	27	10.22	36	0	60	3	0	0	28	0	0
7456	M	5.36	13.4	42	196	0.2	11	24	5.54	38	0	60	2	0	0	28	0	0
7286	F	5.16	12.8	36	195	0.2	14	27	11.8	38	0	62	3	0	0	28	0	0
7381	F	4.9	12.5	36	115	0.5	14	27	10.24	51	0	43	3	0	0	28	0	0
Mean		5.09	12.9	37	195	0.1	13	27	9.58	40	0	57	3	0	0	28	0	0

PC-[illegible]

Study for Subacute Dermal Dermal Toxicity Study.

TABLE [illegible]

Individual Hematological Values - 1 Week.

Group, Number Sex	Erythrocytes $10^6/mm^3$	Hemoglobin g/100 ml	Hematocrit %	Platelets $10^3/mm^3$	Reticulocytes %	Prothrombin Time sec	Activated P.T.T. sec	Leucocytes $10^3/mm^3$	Neutrophils Seg. %	Neutrophils Band-Seg. %	Lymphocytes %	Eosinophils %	Monocytes %	Basophils %	RBC Morph.	WBC Diff.
Controls:																
7361 M	4.80	11.9	38	224	0.2	12	28	6.97	29	0	68	3	0	0	75	25
7365 M	4.71	11.9	38	249	0.3	12	29	11.56	15	0	81	1	0	0	82	35
7366 F	4.29	11.2	37	246	0.2	13	28	7.66	21	0	65	0	0	0	82	27
7367 F	4.03	11.4	36	292	0.3	12	31	8.98	42	0	56	0	0	0	82	27
Mean	4.46	11.7	38	252	0.2	12	29	9.49	26	0	73	1	0	0	82	27
1 mg/kg/day:																
7362 M	4.36	11.6	37	274	0.3	11	27	9.58	16	0	66	3	0	0	85	27
7364 M	4.46	10.7	35	188	0.4	12	28	9.83	19	0	72	4	0	0	85	31
7368 F	4.05	11.9	39	274	0.2	13	29	10.07	22	1	73	3	1	0	82	32
7369 F	4.25	11.2	35	247	0.4	13	29	9.83	18	0	73	4	0	0	82	32
Mean	4.29	11.4	37	233	0.3	12	28	9.78	19	0	74	3	0	0	83	32
10 mg/kg/day:																
7363 M	4.42	12.1	38	246	1.8	13	27	8.88	42	0	57	1	0	0	75	27
7365 M	4.62	12.3	37	241	0.3	13	31	12.98	17	0	80	3	0	1	77	32
7370 F	4.73	12.4	39	283	0.1	13	33	7.45	35	0	63	2	0	0	83	32
7371 F	4.45	12.3	39	255	0.2	13	33	6.64	28	0	70	3	0	0	82	32
Mean	4.55	12.1	38	256	0.5	13	31	9.95	24	0	72	2	0	0	82	32
30 mg/kg/day:																
7367 M	4.54	11.2	36	135	0.8	13	34	7.92	12	0	88	0	0	0	75	25
7369 M	4.65	11.6	37	197	0.2	14	35	11.16	27	0	73	0	0	0	83	25
7372 F	4.51	11.5	37	74	0.3	15	35	6.19	9	0	90	1	0	0	78	25
7373 F	4.56	11.8	37	237	0.2	14	38	8.54	17	0	82	0	0	0	82	25
Mean	4.51	11.7	36	214	0.2	15	35	8.44	15	0	85	0	0	0	80	25
100 mg/kg/day:																
7368 M	Died, week 5															
7369 M	Died, week 2															
7374 F	Died, week 1															
7375 F	Died, week 1															

*[illegible]

[illegible]

000043

FC-119:

Short Term Subacute Rhesus Rodent Fertility Study.

TABLE 2.

Individual Reproductive Values - 3 Weeks.

Group, Number	Sex	Testicular Wt. g/kg	Epididymal Wt. g/kg	Prostate Wt. mg	Pregnancies 10^3/cm	Seminal Vesicle Wt. g	Fertilizable Time min	Activated S.T.C. min	Sperm Count 10^6/cm	Motility Sup. %	Motility Non-Sup. %	Morphology cytol. %	Morphology Intact %	Morphology Fore. cytol. %	Morphology Sperm path. %	[illegible] %	[illegible] mm	[illegible] g/100 g
Controls																		
7362	M	6.99	12.4	37	287	0.2	11	32	7.62	29	0	74	4	2	4	26	4	4
7365	M	5.59	14.1	37	219	0.3	10	23	12.82	18	0	85	4	1	0	26	4	4
7336	F	4.32	12.4	36	174	0.1	11	25	8.42	14	0	74	4	1	0	24	22	A
7356	F	4.69	12.8	6	234	0.3	11	28	8.41	15	1	85	6	1	0	27	23	A
Mean		4.90	12.4	37	234	0.3	11	24	9.49	16	0	85	3	1	0	25	24	A
2 mg/kg/day:																		
7366	M	6.06	12.4	37	299	0.1	11	24	7.53	34	0	71	6	1	0	24	23	A
7346	M	6.24	12.0	31	229	0.2	11	24	5.44	25	0	74	6	0	1	24	23	A
7361	F	4.92	13.0	39	303	0.2	11	25	13.21	14	0	74	5	1	0	25	24	A
7363	F	4.74	13.0	37	245	0.2	11	24	9.35	20	0	80	5	1	0	25	23	A
Mean		4.74	12.7	37	285	0.2	11	24	9.83	19	0	74	3	1	0	24	23	A
10 mg/kg/day:																		
7363	M	5.86	13.4	40	211	0.2	11	24	8.43	34	0	60	4	1	0	24	23	A
7358	M	5.79	12.6	40	218	0.3	11	23	20.18	4	0	94	2	0	0	24	23	A
7335	F	5.62	13.4	40	244	0.3	10	23	16.72	37	0	91	11	5	0	23	24	A
7361	F	5.65	13.5	39	212	0.1	11	27	8.52	36	0	84	3	1	0	24	24	A
Mean		5.47	13.1	40	216	0.2	11	24	11.46	35	0	87	6	2	0	23	24	A
20 mg/kg/day:																		
7367	M	Died, week 7																
7355	M	1.82[b]	9.7	30	241	0.2	10	65	10.16	34	0	34	3	1	0	26	25	27
7360	F	Died, week 13																
7357	F	Died, week 12																
Mean		1.82	9.7	30	241	0.2	10	65	10.16	34	0	34	3	1	0	26	25	27
100 mg/kg/day:																		
7361	M	Died, week 5																
7354	M	Died, week 2																
7337	F	Died, week 3																
7351	F	Died, week 6																

[a] [illegible]

[b] [illegible]

[c] [illegible]

000044

FC-1431 Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 8. Means and Significance of Biochemical Values.

Biochemistry	Month of Study	Control	3 mg/kg/day	10 mg/kg/day	30 mg/kg/day
Glucose, mg/100 ml	1	89	117*	104	122
	3	81	96	88	66[a]
B.U.N., mg/100 ml	1	23.0	21.2	22.5	20.1
	3	27.6	20.2	22.0	22.6[a]
Alk. Phos., Int'l units/l	1	597	847	601	365*
	3	851	783	743	360[a]
S.G.O.T., Int'l units/l	1	29	35	34	59**
	3	45	41	35	88[a]
S.G.P.T. Int'l units/l	1[b]	15	21	34*	44
	3[c]	31	31	34	46[a]
Cholesterol, mg/100 ml	1	165	154	158	174
	3	165	141	154	240[a]
Total Protein, g/100 ml	1	7.94	8.23	8.66	8.36
	3	8.21	8.24	8.43	5.52[a]
Albumin, g/100 ml	1	4.78	5.05	4.65	4.28
	3	4.82	5.12	5.17	2.00[a]
Sodium, meq/liter	1	153	152	155	152
	3	151	154	159**	150[a]
Potassium, meq/liter	1	5.1	5.1	5.2	5.7
	3	5.5	5.6	6.0	5.9[a]
Chloride, meq/liter	1	112	110	113	112
	3	113	112	114	113[a]
γ-G.T.P., Sigma units/ml	1	61	49	47	33
	3	44	38	51	49[a]
C.P.K., Sigma units/ml	1	9	14	16	19*
	3	7	6	9	10[a]
Inorganic Phosphate, mg/100 ml	1	7.9	7.2	7.0	6.7
	3	6.9	6.3	7.3	5.0[a]

*Significantly different from control group, $p<0.05$.
**Significantly different from control group, $p<0.01$.
[a]Value not used in statistical analysis due to only one animal surviving.
[b]I.U./l
[c]Sigma units/ml

137-090

FC-141:

Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 9.

Individual Biochemical Values - Control 1.

Group. Monkey Number	Sex	Glucose mg/100 ml	B.U.N. mg/100 ml	Alk. Phos. Int'l units/l	S.G.O.T. Int'l units/l	S.G.P.T. Int'l units/l	Cholesterol mg/100 ml	Total Protein g/100 ml	Albumin g/100 ml	Sodium meq/l	Potassium meq/l	Chloride meq/l	Inorganic Phosphorus mg/100 ml	T-C.T.P. Sigma u/ml	Creatinine Phosphokinase Sigma u/ml
Control:															
7362	M	96	41.0	780	40	99	219	8.68	5.40	160	5.0	111	6.5	67	15
7365	M	82	19.7	658	61	86	123	9.58	4.30	159	5.3	110	6.7	44	18
7336	F	79	24.0	915	30	80	185	9.52	5.30	156	4.3	110	6.5	41	15
7386	F	85	21.0	960	29	86	196	8.52	5.12	162	5.0	111	6.5	37	16
Mean		85	25.7	829	43	88	179	9.06	5.03	158	4.9	111	6.6	47	16
3 mg/kg/day:															
7364	M	112	19.9	880	42	94	197	9.08	5.28	155	4.3	108	5.0	50	12
7366	M	71	26.7	580	69	89	172	9.12	5.80	157	4.9	109	7.1	38	29
7384	F	96	22.0	570	38	106	133	10.12	5.19	162	6.0	113	6.1	32	16
7385	F	107	22.9	1120	60	76	154	8.72	4.80	158	5.2	116	5.4	41	29
Mean		96	22.9	828	50	97	164	9.26	5.22	158	5.1	111	5.9	38	21
10 mg/kg/day:															
7363	M	89	27.2	1167	46	124	237	9.84	5.10	167	6.2	117	6.7	78	16
7458	M	180	24.2	806	63	136	107	10.08	3.99	159	4.9	107	7.7	55	14
7338	F	98	26.0	726	26	75	189	8.48	5.14	157	4.4	109	6.3	52	34
7383	F	98	27.3	581	31	91	168	8.32	5.25	154	5.1	112	6.0	59	64
Mean		116	24.7	833	42	105	175	9.18	4.87	158	5.2	111	6.7	61	32
30 mg/kg/day:															
7367	M	108	26.4	970	47	114	150	9.38	5.60	170	6.2	116	6.8	65	15
7455	M	100	24.0	687	37	86	205	9.50	5.21	162	5.3	111	6.6	58	9
7382	F	122	27.9	641	40	79	174	11.10	5.22	165	5.5	112	6.8	43	18
7387	F	117	23.5	978	45	138	194	9.44	5.60	155	3.9	113	5.4	39	16
Mean		117	25.7	819	42	104	181	9.86	5.56	163	5.2	113	6.4	52	15
100 mg/kg/day:															
7361	M	93	29.0	590	43	80	155	8.60	5.00	159	5.4	116	6.9	64	17
7456	M	100	23.6	798	40	106	202	9.00	5.69	157	4.5	109	5.7	44	22
7335	F	75	28.0	576	40	46	151	8.96	5.19	157	5.2	111	5.6	54	20
7381	F	119	22.1	1133	40	203	124	9.60	4.89	159	5.2	112	6.7	47	20
Mean		97	25.5	800	41	96	158	9.05	5.19	158	5.2	112	6.2	53	17

000046

137-470

PAGE 22

FC-141:

Ninety Day Subacute Bioassay Toxicity Study.

TABLE 10.

Individual Biochemical Values - 1 Month.

Group, Monkey Number	Sex	Glucose mg/100 ml	B.U.N. mg/100 ml	Alk. Phos. Int'l units/l	S.G.O.T. Int'l units/l	S.G.P.T. Int'l units/l	Cholesterol mg/100 ml	Total Protein g/100 ml	Albumin g/100 ml	Sodium meq/l	Potassium meq/l	Chloride meq/l	Inorganic Phosphate mg/100 ml	γ-G.T.P. Sigma u/ml	Creatinine Phosphokinase Sigma u/ml
Control:															
7362	M	87	31.9	611	27	18	191	7.30	4.82	153	5.4	117	6.6	81	8
7365	M	84	14.2	626	33	17	121	8.40	4.11	153	5.4	111	8.4	50	11
7336	F	87	21.9	622	15	15	142	7.90	4.85	148	4.2	109	8.4	58	7
7386	F	96	34.9	480	31	30	206	8.15	5.30	158	5.4	112	8.1	44	11
Mean		89	21.0	567	24	15	165	7.94	4.78	153	5.1	112	7.9	61	9
3 mg/kg/day:															
7364	M	102	18.8	976	32	36	173	8.15	5.20	152	4.3	106	6.9	22	6
7366	M	132	23.3	693	39	25	148	8.05	5.42	154	4.4	110	6.6	36	7
7334	F	105	24.2	536	30	15	141	8.70	4.80	152	5.6	112	7.5	67	39
7385	F	120	19.1	1185	40	13	153	8.00	4.72	152	5.2	114	7.8	47	7
Mean		117	21.2	847	35	21	154	8.23	5.05	152	5.1	110	7.2	49	14
10 mg/kg/day:															
7363	M	99	24.9	552	40	35	209	9.40	4.92	162	4.3	116	6.9	65	7
7458	M	97	27.5	732	65	43	134	9.05	4.32	151	4.9	109	8.4	44	29
7338	F	90	27.7	840	23	29	145	8.20	4.50	152	4.3	112	5.6	37	24
7383	F	124	20.0	480	31	30	132	8.00	5.19	154	5.2	113	7.2	43	24
Mean		102	22.5	601	34	34	158	8.66	4.66	155	5.2	113	7.0	43	16
30 mg/kg/day:															
7367	M	122	35.2	376	49	38	180	8.20	4.20	157	6.0	120	6.6	40	25
7455	M	86	21.0	322	61	80	177	8.55	3.22	140	5.2	112	6.9	40	34
7332	F	104	25.2	400	83	43	161	8.15	4.21	149	5.9	111	6.8	28	27
7387	F	105	21.8	340	45	23	179	8.55	5.00	153	5.6	114	7.2	24	16
Mean		122	26.1	365	59	41	174	8.36	4.26	152	5.7	112	6.7	33	24
100 mg/kg/day:															
7361	M	Died, week 5													
7456	M	Died, week 2													
7335	F	Died, week 3													
7381	F	Died, week 4													

FC-141:

Bluefly Bay Subacute Toxicity Study.

TABLE II. Individual Biochemical Values - 3 Months.

Group. Monkey Number	Sex	Glucose mg/100 ml	B.U.N. mg/100 ml	Alk. Phos. Int'l units/l	S.G.O.T. Int'l units/l	S.G.P.T. Sigma units/ml	Cholesterol mg/100 ml	Total Protein g/100 ml	Albumin g/100 ml	Sodium meq/l	Potassium meq/l	Chloride meq/l	Inorganic Phosphate mg/100 ml	γ-G.T.P. Sigma u/ml	Creatinine Phosphokinase Sigma u/ml
Control:															
7362	M	95	41.9	[illegible]	55	44	195	7.59	4.99	152	5.5	114	5.6	27	7
7365	M	77	17.4	740	47	30	135	9.18	4.40	151	6.1	113	8.0	53	8
7336	F	67	21.1	781	39	24	150	8.32	4.98	138	5.1	114	7.3	62	7
7386	F	86	18.1	1068	59	17	177	7.76	4.90	153	5.1	109	4.7	65	6
Mean		81	27.6	851	45	32	165	8.21	4.82	151	5.5	113	6.9	44	7
3 mg/kg/day:															
7364	M	106	17.1	1092	41	28	166	7.72	5.09	153	5.4	112	7.0	45	7
7366	M	111	18.1	594	39	3[illegible]	126	8.09	5.32	153	5.5	109	5.3	51	6
7384	F	96	23.4	632	39	33	132	8.93	4.91	154	5.2	112	6.5	27	6
7385	F	76	22.6	1024	43	29	142	8.21	4.97	155	5.0	114	6.4	29	6
Mean		96	20.2	783	41	31	141	8.24	5.12	154	5.6	112	6.3	38	6
10 mg/kg/day:															
7363	M	87	24.9	936	42	42	194	8.44	5.42	164	7.0	119	8.0	43	7
7458	M	88	21.1	936	38	31	119	9.71	4.69	159	6.2	112	9.0	52	11
7328	F	75	21.8	624	30	25	155	7.93	5.27	156	4.8	110	5.6	60	7
7383	F	100	20.0	624	30	37	128	7.42	5.11	158	5.8	113	6.5	48	9
Mean		88	22.0	743	35	34	154	8.43	5.17	159	6.0	114	7.3	51	9
30 mg/kg/day:															
7367	M	Died, week 7													
7455	M	66	22.6	360	88	46	240	5.52	2.08	150	5.9	113	5.0	49	30
7382	F	Died, week 13													
7387	F	Died, week 12													
Mean		66	22.6	360	88	46	240	5.52	2.08	150	5.9	113	5.0	49	30
100 mg/kg/day:															
7361	M	Died, week 5													
7456	M	Died, week 2													
7325	F	Died, week 3													
7381	F	Died, week 4													

000048

Page 4

Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 12. Means and Significance of Urinalysis Values.

Urinalysis	Month of Study	Control	3 mg/kg/day	10 mg/kg/day	30 mg/kg/day
Volume, ml	1	35	33	51	41
	3	71	94	51	40[a]
pH	1	8.5	8.5	8.1	8.1
	3	8.3	7.6	8.2	6.6[a]
Specific Gravity	1	1.028	1.026	1.026	1.026
	3	1.010	1.015	1.024	1.031[a]

[a]Value not used in statistical analysis due to only one animal surviving.

137-090

FC-142: Ninety Day Subacute Rurous Monkey Toxicity Study.

TABLE 13. Individual Urinalysis Values - Control 2.

Group. Monkey Number	Sex	Volume ml	Color and Appear.	pH	Spec. Grav.	Protein	Glucose	Occult Blood	Ketones	Leucocytes	Erythrocytes	Epi. Cells	Bilirubin	Triple Phos.	Cal. Oxal.	Uric Acid Crystals	Bacteria	Casts
Control:																		
7362	M	100	LS-cl	7.6	1.026	N	N	++	N	-	0-2	0-2	T	0-2	-	-	M	-
7363	M	25	LS-cl	7.2	1.037	N	N	N	N	-	1-3	0-2	T	0-2	-	-	M	-
7336	F	27	LS-C	7.0	1.035	N	N	N	1+	-	-	-	0-2	0-2	0-2	-	F	-
7386	F	70	LS-cl	8.6	1.023	N	N	4+	N	-	-	0-2	0-2	0-2	M	-	M	-
Mean		54		7.6	1.027													
3 mg/kg/day:																		
7364	M	25	LS-cl	7.0	1.032	N	N	++	N	-	-	0-2	T	T	T	-	M	-
7365	M	25	LS-cl	7.2	1.035	N	N	++	N	-	-	0-2	T	0-2	0-2	-	M	-
7334	F	215	LS-C	8.3	1.026	N	N	N	N	-	-	0-2	0-2	0-2	-	-	M	-
7335	F	35	LS-cl	8.3	1.029	N	N	N	N	-	-	0-2	T	0-2	-	-	M	-
Mean		75		7.9	1.026													
10 mg/kg/day:																		
7363	M	20	LS-cl	7.7	1.020	N	N	++	N	-	-	0-2	T	T	-	-	M	-
7454	M	50	LS-cl	7.5	1.034	N	N	++	N	-	-	0-2	T	0-2	T	-	M	-
7334	F	75	LS-cl	7.8	1.036	N	N	++	N	-	-	1-3	T	0-2	M	-	F	-
7335	F	35	LS-cl	8.3	1.020	N	N	3+	N	-	-	0-2	0-2	0-2	-	-	F	-
Mean		35		7.8	1.026													
30 mg/kg/day:																		
7367	M	35	LS-cl	7.7	1.052	N	N	++	N	-	1-3	1-3	0-2	0-2	0-2	-	M	-
7455	M	35	LS-cl	8.8	1.030	N	N	++	N	-	1-3	1-3	0-2	T	-	-	M	-
7332	F	20	LS-cl	7.0	1.055	N	N	N	N	-	-	1-3	T	0-2	-	-	F	-
7337	F	45	LS-cl	8.2	1.030	N	N	N	N	-	-	0-2	T	0-2	0-2	-	M	-
Mean		38		7.3	1.041													
100 mg/kg/day:																		
7361	M	21	LS-cl	7.6	1.035	N	N	++	N	-	0-2	-	T	0-2	-	-	M	-
7456	M	25	LS-cl	7.1	1.042	N	N	++	3+	-	-	0-2	T	0-2	T	-	M	-
7335	F	17	LS-cl	7.2	1.041	N	N	++	1+	-	1-3	-	0-2	0-2	T	-	F	-
7381	F	62	LS-cl	8.1	1.042	N	N	1+	1+	-	-	1-3	0-2	0-2	M	-	M	-
Mean		38		7.5	1.040													

Color: [illegible]

[illegible]

[illegible]

Twenty Day Subacute Dermal Rabbit Toxicity Study.

TABLE 16. Individual Urinalysis Values - 1 Month.

Group, Rabbit Number	Sex	Volume ml	Color and Appear.	pH	Spec. Grav.	Protein	Glucose	Occult Blood	Ketones	Leuco-cytes	Erythro-cytes	Epi. Cells	Bacteria	Triple Phos.	Cal. Oxal.	Amo Acid Crystals	Isocrits	Casts
Control:																		
7[illegible]	M	55	LS-C	8.5	1.[illegible]	N	N	N	N	-	[illegible]	-	[illegible]	[illegible]	N	-	N	-
7[illegible]	M	35	LS-C	8.5	1.[illegible]	N	N	N	N	-	-	-	[illegible]	F	[illegible]	-	N	-
7[illegible]	F	20	LS-C	8.5	1.013	N	N	[illegible]	N	-	-	1-3	F	F	F	-	N	-
7[illegible]	F	10	LS-C	8.5	1.0[illegible]	N	N	[illegible]	N	-	-	[illegible]	N	F	N	-	N	-
Mean		35		8.5	1.0[illegible]													
3 mg/kg/day:																		
7[illegible]	M	20	LS-C	8.6	1.0[illegible]	N	N	N	N	-	-	[illegible]	F	N	[illegible]	-	N	-
7[illegible]	M	30	LS-C	8.5	1.0[illegible]	N	N	N	N	-	-	[illegible]	F	F	F	-	N	-
7[illegible]	F	10	LS-C	8.0	1.0[illegible]	+	N	+	+	-	[illegible]	-	F	[illegible]	F	-	N	-
7[illegible]	F	30	LS-C	8.5	1.0[illegible]	N	N	N	N	-	-	[illegible]	F	[illegible]	N	-	N	-
Mean		30		8.5	1.0[illegible]													
10 mg/kg/day:																		
7[illegible]	M	60	LS-C	7.5	1.0[illegible]	N	N	N	N	-	[illegible]	-	F	[illegible]	N	-	N	-
7[illegible]	M	35	LS-C	8.0	1.0[illegible]	N	N	N	N	-	-	-	[illegible]	[illegible]	N	-	N	-
7[illegible]	F	35	LS-C	8.5	1.0[illegible]	N	N	N	N	-	-	1-3	[illegible]	[illegible]	N	-	N	-
7[illegible]	F	50	LS-C	8.5	1.0[illegible]	N	N	+	N	-	[illegible]	[illegible]	F	[illegible]	N	-	N	-
Mean		51		8.1	1.0[illegible]													
30 mg/kg/day:																		
7[illegible]	M	30	LS-C	7.5	1.0[illegible]	N	N	N	N	-	-	[illegible]	[illegible]	[illegible]	-	-	N	-
7[illegible]	M	30	LS-C	8.0	1.0[illegible]	N	N	N	N	-	[illegible]	[illegible]	N	F	-	-	N	-
7[illegible]	F	60	LS-C	8.5	1.0[illegible]	N	N	N	N	-	[illegible]	-	F	F	-	-	N	-
7[illegible]	F	45	LS-C	8.5	1.0[illegible]	N	N	N	N	-	-	[illegible]	F	[illegible]	[illegible]	-	N	-
Mean		41		8.1	1.0[illegible]													
30 mg/kg/day:																		
7[illegible]	M	[illegible], [illegible] 5																
7[illegible]	M	[illegible], [illegible] 2																
7[illegible]	F	[illegible], [illegible] 3																
7[illegible]	F	[illegible], [illegible] 4																

Key: [illegible]

Urine: [illegible]

FC-144:

Rocky Bay Subacute Dermal Kidney Toxicity Study.

TABLE 15.

Individual Urinalysis Values - 3 Months.

Group. Number Number	Sex	Volume ml	Color and Appear.	pH	Spec. Grav.	Protein	Glucose	Occult Blood	Ketones	Leucocytes	Erythrocytes	Epi. Cells	Urates	Triple Phos.	Cal. Oxal.	Uric Acid Crystals	Bacteria	Casts
Control:																		
7362	M	110	LS-C	8.2	1.012	S	S	S	S	-	-	occ	F	occ	-	-	M	-
7365	M	40	LS-C	8.7	1.029	S	S	S	1+	-	occ	1-3	F	F	-	-	M	-
7336	F	85	LS-C	8.3	1.025	S	S	S	tr	-	-	occ	F	occ	F	-	M	-
7356	F	50	LS-C	8.9	1.016	S	S	3+	S	occ	-	occ	F	F	F	-	M	-
Mean		71		8.3	1.018													
3 mg/kg/day:																		
7364	M	50	LS-C	6.9	1.020	S	S	S	tr	-	-	-	F	occ	-	-	M	-
7366	M	150	LS-C	7.4	1.007	S	S	S	S	-	-	occ	F	occ	-	-	M	-
7374	F	115	LS-C	8.1	1.030	S	S	S	S	-	-	occ	F	S	F	-	M	-
7385	F	50	LS-C	8.5	1.021	S	S	tr	S	-	occ	1-3	M	F	M	-	M	-
Mean		94		7.6	1.015													
10 mg/kg/day:																		
7363	M	40	LS-C	8.8	1.027	S	S	S	S	-	-	occ	F	occ	occ	-	M	-
7458	M	35	LS-cl	8.1	1.022	S	S	S	S	-	-	-	F	occ	-	-	M	-
7328	F	50	LS-C	9.0	1.029	S	S	S	S	-	occ	occ	F	occ	-	-	M	-
7361	F	80	LS-cl	7.0	1.019	S	S	S	S	-	occ	occ	F	-	-	-	M	-
Mean		51		8.2	1.024													
30 mg/kg/day:																		
7367	M	Died, week 7																
7455	M	40	S-C	6.6	1.031	S	S	3+	S	1-3	occ	-	F	M	occ	-	M	-
7352	F	Died, week 13																
7357	F	Died, week 12																
Mean		40		6.6	1.031													
300 mg/kg/day:																		
7351	M	Died, week 5																
7456	M	Died, week 3																
7355	F	Died, week 3																
7381	F	Died, week 4																

Color: tr - Trace
1+ - Trace to slight
2+ - Slight to moderate
3+ - Moderate
4+ - Marked

S - Straw
LS - Light Straw
DS - Dark Straw
LA - Light Amber
DA - Dark Amber
Cl - Cloudy

B - Bright
F - Foul
L - Lysed
M - Many
R - Rare
occ - Occasional

QNS - Quantity not sufficient
mcm - Normal
- Zero

FD-690

0000552

Protocol: Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 16. Summary of Gross Necropsy Observations, Terminal Sacrifice.

		0 mg/kg/day			1.5 mg/kg/day			10 mg/kg/day			30 mg/kg/day			100 mg/kg/day		
Sex		M		F	M		F	M		F	M		F	M		F
Lesion		2	2	2	1	1	1	2	2	2	1	1	1	2	2	2
No Gross Lesions			1			1										
External																
swelling, eye area													1			
alopecia														1		
dehydrated														1		
unevaluated														1		
red vaginal discharge														1		
scab, facial area														1		
Lung																
site lesion		1	1	1			1	1	1	1	1		1	1		
adhesions			1				1	1		1			1		1	
dark red foci/reddish purple area						1			1	1						1
yellow, white foci											1					1
nodules																1
Heart																
hemorrhage, subendocardial															1	1
gelatinized fat, endocardial																1
atrophy																1
Lymph Nodes																
enlarged			1													
reddish black in color															1	
Thymus																
atrophy												1				
Abdominal Cavity																
depletion of fat																1
Stomach																
dark red foci									1		1	1	1			
erosion, mucosa, pyloric portion											1					
mucosal hyperemia												1				
yellowish colostrum material, fundic portion																
hemorrhage, fundic mucosa														1		
ulcers															1	1
Small Intestine																
greenish-gray mucoid material												1				
dark red/brown mucoid material														1	1	1
liquid, blood tinged fluid															1	
reddish brown in color															1	
congestion, mucosa															1	
hemorrhage, mucosa																1
Large Intestine																
congestion, mucosa											1			1		
hemorrhage, mucosa														1	1	
dark reddish black foci															1	
semi solid, blood stained contents															1	

*Died on study

AT-070

FC-143: Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 10. Cont. Summary of Gross Necropsy Observations.

Site / Lesion	Control	0 mg/kg/day	2 mg/kg/day	10 mg/kg/day	40 mg/kg/day	100 mg/kg/day
Pancreas						
accessory spleen			X			
Liver						
cyst				X		
brownish color					X	
accentuated lobulations					X	X
granular surface					X	
yellowish mottling						X
reddish yellow color						X
Kidneys						
brownish discoloration					X	
Skin						
subcutaneous edema, abdomen						X
subcutaneous hemorrhage, abdomen						X

*Died on study

TABLE 17. Mortality (Count) and Relative (% Body Weight) Organ Weights, Terminal Sacrifice and Recovery.

Group, Sex, Number	Sex	Body Wt. (g)	Spleen		Liver		Kidneys		Testes		Brain	
			g	%	g	%	g	%	g	%	g	%
Terminal Sacrifice:												
Controls:												
7362	M	1.25	2.35	0.07	76.73	2.13	0.65	0.26	11.85	0.3[illegible]	0.85	0.01
7365	M	1.85	2.87	0.20	79.15	2.06	0.71	0.18	17.06	0.44	2.23	0.05
Mean		2.55	5.21	0.14	74.54	2.12	0.68	0.19	14.46	0.40	1.54	0.05
7336	F	1.60	5.03	0.15	84.79	2.49	-	-	13.84	0.41	0.29	0.02
7340	F	1.90	3.67	0.11	77.77	2.22	0.62	0.18	19.58	0.56	0.37	0.11
Mean		1.45	4.45	0.13	81.32	2.36	0.62*	0.18*	16.60	0.48	0.25	0.06
3 mg/kg/day:												
7364	M	1.30	4.40	0.11	91.67	2.23	0.77	0.19	19.76	0.48	1.46	0.04
7366	M	1.65	1.82	0.07	83.17	2.38	0.62	0.31	22.60	0.67	0.85	0.03
Mean		3.38	3.27	0.09	77.25	2.31	0.69	0.25	16.08	0.47	1.14	0.04
7334	F	1.70	6.82	0.19	102.61	2.77	0.78	0.22	17.66	0.45	0.19	0.05
7335	F	1.45	2.74	0.09	67.23	1.95	0.55	0.16	14.44	0.42	0.16	0.04
Mean		1.58	4.88	0.17	84.91	2.36	0.67	0.19	16.02	0.45	0.17	0.05
10 mg/kg/day:												
7361	M	1.80	2.34	0.06	87.25	2.32	0.74	0.19	24.84	0.61	1.73	0.05
7458	M	1.25	1.91	0.15	82.30	2.53	0.67	0.21	16.54	0.51	1.14	0.04
Mean		3.53	3.65	0.11	86.73	2.41	0.71	0.20	24.69	0.68	1.67	0.05
7335	F	1.55	4.86	0.11	81.60	2.34	0.46	0.19	15.32	0.43	0.29	0.02
7341	F	1.70	3.94	0.11	85.15	2.31	0.46	0.17	13.50	0.37	0.79	1.05
Mean		3.63	4.40	0.11	84.18	2.32	0.46	0.21	14.41	0.40	0.54	0.54
30 mg/kg/day:												
7455	M	2.40	3.50	0.13	76.78	2.45	0.66	0.15	14.35	0.32	1.24	0.03
Brother:												
30 mg/kg/day:												
7367	M	2.10	1.45	0.07	75.33	3.54	1.03	0.29	15.36	0.76	1.46	0.05
7382	M	1.75	1.07	0.19	102.67	5.80	1.34	0.77	19.03	0.95	0.51	0.03
7367	M	2.25	1.97	0.08	85.17	3.74	1.24	0.53	15.96	0.71	0.37	2.40
100 mg/kg/day:												
7361	M	2.40	1.65	0.07	79.02	3.29	1.59	0.66	21.65	0.91	1.37	0.06
7456	M	2.30	1.36	0.07	85.60	3.55	1.45	0.54	17.77	0.73	0.72	0.03
7335	F	1.55	1.49	0.22	71.24	3.42	1.03	0.50	15.40	0.73	0.26	0.12
7341	F	2.40	3.07	0.12	82.56	3.44	1.34	0.55	15.24	0.63	0.33	0.02

Group means relative organ weights given in this table are calculated by averaging the individually calculated relative organ weights.

* Significantly different from control group at $p < 0.05$.

** Significantly different from control group at $p < 0.01$.

Sex indicated in subheadings.

- = not applicable.

0000035

Ninety Day Subacute Dermal Rabbit Toxicity Study.

TABLE 17. Cont.: Absolute (Grams) and Relative (% Body Weight) Organ Weights, Terminal Sacrifice and Deaths.

Group, Animal Number	Sex	Body Wt. kg	Heart g	Heart %	Thyroid/ Parathyroid g	Thyroid/ Parathyroid %BW	Brain g	Brain %	Pituitary g	Pituitary %BW
Terminal Sacrifice:										
Control:										
7182	M	3.75	21.69	0.58	1.050	0.32	97.64	2.68	0.953	0.26
7185	M	3.85	18.17	0.47	0.296	0.08	98.39	2.75	0.863	0.24
Mean		3.55	14.93	0.42	0.673	0.20	88.22	2.51	0.856	0.24
7186	F	3.40	15.30	0.45	-	-	82.64	2.47	0.856	0.25
7184	F	3.50	14.75	0.42	0.839	0.24	81.55	2.33	0.873	0.25
Mean		3.45	15.03	0.44	0.839*	0.24*	82.10	2.38	0.865	0.25
3 mg/kg/day:										
7186	M	4.30	18.90	0.44	0.893	0.22	96.81	2.24	0.980	0.23
7186	M	2.65	12.70	0.48	0.378	0.14	83.50	3.15	0.851	0.32
Mean		3.38	15.80	0.47	0.636	0.18	89.16	2.75	0.866	0.27
7182	F	3.70	16.87	0.46	0.694	0.19	78.64	2.13	0.886	0.23
7185	F	3.45	15.19	0.44	0.563	0.16	80.21	2.32	0.853	0.25
Mean		3.58	16.03	0.45	0.629	0.17	79.44	2.23	0.870	0.24
10 mg/kg/day:										
7183	M	3.80	15.86	0.40	1.211	0.32	77.73	2.05	0.861	0.17
7456	M	3.25	14.14	0.43	0.488	0.15	83.38	2.57	0.847	0.26
Mean		3.53	14.62	0.42	0.850	0.23	80.56	2.31	0.855	0.26
7188	F	3.55	11.85	0.33	0.461	0.13	77.19	2.17	-	-
7181	F	3.70	11.69	0.32	0.537	0.15	75.88	2.05	0.831	0.22
Mean		3.63	11.77*	0.32**	0.499	0.14	76.54**	2.11	0.831*	0.22*
30 mg/kg/day*:										
7455	M	2.40	10.50	0.44	0.392	0.17	73.01	3.13	0.809	0.34
Deaths:										
30 mg/kg/day:										
7187	M	2.30	10.79	0.46	0.532	0.23	83.27	3.62	0.866	0.37
7182	F	2.35	11.93	0.51	0.543	0.23	83.22	3.70	0.876	0.37
7187	F	2.35	10.21	0.43	0.645	0.34	91.45	4.66	0.850	0.36
100 mg/kg/day:										
7181	M	2.40	14.54	0.61	0.791	0.33	92.43	3.85	0.972	0.40
7454	M	2.30	13.55	0.58	0.708	0.31	95.42	3.53	0.868	0.37
7175	F	2.05	11.44	0.56	0.474	0.23	74.28	3.62	0.758	0.37
7181	F	2.60	12.95	0.50	0.411	0.16	84.29	3.32	0.882	0.33

Group means relative organ weights above 0.10% rather than calculated by averaging the individually calculated relative organ weights.

*Significantly different from Control group mean, p 0.05.

**Significantly different from Control group mean, p 0.02.

*Not included in means.

- - Not applicable.

000056

FC-163: Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 18. Microscopic Observations.

Tissue / Lesion	Control				1 mg/kg/day				10 mg/kg/day				30 mg/kg/day				100 mg/kg/day			
Brain	1	1	1	1	1	1	1	1	1		1	1	1	1	1	1	1	1	1	1
focal perivascular lymphoid infiltrates										3										
Spinal cord	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Peripheral nerve	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Eye	1		1	1	1	1						1		1	1	1	1	1		1
Sarcocystis sp. in ocular muscle		*					*												*	
focal lymphoid infiltrates in sclera											3									
focal lymphoid infiltrates in lacrimal gland								3					3							
focal lymphoid infiltrate in palpebral conjunctiva									3	3										
cystic tarsal gland										3										
Pituitary	1	1	1	1	1	1	1	1	1		1	1	1		1			1		
diffuse congestion														3		3	3		3	3
small parenchymal cyst										*										
Thyroid	1	1	1		1		1	1	1	1	1	1	1	1				1	1	
foci of interstitial lymphoid infiltrates				3		2										2				
focal interstitial fibrosis				3												2				
diffuse congestion															3		3			3
Parathyroid	1	1	3	1	1	1	*	*	*	*	*	*	*	1	*	*		*	*	1
diffuse congestion																	3			
Tongue	1								1			1			1	1	1	1		
foci of inflammatory cell infiltrates in lamina propria and mucosal epithelium		3	3	4	2	3	2	3		3	3		2	3					2	2
foci of inflammatory cell infiltrates in muscle		2					3			3	2		3						3	
Sarcocystis sp.							*				*									

Code:
4 = condition present
a = autolysed
1 = not remarkable
2 = very slight
3 = slight
4 = moderate
5 = marked
6 = extreme
* = not available
Killed or sacrificed in extremis

137-090

FG-143: Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 18. Cont. Microscopic Observations.

Tissue / Lesion	Control				1 mg/kg/day				10 mg/kg/day				30 mg/kg/day				120 mg/kg/day			
	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Tonsil																				
foci of inflammatory cell infiltration in mucosal epithelium and tonsillar crypt	3	4	2	3		4	3	3	3	3	4	4		2		3				4
Sarcocystis sp. in muscle		x																		
Conyloneme sp. in mucosal epithelium				x																
atrophy of lymphoid follicles																4				4
Adrenal																				
focal of dystrophic mineralization	3	3	2	2	3		2			3	2	2					2			
diffuse congestion														3	4	3	3		4	3
diffuse lipid depletion														3	3	3	3	3	3	3
focal of lymphoid infiltrates in sinusoids			3		2		2	3	3	3		2								
acidophilic degeneration of individual to small groups of cells													2			3				
Trachea																				
focal of inflammatory cell infiltrates in lamina propria	3		3	3	3	2	2	3	3	3	3	2	2		3				3	3
Salivary gland																				
focal interstitial lymphoid infiltrates	2	3		2		3	4	3		2	2	3	3		2	3				
diffuse congestion														3	3		3			3
decreased cell size, loss of cytoplasmic granules														4			4			
Lung																				
acarian pigment (peribronchial, peribronchiolar, perivascular)	3	2	3	2	3	2	2	2	2	3	3	2	3	3	2	4	2		2	3
focal perivascular lymphoid infiltrates					3					3	3									
focal peribronchial/peribronchiolar lymphoid aggregates	4	4	3	4	3	3	4	3	3	4	4	3	3		2	2			3	3
lung mite in bronchiolar lumen	x			x																
interstitial pneumonia	3	4		4	3		3	4	3			3	4		4			3		
diffuse congestion														3	3	3	4			
foreign body pneumonia		5						5												
focal hemorrhage		3															3			
acute focal bronchopneumonia	4				3						4									
mucorous aggregation of pigment laden alveolar macrophages																3				

Code: x = condition present; 4 = autolysed; 1 = not remarkable; 2 = very slight; 3 = slight; 4 = moderate; 5 = marked; 6 = extreme; - = not available; *Died or sacrificed in moribund

137-090

FC-143: Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 10, Cont. Microscopic Observations.

Tissue Lesion	Control				1 mg/kg/day				10 mg/kg/day				30 mg/kg/day				100 mg/kg/day			
	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Heart																				
focal interstitial lymphoid infiltrates		1			1				1	1		1	1		1			1		
focus of lymphoid infiltrate in endocardium	1		1	1		1	1	1						1			1		1	
focal subendocardial hemorrhage											1									
atrophy of epicardial fat																	1			
Aorta	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Spleen	1	1	1	1	1	1	1	1	1	1										
atrophy of lymphoid follicles													1	1	1	1	1	1	1	1
diffuse congestion											1	1	1	1	1	1	1	1	1	1
focal amyloidosis in lymphoid follicles																			1	
increased amount of hemosiderin pigment																1				
Lymph nodes	1		1	1	1	1	1	1	1	1	1	1	1	1						
atrophy of lymphoid follicles																1	1	1	1	1
increased amount of hemosiderin pigment		1															1			
neutrophil infiltrate in sinuses															1			1	1	
diffuse congestion															1					1
lymphoid hyperplasia		1																		
Esophagus	1			1		1									1		1		1	1
foci of inflammatory cell infiltrates in lamina propria		1	2		2		1	2		1	2	2	1	2		2		2		
foci of interstitial lymphoid infiltrates in muscularis		2					2			2	2	2								
Congestion sp. in mucosal epithelium									1											
Stomach																				
foci of inflammatory cell infiltrate in lamina propria	1	4	1	1	1	1	4	4	4	1	4	1	1			1	1	2	4	1
diffuse congestion											2			1	1				1	
foci of inflammatory cell infiltrates in submucosa					4				4		4	1								
foci of inflammatory cell infiltrates in muscularis							1			1										
foci of inflammatory cell infiltrates in serosa										1										
parasitic granulomas in omentum										1										
focal mucosal hemorrhage											1		1						1	
focal coagulation necrosis in mucosa																			1	

Code: 4 = condition present; 3 = autolysed; 2 = very slight; 1 = slight; 5 = severe; 6 = extreme; - = not available; M(ale) or F(emale) 10 weeks

137-090

FC-143: Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 18. Cont. Microscopic Observations.

Tissue / Lesion	Control				1 mg/kg/day				10 mg/kg/day				10 mg/kg/day				100 mg/kg/day			
Small Intestine	1	1	1	1	1	1	1	1	1	1	1	1	1							
diffuse villous atrophy																	1	1		
focal hemorrhage														1				1	1	
diffuse congestion														1	1	1			1	1
focal aggregate of brown pigment-laden foamy macrophages in mesentery																				1
inflammatory cell infiltrates in serosa																	1			
atrophy of lymph nodule														1			1		1	
Cecum	1	1	1	1	1	1	1	1		1	1	1					1			1
transmural inflammatory cell infiltrates																		1		
diffuse congestion														1	1	1		1	1	
focal mucosal hemorrhage														1				1	1	
inflammatory cell infiltrates in serosa									1											
parasitic granuloma in muscularis													1							
atrophy of lymph nodule																1			1	
Colon	1	1	1	1	1	1	1	1	1	1	1	1	1							1
diffuse congestion														1	1	1		1	1	
parasitic granuloma in submucosa																	1			
transmural inflammatory cell infiltrates																	1			
focal mucosal hemorrhage														1						
atrophy of lymph nodule																1			1	
Rectum	1	1	1	1	1	1	1	1	1	1	1	1	1				1	1		1
diffuse congestion														1	1	1			1	
inflammatory cell infiltrates in muscularis																			1	
atrophy of lymphoid nodule																1			1	
Pancreas	1	1				1			1		1	1					1	1	1	1
focal periductal lymphoid infiltrates			1	1	1		1			1			1							
focal interstitial lymphoid infiltrates							1	1												
diffuse congestion														1	1	1				
Thymus	1	1	1	1	1	1	1	1	1	1	1	1	*	*	*	*	*	*	*	*

Code: M = condition present; A = autolysed; 1 = not remarkable; 2 = very slight; 3 = slight; 4 = moderate; 5 = marked; 0 = absent; * = not available; *Died or sacrificed in anesthesia

137-090

FC-143: Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 1A, Cont. Microscopic Observations.

Tissue Lesion	Control S	Control U	Control M	Control H	3 mg/kg/day S	3 mg/kg/day U	3 mg/kg/day M	3 mg/kg/day H	10 mg/kg/day S	10 mg/kg/day U	10 mg/kg/day M	10 mg/kg/day H	30 mg/kg/day S	30 mg/kg/day U	30 mg/kg/day M	30 mg/kg/day H	100 mg/kg/day S	100 mg/kg/day U	100 mg/kg/day M	100 mg/kg/day H
Liver																				
portal inflammatory cell infiltrates	3	3	3	3			3	2	3	3	2	2		2			2		1	
parenchymal inflammatory cell infiltrates	3	2	2	3	3	3	3	3	3	3	2									
diffuse congestion																			1	2
acidophilic degeneration of individual to small groups of hepatocytes														4	3	3	3		3	3
diffuse hepatocellular hypertrophy with cytoplasmic vacuolation								3								3				
neutrophil infiltrates in sinusoids													3		3					
													3							
Gallbladder																				
foci of inflammatory cell infiltrates in lamina propria	3	3	4	3	3	2	2	3	2	3	3	3	1	4	4	4	4	4	1	4
Kidney																				
focal interstitial lymphoid infiltrates	3	2		2	3	3	4	2	2	3	2	3	2		2	2		2	2	
multinucleated lining epithelium in papillary ducts		x	x				x					x								
cyst in medulla		x																		
chronic interstitial nephritis			3																	
diffuse congestion														4	3	3	3	3	3	3
microlith in renal tubules																	x			
small foci of dystrophic mineralization			2										2			2		2	2	
Urinary bladder																				
foci of inflammatory cell infiltrates in lamina propria	3	2	3	2	2	3	2	3	3	3		1	1	1	1	1		1		
diffuse congestion															3		3		3	3
Testes																				
prepubertal development	x	x			x	x			x	x			x	x			x	x		
chronic focal vasculitis		4																		
focal perivascular lymphoid infiltrate										2										
Ovaries																				
small foci of dystrophic mineralization				1			1	1				1	1			1			1	1
diffuse congestion			2												2					
															3					

Code: x = condition present; 4 = autolysed; 1 = not remarkable; 2 = very slight; 3 = slight; 4 = moderate; 5 = marked; 6 = extreme; - = not available; *Died or euthanized in extremis

137-090

FC-143: Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 16. Cont. Microscopic Observations.

Tissue Lesion	Control				1 mg/kg/day				10 mg/kg/day				30 mg/kg/day				100 mg/kg/day			
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Prostate																				
focal interstitial lymphoid infiltrates	3	3			2	3			2	3			2					1		
focal lymphoid infiltrate in corpus cavernosum		3				2			2				3							
Uterus																				
diffuse congestion											1	1				1				
blood in uterine glands			3	2			2								3				3	3
small foci of hemorrhage in endometrium			2	3			3								2				3	
brown pigment-laden macrophages in endometrium								3												
inflammatory cell infiltrates in endometrium			3	2			4	2												
proteinaceous fluid and inflammatory cells in uterine lumen																			3	
Vagina																				
foci of lymphoid infiltrates in lamina propria and mucosal epithelium			3	4			3	3			4	4			2	3			2	3
foci of lymphoid infiltrates in muscularis				2				2				3								3
Sarcocystis sp.							4													
focal lymphoid infiltrate in tunica adventitia							3													
diffuse congestion															3					
focal neutrophil infiltrate in mucosa											3									
Skeletal muscle	1		1	1	1	1			1		1						1			
Sarcocystis sp.		4					4	4						4					4	
focal interstitial inflammatory cell infiltrates		3					4	2		3		2								
interstitial fibrosis																4				3
focal/multifocal atrophy of muscle														4		4				4
increased sarcolemmal nuclei													4		3			4		
Skin																				
brown/black pigment in dermis	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
dermal inflammatory cell infiltrates		2				3	3													
diffuse acanthosis	3		3																	
diffuse congestion																	3			
hyperkeratosis					3	3		3		3	3			3	3	3			3	3
few large areas of hemorrhage in subcutis							3										3			

Code: 4 = condition present; a = autolysed; 1 = not remarkable; 2 = very slight; 3 = slight; 4 = moderate; 3 = marked; 6 = extreme; - = not available; *Died or sacrificed in extremis

137-090

FC-143: Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 10. Cont. Microscopic Observations.

Tissue / Lesion	Control				1 mg/kg/day				10 mg/kg/day				30 mg/kg/day				100 mg/kg/day			
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Mammary gland																				
brown pigment in dermis								1												
hyperkeratosis	x	x		x	x				x	x	x		x			x	x	x	x	x
dermal inflammatory cell infiltrates	1		1	1	1	1	1			1		1	1	1	1					
inflammatory exudate in acinar lumen/ducts			1	1	2		1		1		1	1	2							
inflammatory cell infiltrates in intralobular connective tissue		2		2												2				
diffuse congestion		1							2											
intraepidermal microabscess																	1			
													x							
Penis	x	x	x	x	x	x	x	x	x	x	x	x	x	1	x	1	1	1	1	1
Bone marrow (Rib junction)	1	1	1	1	1	1	1	1	1	1	1	1								
hypocellular marrow													1	4	4	1	4	4	4	4
congestion														1	1	4	1	1	4	1
Miscellaneous																				
acute focal cheilitis, lip																4				

Comment:
x = condition present
A = autolysed
? = not remarkable
1 = very slight
2 = slight
3 = moderate
4 = marked
5 = extreme
x = not available
*Died or sacrificed in extremis

137-096

Oral Teratology Study of T-3141CoC in Rabbits

Experiment No.: 0681TB0398

Conducted At: Safety Evaluation Laboratory

St. Paul, Minnesota

Dosing Period: September 21, 1981 through November 6, 1981

Study Director:

2-19-82
Date

2-19-82
Date

2/23/82
Date

Summary

Oral administration of distilled water solutions of T-3141CoC at doses of 0, 50, 5 and 1.5 mg/kg/day to pregnant New Zealand White/Minikin rabbits during gestational days 6 through 18 (period of organogenesis) was not embryotoxic and did not affect the ovaries or reproductive tract contents of the does. The compound did not cause gross, internal, or skeletal malformations of the fetuses. T-3141CoC was not teratogenic in the rabbit.

T-3141CoC administration was toxic to only the 50 mg/kg/day dose group pregnant animals. The high dose does, as a group, lost significantly more weight than the 0 mg/kg/day group does. No compound-related toxic clinical signs or deaths occurred in any of the compound-treated groups.

Introduction

A rangefinding study[a] determined that the T-3141CoC[b] was very toxic to pregnant rabbits at dose levels of 100 mg/kg/day and higher. The toxicity resulted in deaths within the first four days of dosing. The 50 mg/kg/day rabbits survived 13 days of dosing but lost a significant amount of body weight early in the dosing interval (days 6-9 gestation). The high dose level for a rabbit teratology study was set at 50 mg/kg/day based on the results of the rangefinding study.

This teratology study[c] in rabbits was conducted to evaluate the embryotoxic and teratogenic effects of orally administered T-3141CoC. The study was sponsored by 3M Commercial Chemical Division, St. Paul, Minnesota and was conducted by the Safety Evaluation Laboratory, St. Paul, Minnesota. Two sets of compound administration groups were dosed between September 21 and November 6, 1981. The protocol and list of the principal participants and supervisory personnel can be found in Appendices I and II respectively.

All portions of this study were conducted according to the Good Laboratory Practice (GLP) regulations and the Safety Evaluation Laboratory Standard Operating Procedures (see Appendix III for Quality Assurance Unit statement). The storage location for specimens, raw data and a copy of the final report is maintained in the Safety Evaluation Laboratory's record archives.

Methods

Sexually mature New Zealand White/Minikin female rabbits were obtained from Dutchland Laboratories, Inc., Denver, PA, and assigned cages according to a computer-generated random numbers table. The rabbits, ranging in weight from 1941 to 2013 grams, were then divided into four groups of 18 animals each. The rabbits were housed individually in stainless steel cages in a temperature and humidity controlled room. Food[d] and water were available ad libitum. The lights were on a 12 hour light/dark cycle. Each female rabbit was injected with 1 mg of pituitary luteinizing hormone via the ear vein before insemination. The does were then artificially inseminated with 0.5 ml of pooled diluted semen. The day of insemination was designated day 0 of pregnancy.

The animals were observed daily from days 3 through 29 of gestation for abnormal clinical signs. Body weights were recorded on gestational days 3, 6, 9, 12, 15, 18 and 29. The four groups were dosed with T-3141CoC dissolved in distilled water at 0, 50, 5 and 1.5 mg/kg/day. The solutions were administered daily using a constant dose volume of 1 ml/kg by oral intubation with a syringe and rubber catheter on gestational days 6 through 18. T-3141CoC characterization was provided by 3M Commercial Chemical Division, St. Paul, Minnesota (Appendix IV).

[a] Experiment Number 0681RB0131
[b] PC-143
[c] Experiment Number 0681TR0190
[d] Purina Rabbit Chow, Ralston Purina Co., St. Louis, MO

All surviving animals were euthanatized on gestational day 29. The ovaries and uterus, including its contents, were examined immediately to determine the following: number of corpora lutea, number of viable fetuses, number of resorption sites, fetal weights and sex, and gross fetal abnormalities. The fetuses were then placed in an incubator at 37°C for a 24-hour incubation period. Following the incubation period, all fetuses were killed and examined for internal abnormalities. The fetuses were then preserved in ethyl alcohol for clearing and staining of the skeleton with alizarin red to detect skeletal abnormalities.

Results and Discussion

The oral administration of T-3141GOC to pregnant rabbits during the period of organogenesis caused maternal toxicity only in the high dose group (50 mg/kg/day). The high dose does, as a group, lost significantly more weight than the control group (0 mg/kg/day) between day 6 (the initiation of dosing) and day 9 of the study (Table 1, Appendix V). The high dose does gained weight comparable to the controls after day 9 of gestation. The mid (5 mg/kg/day) and low (1.5 mg/kg/day) dose group mean maternal body weight gains were never significantly different from the control group. The failure of the low dose does to gain body weight comparable to the control group was due to one animal (#B2336) which consistantly lost weight during the dosing interval and eventually aborted and died. No compound-related clinical signs were unique to T-3141GOC treatment of pregnant rabbits.

Maternal deaths occurred during the study. Five of the six deaths were terminations due to broken backs or were due to intubation error. No deaths could be attributed directly to compound-related toxicity.

Reproductive function of the does and fetal survival were not affected by compound administration. The conception incidence, the incidences of abortions or does delivering early and the 24 hour incubation mortality incidence of the treated groups were not different from the control group (Table 2).

T-3141GOC was not embryotoxic and did not affect the ovaries or reproductive tract contents of the does. The number of male, female, total and dead fetuses, the mean number of resorption sites, implantation sites, corpora lutea and mean fetus weights of the three compound dosed groups were not significantly different from the control group (Table 3, Appendix VI).

No compound-related major gross fetal malformations were observed in any compound-dosed group. One low dose fetus had a clubbed forepaw (Table 4, Appendix VII). No internal fetal findings were observed (Table 5, Appendix VIII).

T-3141GOC treatment did not cause compound-related fetal skeletal malformations. The incidences of 13 ribs in the high dose group and 13 ribs spurred in the mid dose group were significantly higher than in the control

group (Table 6, Appendix IX). The findings involving the 13th rib are naturally occurring and were not considered malformations. Findings associated with skeletal ossifiction were not different among the four treatment groups. The oral administration of T-3141CoC to pregnant rabbits was not teratogenic at the dose levels tested.

Table 1

Oral Teratology Study of T-3141CoC in Rabbits
Mean Body Weight Gain or Loss (g) With Standard Deviations
of Pregnant Rabbits Between Gestational Day Weighings

Dose Group		DAY	1-6	9	12	15	18	29
0 mg/kg/day		Mean	43	[illegible]	19	50	49	101
	Strain	DEV	51.2	[illegible]	[illegible]	26.4	25.[illegible]	[illegible]
50 mg/kg/day		Mean	56	[illegible]A	31	79	53	186
	Strain	DEV	29.9	73.8	79.0	56.4	50.0	[illegible]
5 mg/kg/day		Mean	91	[illegible]	6	36	44	[illegible]
	Strain	DEV	35.1	54.2	83.1	79.1	51.0	45.4
1.5 mg/kg/day		Mean	45	[illegible]	41	29	4	206
	Strain	DEV	41.3	40.1	[illegible]	[illegible]	[illegible]	[illegible]

A significantly lower than the control (Dunnett's t test $p < 0.05$)

Table 2

Oral Teratology Study of T-3141OOC in Rabbits
Reproductive Performance and Fetal Mortality[a]

Dose Group	Total Number of Animals	Conception Incidence	%	Incidence of Abortions or Premature Deliveries	%	24 Hour Incubation Mortality Incidence	%
0 mg/kg/day	18	15/18	83	4/15	27	0/49	0
50 mg/kg/day	18	17/18	94	2/17	12	1/74	1
5 mg/kg/day	18	11/17[b]	64	0/11	0	0/71	0
1.5 mg/kg/day	17[c]	9/15[b]	60	1/9	11	1/45	2

[a] Treatment groups were not significantly different from the control group (Chi-square with Yates correction $p < 0.05$)
[b] One or more animals died early in study and pregnancies could not be determined
[c] One of 18 animals was terminated before the initiation of dosing because of a debilitating condition

000007

Table 3

Oral Teratology Study of T-3151COC in Rabbits
Mean Litter Data and Fetal Weights With Standard Deviations[a]

Dose Group	No. of Fetuses Examined	Fetal Sex M	Fetal Sex F	Fetal Sex Total	Litter Fetal Weight	Resorption Sites	Implantation Sites	Live Fetuses	Fetal Weight
0 mg/kg/day	12	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Std. Dev	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
30 mg/kg/day	12	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Std. Dev	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
5 mg/kg/day	11	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Std. Dev	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.5 mg/kg/day	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Std. Dev	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[a] Treatment groups were not significantly different from control group (Dunnett's t test $p < 0.05$)

Table 4

Oral Teratology Study of T-3141CoC in Rabbits
Number of Fetuses With Gross Findings[a]

	Dose Groups			
	0 mg/kg/day	50 mg/kg/day	5 mg/kg/day	1.5 mg/kg/day
Total fetuses examined	49	74	71	45
Small	3 (6)	1 (1)	2 (3)	2 (4)
Clubbed forepaw				1 (2)

Table 5

Oral Teratology Study of T-3141CoC in Rabbits
Number of Fetuses With Internal Findings[b]

	Dose Groups			
	0 mg/kg/day	50 mg/kg/day	5 mg/kg/day	1.5 mg/kg/day
Total fetuses examined	49	74	71	45

[a] Treatment groups were not significantly different from the control group (Dunnett's t test $p < 0.05$)
[b] No findings were observed in the fetuses examined
( ) = percent of total examined

Table 6

Oral Teratology Study of T-1161CoC in Rabbits
Numbers of Fetuses With Skeletal Findings

	0 mg/kg/day	50 mg/kg/day	5 mg/kg/day	1.5 mg/kg/day
No. of fetuses examined	49	74	71	45
Fontanelle not closed	19 (30)	16 (22)	24 (34)	11 (24)
Holes in parietal	1 (2)	1 (1)		
Holes in both parietals				1 (2)
One sternebrae missing			1 (1)	
Sternebrae not ossified	7 (14)	16 (22)	11 (16)	10 (22)
Sternebrae asymmetrical	4 (8)	3 (4)	5 (7)	5 (11)
Extra sternebrae	1 (2)	1 (1)		1 (2)
Sternebrae fused	1 (2)	1 (1)		
13 ribs	8 (16)	20 (30)[a]	21 (30)	9 (20)
13 ribs spurred	3 (6)	12 (16)	18 (25)[a]	7 (16)
13 ribs floating	1 (2)		1 (1)	1 (2)

[a] Significantly higher than the control (Dunnett's t test $p \leq 0.05$)
( ) = percent of total examined

Appendix I

TITLE: Protocol for Oral Teratology Study of T-3141CoC[A] in Rabbits (Experiment Number 0661T30198).

OBJECTIVE: A teratology study will be used to evaluate the embryotoxic and teratogenic effects of orally administered T-3141CoC to pregnant rabbits during the period of organogenesis. The procedure complies with the general recommendations of the FDA issued in January, 1963 ("Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use"). The study will be conducted according to the 1978 Good Laboratory Practice Regulations and Safety Evaluation Laboratory's Standard Operating Procedures.

SPONSOR: 3M Commercial Chemical Division, St. Paul, Minnesota.

TESTING FACILITY: Safety Evaluation Laboratory, St. Paul, Minnesota.

STUDY DIRECTOR:

START OF DOSING: September, 1981.

TEST SYSTEM: Seventy-two sexually mature New Zealand White/Minikin rabbits from Dutchland Laboratories, Inc., will be housed in stainless steel cages with wire mesh floors in a temperature and humidity controlled room. This strain of rabbit will be used because historical control data is available. Purina Rabbit Chow and water will be available ad libitum. The lights will be on a 12 hour/dark cycle.

TEST SYSTEM IDENTIFICATION: Each animal will be ear tagged and that number will be indicated on the outside of the cage.

RANDOMIZATION: The animals will be assigned cages according to a computer-generated random numbers table.

CONTROL ARTICLE: Distilled water.

TEST ARTICLE: T-3141CoC.

ANALYTICAL SPECIFICATIONS: The test article composition and purity will be determined by the Sponsor (3M Commercial Chemical group) prior to the start of the study and at the end of dosing. The sponsor is responsible for retaining a reference sample of the test and control article, as required, for GLP compliance.

[A] FC-143

DOSAGE LEVELS AND EXPERIMENT DESIGN: The test article will be dissolved in distilled water daily. The test article solution and control article will be administered by oral intubation to the rabbits on days 6 through 18 of gestation according to the following:

Dose Level	Group Size
50 mg/kg/day	18
5 mg/kg/day	18
1.5 mg/kg/day	18
0 mg/kg/day	18

The oral route of administration will be used because toxicity has been defined by this route in a rangefinder study. No dietary contaminants are known to interfere with the test article.

On the day of breeding, each female will be given an intravenous injection of 1 mg of pituitary luteinizing hormone to induce ovulation. The does will then be artificially inseminated with 0.5 ml of pooled diluted semen collected from New Zealand White/Minikin male rabbits.

The animals will be observed daily from day 1 through day 29 of gestation for abnormal clinical signs. Body weights will be recorded on days 3, 6, 9, 12, 15, 18 and 29 of pregnancy and the rabbits dosed accordingly using a constant dose volume of 1 ml/kg of body weight.

The females will be killed on day 29 and the ovaries, uterus and its contents will be examined to determine: number of corpora lutea, number of fetuses (live and dead), number of resorption sites, number of implantation sites, pup weight and gross abnormalities. The pups will be placed in an incubator at 37° C for a 24 hour survival check. The following day the pups will be terminated and their viscera examined for any internal abnormalities. The pups will then be fixed in ethyl alcohol for subsequent skeletal examination after clearing and staining with alizarin red.

SAMPLES FOR POSSIBLE SPONSOR COMPOUND LEVEL ANALYSIS: A blood sample will be taken pre-dose, on day 18 and on day 29 of gestation from the same six rabbits at each dose level. A liver sample will be taken from the same rabbits on day 29 of gestation. The samples will be labeled, frozen and transferred to the sponsor.

These samples are not considered an integral part of the teratology evaluation of T-1141CoC in rabbits, that is they are not per se a part of the teratology study. They were taken at the sponsor's request because they may supply useful supplemental information. The samples will not necessarily be analyzed; the extent to which they are analyzed is a matter of scientific judgement by the sponsor. The results of these analyses, if and when they are completed, will be reported in a separate report. A copy of such report will be sent to the study director for inclusion in the Safety Evaluation Laboratory record archive file. Alternatively, the sponsor can notify Safety Evaluation that the samples will not be analyzed.

DATA ANALYSIS AND FINAL REPORT: The proposed statistical methods to be used for analysis of the data are: Dunnett's t test for dam and pup weights, number of fetuses, number of resorption sites, number of implantation sites and number of corpora lutea; Chi square for percent abnormalities. The proposed date for the final report is 2-3 months after detailed pup examinations have been completed (approximately first quarter, 1982).

Sanitized

CS 808 3/26/
Rec'd 5/4/82

(43)

Oral Teratology Study of T-2998COC in Rats

Experiment No.:	0681TR0110
Conducted At:	Safety Evaluation Laboratory
	St. Paul, Minnesota
Dosing Period:	April 6, 1981 through April 16, 1981
Study Director:	

Summary

Oral administration of T-2990CoC at doses of 0, 150, 50, 1.5 and 0.05 mg/kg/day to pregnant Sprague-Dawley rats during days 6 through 15 of gestation (period of organogenesis) was not embryotoxic and did not affect the ovaries or reproductive tract contents of the dams. The compound did not cause abnormal gross, internal, or skeletal malformations of the fetuses. T-2990CoC was not teratogenic in the rat.

T-2990CoC administration was maternally toxic to the 150 mg/kg/day dose group animals. It caused significantly low mean body weight during the dosing interval. Toxic clinical signs and deaths occurred in only the 150 mg/kg/day dose group.

Introduction

This teratology study[a] in rats was conducted to evaluate the embryotoxic and teratogenic effects of orally administered T-2998CoC[b]. The study was sponsored by 3M Commercial Chemical Division, St. Paul, Minnesota and was conducted by the Safety Evaluation Laboratory, , Inc., St. Paul, Minnesota. Two sets of compound administration groups were dosed between April 8 and April 16, 1981. The protocol and list of the principal participants and supervisory personnel can be found in Appendices I and II respectively.

All portions of this study were conducted according to the Good Laboratory Practice (GLP) regulations and the Safety Evaluation Laboratory Standard Operating Procedures (see Appendix . for Quality Assurance Unit statement). The storage location for specimens, raw data and a copy of the final report is maintained in the Safety Evaluation Laboratory's record archives.

Methods

Time mated Sprague Dawley derived CD rats were obtained from Charles River Breeding Laboratory, Wilmington, Massachusetts, and assigned cages according to a computer-generated random numbers table. The rats, ranging in weight from 167 to 230 grams, were then divided into four groups of 22 animals each. The rats were housed individually in hanging stainless steel cages with wire mesh floors and fronts in a temperature and humidity controlled room. Food[c] and water were available ad libitum. The lights were on a 12 hour light/dark cycle.

The animals were observed daily from day 3 through day 20 of gestation for abnormal clinical signs. Body weights were recorded on days 3, 6, 9, 12, 15 and 20 of gestation and the rats dosed accordingly using a constant dose volume of 5 ml/kg of body weight. The five groups were dosed with T-2998CoC dissolved in distilled water daily at 0, 150, 5, 1.5 or 0.05 mg/kg/day. T-2998CoC was administered daily by oral intubation with a syringe equipped with a ball-tipped intubation needle to the rats on days 6 through 15 of gestation (day 0 indicated by sperm-positive vaginal smear). T-2998CoC analysis was provided by 3M Commercial Chemical Division, St. Paul, Minnesota (Appendix IV).

All surviving animals were sacrificed on day 20 by cervical dislocation and the ovaries and uterus, including its contents, were examined immediately to determine the following: number of corpora lutea, number of viable fetuses, number of resorption sites, pup weights and sex, and any gross fetal abnormalities. Approximately two-thirds of the fetuses were preserved in alcohol for clearing and staining of the skeleton with alizarin red to detect skeletal abnormalities. Approximately one-third of the fetuses were fixed in Bouin's solution for subsequent free-hand sectioning by the Wilson technique to determine visceral abnormalities. In order to evaluate lens findings seen under the dissecting microscope, all eye sections with findings, plus select eye sections without lens findings were inbedded in paraffin, sectioned at 5-6 microns, stained with hematoxylin and eosin and examined histologically.

a Experiment No. 0601TR0110
b FC-143
c Purina Laboratory Chow, Ralston Purina Co., St. Louis, MO

Results and Discussion

T-2998CoC administered during the period of organogenesis was toxic to the high dose group (150 mg/kg/day) rats in causing low mean body weights during the dosing period. At gestational days 9, 12 and 15 (Table 1, Appendix V), the high dose group rats weighed significantly less than controls (0 mg/kg/day). The mean maternal body weights of the intermediate (5 mg/kg/day), mid (1.5 mg/kg/day), and low (0.05 mg/kg/day) dose groups were not different from the controls throughout the study.

Abnormal clinical signs were observed and deaths occurred only in the high dose group. Three rats in the high dose group died. All three of the rats that died were ataxic and two of the rats were pale for one to two days before death. The surviving high dose rats did not have abnormal clinical signs and signs of toxicity did not occur in lower dose animals.

T-2998CoC was not embrystoxic and did not affect the ovaries or reproductive tract contents of the dams. The mean number of male, female, total and dead fetuses, the mean number of resorption sites, implantation sites, corpora lutea and mean fetus weights of the four T-2998CoC dose groups were not significantly different from the control (Table 2, Appendix VI).

T-2998CoC did not cause compound-related abnormal gross fetal findings (Table 3), nor did T-2998CoC treatment produce fetal skeletal malformations (Table 4, Appendix VII). A significant higher incidence of the skeletal finding of one sternebrae missing occurred in the high dose group. One sternebrae missing is a minor skeletal aberration and was not considered a malformation in this study. Further, the incidence of the finding of one sternebrae missing was not different among the control group and the lower three treatment groups. The incidences of skeletal findings associated with delayed ossification and rib aberrations were not different among the five treatment groups.

A fetal lens finding was observed to occur in individual fetuses of all dose groups including the control group. The lens findings were localized to the area of the embryonal nucleus, although a variety of morphological appearances were present within that location. The range of morphological appearances as observed under the dissecting microscope included: a discoloration of the lens near the anteriocentral region extending from beneath the lens epithelium to half-way through the lens posteriorly, a cleft at the anteriocentral lens region or a combination of lens discoloration and the presence of a cleft.

The lens findings observed under the dissecting microscope were interpreted histopathologically as either a freehand sectioning artifact of a normal area of primary lens fiber degeneration. The cleft was a space opened up at the vestage of the lens vesicle remnant and consisted of a separation of primary lens fibers of the embryonal nucleus from the lens epithelial cells. The dark streak discoloration of the embryonal nucleus resulted from either the lens being freehand sectioned across the area of normal primary lens fiber degeneration or an artifact being created in the lens during freehand and sectioning accentuating the area of normal primary lens fiber degeneration. The

Amendment to the Final Report of the Oral Teratology
Study of T-29990CoC in Rats
Experiment No.: 0681TR0110
Issued 12/18/81

Please insert the amended page 3 to the above report. Five word changes were made in the last two paragraphs. The study conclusions are not changed by this amendment to the results and discussion section of the report.

Amended page 3 to the Oral Teratology Study
of T-2998CoC in Rats - Experiment No. 0601TR0110

Results and Discussion

T-2998CoC administered during the period of organogenesis was toxic to the high dose group (150 mg/kg/day) rats in causing low mean body weights during the dosing period. At gestational days 9, 12 and 15 (Table 1, Appendix V), the high dose group rats weighed significantly less than controls (0 mg/kg/day). The mean maternal body weights of the intermediate (5 mg/kg/day), mid (1.5 mg/kg/day), and low (0.05 mg/kg/day) dose groups were not different from the controls throughout the study.

Abnormal clinical signs were observed and deaths occurred only in the high dose group. Three rats in the high dose group died. All three of the rats that died were ataxic and two of the rats were pale for one to two days before death. The surviving high dose rats did not have abnormal clinical signs and signs of toxicity did not occur in lower dose animals.

T-2998CoC was not embryotoxic and did not affect the ovaries or reproductive tract contents of the dams. The mean number of male, female, total and dead fetuses, the mean number of resorption sites, implantation sites, corpora lutea and mean fetus weights of the four T-2998CoC dose groups were not significantly different from the control (Table 2, Appendix VI).

T-2998CoC did not cause compound-related abnormal gross fetal findings (Table 3), nor did T-2998CoC treatment produce fetal skeletal malformations (Table 4, Appendix VII). A significant higher incidence of the skeletal finding of one sternebrae missing occurred in the high dose group. One sternebrae missing is a minor skeletal aberration and was not considered a malformation in this study. Further, the incidence of the finding of one sternebrae missing was not different among the control group and the lower three treatment groups. The incidences of skeletal findings associated with delayed ossification and rib aberrations were not different among the five treatment groups.

Fetal lens findings were observed to occur in individual fetuses of all dose groups including the control group. The lens findings were localized to the area of the embryonal nucleus, although a variety of morphological appearances were present within that location. The range of morphological appearances as observed under the dissecting microscope included: a discoloration of the lens near the anteriocentral region extending from beneath the lens epithelium to half-way through the lens posteriorly, a cleft at the anteriocentral lens region or a combination of lens discoloration and the presence of a cleft.

The lens findings observed under the dissecting microscope were interpreted histopathologically as a freehand sectioning artifact of a normal area of primary lens fiber degeneration. The cleft was a space opened up at the vantage of the lens vesicle remnant and consisted of a separation of primary lens fibers of the embryonal nucleus from the lens epithelial cells. The dark streak discoloration of the embryonal nucleus resulted from either the lens being freehand sectioned across the area of normal primary lens fiber degeneration or an artifact being created in the lens during freehand sectioning accentuating the area of normal primary lens fiber degeneration. The

differences in the appearance of the lens artifact in individual fetuses and even among dose groups were largely due to the manner and frequency in which the artifact was created and the limitations inherent in visualizing the artifact under the dissecting microscope. Histologically, the lens artifact was the same in all dose groups regardless of the morphological appearance described under the dissecting microscope. T-2998CoC in utero exposed fetuses did not have compound-related changes in their lenses.

Table 1

Oral Teratology Study of T-2990CoC in Rats
Mean Material Body Weights with Standard Deviations

Dose Groups	DAY	3	6	9	12	15	20
0 mg/kg/day	MEAN	196	223	251	276	310	360
	STAN. DEV	16.1	23.4	21.4	20.6	22.3	27.6
150 mg/kg/day	MEAN	202	229	226[a]	259[a]	262[a]	361
	STAN. DEV	14.0	16.1	29.5	20.6	22.5	28.4
5 mg/kg/day	MEAN	201	229	252	277	312	370
	STAN. DEV	12.1	13.9	14.6	17.1	20.0	30.0
1.5 mg/kg/day	MEAN	196	219	245	269	301	369
	STAN. DEV	15.3	14.6	18.5	15.5	17.9	26.7
0.05 mg/kg/day	MEAN	199	225	247	273	306	360
	STAN. DEV	13.8	16.2	19.2	21.0	23.2	32.4

[a] Significantly lower than the control group (Dunnett's t test $p < 0.05$)

Table 2

Oral Teratology Study of T-2998CoC in Rats
Mean Litter Data and Fetus Weights with Standard Deviations[a]

Dose Groups	GRP NO. OF ANIMALS	WHOLE FETUSES M	F	TOTAL	LEAD FETUSES	RESORPTION SITES	IMPLANTATION SITES	CORPORA LUTEA	MEAN WT FETUSES(G)
0 mg/kg/day	28	4.9	4.9	9.8	0.0	0.5	10.3	11.4	4.4
	STAN DEV	1.8	1.8	2.9	0.0	0.9	2.5	1.6	0.4
150 mg/kg/day	14	4.9	5.1	10.0	0.0	0.6	10.6	11.1	4.2
	STAN DEV	2.1	2.2	3.3	0.0	1.0	3.1	1.9	0.3
5 mg/kg/day	21	5.2	5.2	10.4	0.0	0.5	11.0	11.3	4.3
	STAN DEV	2.2	2.4	1.9	0.0	0.8	1.7	1.6	0.3
1.5 mg/kg/day	19	3.7	4.8	8.5	0.0	1.1	9.6	10.1	4.3
	STAN DEV	1.8	2.5	3.6	0.0	1.5	2.8	2.5	0.4
0.05 mg/kg/day	21	5.1	4.9	10.0	0.0	0.4	10.5	11.0	4.3
	STAN DEV	1.9	2.2	2.8	0.2	1.2	2.2	1.0	0.2

[a] Treatment groups were not significantly different from the control group (Dunnett's t test $p < 0.05$)

010000

Table 3

Oral Teratology Study of T-2990CoC in Rats
Number of Fetuses with Gross Findings[a]

Findings	0 mg/kg/day	150 mg/kg/day	5 mg/kg/day	1.5 mg/kg/day	0.05 mg/kg/day
Total Fetuses Examined	196	140	219	162	211
Runted	1	---	---	---	---
Small	---	---	---	2	---
Umbilical hernia	---	---	---	---	1
Total Normal Fetuses	195	140	219	160	210
Total Abnormal Fetuses	1	0	0	2	1

[a] Treatment groups were not significantly different from the control group (Chi-square $p < 0.05$)

Table 4

Oral Teratology Study of T-2990CoC in Rats
Number and Percent of Fetuses with Skeleton Findings

Skeleton Findings	0 mg/kg/day		150 mg/kg/day		5 mg/kg/day		1.5 mg/kg/day		0.05 mg/kg/day	
Fontanelle not closed	24	(18)	18	(19)	17	(11)	15	(13)	20	(14)
Hole in frontal							1	(1)		
Frontals not ossified	17	(13)	14	(15)	5	(3)	9	(8)	9	(6)
Parietals not ossified	17	(13)	14	(15)	5	(3)	9	(8)	9	(6)
Interparietals not ossified	17	(13)	13	(14)	2	(1)	6	(5)	3	(2)
Sternebrae not ossified	40	(67)	64	(67)	101	(67)	71	(63)	95	(4)
Sternebrae bipartite			1						5	(3)
Sternebrae asymmetrical	14	(10)	10	(10)	12	(8)	13	(12)	10	(12)
One sternebrae missing	20	(15)	30	(31)[a]	29	(19)	19	(17)	22	(15)
Two sternebrae missing	9	(7)	7	(7)	8	(5)	2	(2)	3	(2)
Four sternebrae missing							1	(1)		
13 ribs	2	(1)	2	(2)	4	(3)	1	(1)	6	(4)
13 ribs spurred	4	(3)	10	(10)	5	(3)	6	(5)	6	(4)
Wavy ribs	7	(5)	7	(7)	3	(2)	6	(5)	3	(2)
Protrusion on ribs	6	(4)	7	(7)	4	(3)	4	(4)	3	(2)
One body vertebrae bipartite	35	(26)	15	(16)	30	(20)	17	(15)	25	(17)
Two bodies vertebrae bipartite	6	(4)	2	(2)	14		2	(2)	6	(4)
Three bodies vertebrae bipartite	1				3	(2)			2	(1)
One body of vertebrae missing			1							
Total Number of Fetuses	136		97		150[b]		112[b]		140	
Total Abnormal Fetuses	126	(93)	88	(92)	136	(91)	93	(83)	127	(86)
Total Normal Fetuses	10	(7)	9	(8)	14	(9)	19	(17)	21	(14)

[a] Significantly higher than the control group (Chi-square $p < 0.05$)
[b] Results from one fetus are missing
( ) = percent of total examined

Table 5

Oral Teratology Study of T-2998CoC in Rats
Number and Percent of Fetuses with Internal Findings

Internal Findings	0 mg/kg/day		150 mg/kg/day		5 mg/kg/day		1.5 mg/kg/day		0.05 mg/kg/day	
Fetuses with lens findings	5	(8)	11	(26)[a]	2	(3)	6	(12)	5	(8)
A dark streak in the lens of one eye			2	(9)					2	(3)
A dark streak & cleft in the lens of one eye			3	(7)						
A cleft in the lens of one eye	5	(8)	5	(12)	1	(1)	6	(12)	2	(3)
A clefts in the lens of both eyes			1	(2)	1	(1)			1	(2)
Hydronephrosis					1	(1)				
Enlarged renal pelvis	17	(29)	1	(2)[a]	4	(6)[a]	9	(18)	10	(16)
Abdominal cavity full of blood	2	(3)	3	(7)	3	(4)	1	(2)	3	(5)
Total Normal Fetuses	38	(63)	30	(70)	59	(87)	37	(76)	46	(73)
Total Abnormal Fetuses	22	(37)	13	(30)	9	(13)	12	(24)	17	(27)
Total Fetuses Examined	60		43		68		49		63	

[a] Significantly different from the control group (Chi-square $p < 0.05$)
( ) = percent of total examined

Appendix I

TITLE: Protocol for Oral Teratology Study of T-2998CoC[a] in Rats Experiment Number 0581TR0110).

OBJECTIVE: A teratology study will be used to evaluate the embryotoxic and teratogenic effects of orally administered T-2998CoC to pregnant rats during the period of organogenesis. The procedure complies with the general recommendations of the FDA issued in January, 1966 ("Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use"). The study will be conducted according to the 1978 Good Laboratory Practice Regulations and Safety Evaluation Laboratory's Standard Operating Procedures.

SPONSOR: 3M Commercial Chemical Division, St. Paul, Minnesota.

TESTING FACILITY: Safety Evaluation Laboratory, St. Paul, Minnesota.

STUDY DIRECTOR:

START OF DOSING: April, 1981.

TEST SYSTEM: One hundred and ten sexually mature, time mated Sprague-Dawley derived female rats from Charles River Breeding Laboratory will be housed in hanging stainless steel cages with wire mesh floors and fronts in a temperature and humidity controlled room. This strain of rat will be used because of historical control data and time mated females are readily available. Purina Laboratory Chow and water will be available ad libitum. The lights will be on a 12 hour light/dark cycle.

TEST SYSTEM IDENTIFICATION: Each animal will be ear tagged and that number will be indicated on the outside of the cage.

RANDOMIZATION: The animals will be assigned cages according to a computer-generated random numbers table.

CONTROL ARTICLE: Corn oil.

TEST ARTICLE: T-2998CoC.

ANALYTICAL SPECIFICATIONS: The test article, composition and purity will be determined by the Sponsor (3M Commercial Chemical group) prior to the start of the study and at the end of dosing.

DOSAGE LEVELS AND EXPERIMENT DESIGN: The test article will be suspended in corn oil daily. The test article suspension and control article will be administered by oral intubation to the rats on days 6 through 15 of gestation according to the following:

[a] FC-143

Dose Level	Group Size
150 mg/kg/day	22
5 mg/kg/day	22
1.5 mg/kg/day	22
0.05 mg/kg/day	22
0 mg/kg/day	22

The oral route of administration will be used because metabolism studies showed radiolabeled T-2990CoC was well absorbed. No dietary contaminants are known to interfere with the test article.

The animals will be observed daily from day 3 through day 20 of gestation for abnormal clinical signs. Body weights will be recorded on days 3, 6, 9, 12, 15 and 20 of pregnancy and the rats dosed accordingly using a constant dose volume of 5 ml/kg of body weight.

The females will be killed on day 20 and the ovaries, uterus and its contents will be examined to determine: number of corpora lutea, number of fetuses (live and dead), number of resorption sites, number of implantation sites, pup weight and gross abnormalities. Approximately one-third of the pups will be fixed in Bouin's solution for subsequent free-hand sectioning by the Wilson technique to determine any visceral abnormalities using a dissecting stereoscope. Select eye sections can be sent to histopath for stereomicro examination as deemed necessary by the study director. The remaining approximately two-thirds of the pups will be fixed in ethyl alcohol for subsequent skeletal examination after clearing and staining with alizarin red.

DATA ANALYSIS AND FINAL REPORT: The proposed statistical methods to be used for analysis of the data are: Dunnett's t test for dam and pup weights, number of fetuses, number of resorption sites, number of implantation sites and number of corpora lutea; chi square for percent abnormalities. The proposed date for the final report is 2-3 months after detailed pup examinations have been completed (approximately third quarter, 1981).

Abandoned in Protocol

The control article for Experiment Number Deuterium (oral toxicity study of T-2990CoC in rats) will be changed from corn oil to water. The test article will not be suspended in corn oil daily as noted in the protocol; but solutions will be made by dissolving T-2990CoC in water by and the solution for the whole study will be submitted to a commercial analytical group for clearance before the start of the study and at the end of dosing.

Appendix II

List of Principal Participating Personnel

NAME	FUNCTION

STATEMENT OF QUALITY ASSURANCE

STUDY NUMBER: 0681TR0110

TITLE: Oral Teratology Study of T 2998CoC in Rats

Audits and/or inspections were performed by the Compliance Audit unit for the above titled study, and reported to the study director and to management as follows:

Date Performed	Date Reported
9,14,16 April 1981	21 April 1981
20 April 1981	21 April 1981
20 August 1981	28 August 1981
7 December 1981	14 December 1981
14 December 1981	14 December 1981

Date 14 December 1981

Appendix IV

Prestudy and Poststudy Analysis of T-2990CoC Distilled Water Solutions[a]

Dose Level	Expected	Analysis: Prestudy[b]	Analysis: Poststudy
0 mg/kg	0.0 mg/ml	0.0 ppm	0.00 ppm
150 mg/kg	30.0 mg/ml	30.328 mg/ml	31.0 mg/ml
5 mg/kg	1.0 mg/ml	0.983 mg/ml	0.92 mg/ml
1.5 mg/kg	0.3 mg/ml	0.268 mg/ml	0.33 mg/ml
0.05 mg/kg	0.91 mg/ml	0.0087 mg/ml	0.0092 mg/ml

[a] Pregnant rats were dosed at 5 ml/kg T-2990CoC

Appendix V

Oral Teratology Study of T-2998CoC in Rats
Individual Body Weights (g) and Mean Body Weights
With Standard Deviations For Pregnant Rats

DAY		3	6	9	12	15	20
0 MG/KG/DAY							
N1R	2997	223	243	264	292	321	402
N1R	2998	194	219	247	281	315	385
N1R	3000	186	214	243	261	292	350
N1R	3001	199	222	252	288	325	414
N1R	3002	193	216	229	261	291	356
N1R	3003	170	222	261	290	331	415
N1R	3004	167	144	188	218	254	341
N1R	3005	186	217	251	276	312	374
N1R	3006	185	208	244	266	297	372
N1R	3007	177	211	236	259	297	365
N1R	3052	191	220	252	277	314	401
N1R	3053	216	253	278	301	327	366
N1R	3054	226	261	289	303	347	432
N1R	3055	202	235	267	291	327	399
N1R	3056	206	237	268	292	327	396
N1R	3057	207	236	270	295	329	397
N1R	3058	194	222	243	267	289	354
N1R	3059	191	216	232	251	286	345
N1R	3061	204	239	265	288	330	405
N1R	3062	191	214	236	261	286	341
MEAN		196	225	251	276	310	390
STAN. DEV		16.1	23.4	21.4	22.6	22.3	27.6
NON PREGNANT ANIMALS							
N1R	2999	196	219	231	248	258	280
N1R	3060	188	207	225	235	236	253

International Research and Development Corporation

SPONSOR: 3M Company

COMPOUND: Fluorad® Fluorochemical FC-143

SUBJECT: Ninety Day Subacute Rat Toxicity Study.

Edwin I. Goldenthal, Ph.D.
Vice President and
Director of Research

Collaborators:

D. C. Jessup, Ph.D., Associate
Director of Research
R. G. Gall, D.V.M., Vice President
and Director of Pathology
N. D. Jefferson, B.A., Acting Director
of Small Animal Toxicology
R. J. Arceo, M.D., Staff Pathologist

Date: November 6, 1978

137-089

TABLE OF CONTENTS

		Page
I.	Synopsis	1
II.	Compound	3
III.	Clinical Methods	4
	A. Method	4
	1. General Procedure	4
	2. Compound Administration	4
	3. Observations	5
	4. Laboratory Tests	5
	a. Hematology	5
	b. Biochemistry	5
	c. Urinalysis	5
	d. Serum Samples	5
	5. Statistical Analysis	6
	B. Results	6
	1. General Behavior, Appearance and Survival	6
	2. Body Weights	6
	3. Food and Compound Consumption	7
	4. Laboratory Tests	7
	a. Hematology	7
	b. Biochemistry	8
	c. Urinalysis	8
IV.	Pathological Studies	9
	A. Methods	9
	1. Gross Pathology	9
	2. Histopathology	9
	B. Results	9
	1. Gross Pathology and Organ Weights	9
	2. Histopathology	10

137-089

TABLE OF CONTENTS
(Continued)

Table No.		Page
1.	Group Mean Body Weights; Weight Ranges; and Survival	12
2.	Individual Weekly Body Weights	13-14
3.	Mean Food Consumption	15
4.	Summary of Means and Significance of Hematological Values	16-17
5- 7.	Individual Hematological Values	18-23
8.	Summary of Means and Significance of Biochemical Values	24-25
9-11.	Individual Biochemical Values	26-31
12-14.	Individual Urinalysis Values	32-37
15.	Summary of Gross Necropsy Observations	38
16.	Absolute and Relative Organ Weights	39
17.	Individual Organ Weights	40-41
18.	Histomorphologic Observations	42-47

137-089

I. SYNOPSIS

Fluorad® Fluorochemical FC-143 was fed in the diet at levels of 10, 30, 100, 300 and 1,000 ppm to Charles River CD rats for 90 days. Five male and five female rats were initiated at each dosage level and in the control group. The rats were observed twice daily for overt signs of toxicity and mortality. Individual body weights and sex-group food consumption were recorded weekly. Hematologic, biochemical and urinalysis studies were conducted during the pretest period and at 1 and 3 months of study.

No changes considered to be directly related to the compound were seen in general behavior, appearance or survival. A slight decrease in body weight gain and food consumption was seen for male rats at the 300- and 1,000-ppm dosage levels.

Hematologic, biochemical and urinalysis values for the female rats showed no changes considered to be related to the compound. A few values obtained for the males showed a slight deviation from the control values (i.e. slightly lower erythrocyte count, and elevated blood urea nitrogen and alkaline phosphatase values).

Compound-related gross observations such as enlargement and varying degrees of discoloration on the surface of the liver were observed among male rats in the 1,000-ppm group. There were no such observations amoung female rats from the 1,000-ppm group or in males or females from lower dietary levels.

Statistically significant variations in sex-group mean organ weights, which were considered compound related, occurred in the liver of rats in the 300- and 1,000-ppm dosage groups. All other variations were unaccompanied by any morphologic alterations.

Microscopically, compound-related lesions were confined to the liver. The lesions consisted of focal to multifocal, very slight to slight, cytoplasmic enlargement of hepatocytes located in centrilobular-midzonal regions of the affected liver lobules, accompanied in some instances by increased amount of yellowish-brown pigment resembling lipofuscin in cytoplasm of hepatocytes and occasionally in sinusoidal lining cells. The incidence and relative severity of the above lesions were predominantly amoung males and more pronounced among rats at 1,000 ppm. The other changes recorded in the liver and other tissues were lesions of naturally occurring diseases and they were present in most instances among the control and test rats.

II. COMPOUND

The compound was received from 3M Company, Saint Paul, Minnesota on October 24, 1977 as indicated below:

Label	Description
Fluorad® Flurochemical FC-143 3M Stock No. 98-0211-0008-0 Lot 340	white powder

137-089

III. CLINICAL METHODS

A. METHOD:

1. General Procedure:

Thirty male (222 to 254 grams) and 30 female (151 to 179 grams) Charles River CD rats purchased from The Charles River Breeding Laboratories, Inc., Portage, Michigan were used in this study. The rats were distributed among the groups, based upon a computer-generated table of random numbers. The rats were housed individually in suspended wire-mesh cages and maintained in a temperature-, humidity- and light-controlled room. During the pretest period, rats were provided Purina® Laboratory Chow® and water *ad libitum*. During the test period, the rats were provided the appropriate test diet and water *ad libitum*.

This study was initiated on November 1, 1977 and terminated by sacrifice of all remaining rats on January 30, 1978.

2. Compound Administration:

The test compound was mixed weekly with ground Purina® Laboratory Chow® (i.e., ground basal diet) to provide dosage levels of 10, 30, 100, 300 and 1,000 ppm. Five male and five female rats were used at each dosage level and in a control group. The control rats received the basal diet only, on the same regimen as treated rats. Samples of diet (100 grams each) were taken immediately after preparation and after 7 days standing in weeks 1, 4 and 12. The samples were frozen and subsequently shipped to the sponsor. Diets were prepared in the following manner: to produce a premix, the required amount of Fluorad® Fluorochemical FC-143 was mixed with 500 grams of Purina® Laboratory Chow® using a Hobart blender. To provide the proper dosage level diets, appropriate quantities of the premix were combined with additional ground basal diet in a twin-shell blender. The diets were prepared weekly.

3. Observations:

The rats were observed twice daily for overt signs of toxicity and for mortality. Detailed observations were recorded weekly. Individual body weights and food consumption were recorded weekly during the pretest and treatment periods.

4. Laboratory Tests:

Once during the pretest period and at 1 month and 3 months of the study, blood (orbital sinus puncture technique) and urine samples were obtained for analysis from all surviving rats. Food and water were withheld overnight prior to the sample collection.

a. Hematology:

Hematological studies included: hemoglobin[1], hematocrit[2], total erythrocytes[3], reticulocytes[4], and total[3] and differential leucocyte counts.

b. Biochemistry:

Biochemical studies included: fasting glucose[5], blood urea nitrogen (BUN)[5], plasma glutamic pyruvic transaminase (PGPT)[5] and plasma glutamic oxalacetic transaminase (PGOT)[5] activity, plasma alkaline phosphatase[5] activity, γ-glutamyl peptidase[6], creatinine phosphokinase[7] and calcium[8]. Alkaline phosphatase activity was not determined in the pretest period because of interference by the anticoagulant.

c. Urinalysis:

Urinalysis included: description of color and appearance; measurement of volume, pH[9], and specific gravity[9]; qualitative tests for protein[9], glucose[9], ketone[9], bilirubin[9], and occult blood; and microscopic examination of the sediment.

d. Serum Samples:

Serum samples were obtained for all surviving rats at 13 weeks of study. The samples were pooled by sex and group, frozen, and subsequently shipped to the sponsor.

5. Statistical Analysis:

All statistical analyses compared the treatment groups with the control group, by sex. Body weights (week 13) food consumption (weeks 1-13), hematological, biochemical and urinalysis parameters and abso- l and relative organ weights were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie[10] using Dunnett's[11] multiple comparison tables to judge significance of differences.

B. RESULTS:

1. General Behavior, Appearance and Survival:

No changes considered to be related to the compound were observed in general behavior or appearance. Incidental findings noted for control and treated rats included ocular discharge and pupil dila- tion.

Survival (prior to sacrifice) after 3 months of compound con- sumption was as follows:

Dosage Level	No. Surviving/No. Initiated Male	Female
Control	5/5	5/5
10 ppm	5/5	5/5
30 ppm	5/5	5/5
100 ppm	5/5	4/5
300 ppm	5/5	4/5
1,000 ppm	5/5	5/5

Both deaths occurred following collection of blood. Neither death was preceded by any signs of toxicity.

2. Body Weights (Tables 1-2):

Comparison of group mean body weights, by sex showed a decrease in body weight gain for male rats at the 300- and 1,000-ppm dosage

levels. Changes in body weight were similar for control and treated female rats. At 13 weeks, the group mean body weight for male rats was significantly ($p<0.05$) lower than the control group mean. The group mean body weights at 13 weeks of study were as follows:

Dosage Level	Mean Body Weights, g Male	Female
Control	460	259
10 ppm	478	260
30 ppm	500	268
100 ppm	457	278
300 ppm	431	263
1,000 ppm	362	255

3. Food Consumption (Table 3):

Declines in food consumption were noted at the higher dosage levels for male rats (100, 300 and 1,000 ppm). Food consumption values were similar for control and treated female rats. The average food consumption through the 13-week study were as shown below:

Dosage Level	Average Food Consumption (g/rat/day) Male	Female
Control	27.0	19.7
10 ppm	26.7	19.2
30 ppm	28.7	20.7
100 ppm	25.8	21.5
300 ppm	25.7	19.6
1,000 ppm	23.0	19.5

4. Laboratory Tests (Tables 5-14):

a. Hematology:

A comparison of male rats, by dosage group, to the control group, showed a slight decrease in erythrocytes at 3 months of study. However, the individual values were within the normal range for Charles River CD rats of this age in this laboratory. A similar comparison of female rats showed no variations that could be attributable to compound consumption.

b. Biochemistry:

A comparison of male rats, at the higher dosage levels, to the control male rats, showed a slight increase in the BUN and alkaline phosphatase values. A similar comparison of female rats showed no variations that could be attributable to compound consumption.

c. Urinalysis:

The presence of occult blood was of a higher frequency in females than males at all dosage levels. No changes considered to be related to compound consumption were observed in urinalysis values.

IV. PATHOLOGICAL STUDIES:

A. METHODS:

1. Gross Pathology:

After 90 days of compound administration, five rats/sex/group were sacrificed with carbon dioxide and were necropsied. At necropsy, organs and tissues were examined for gross abnormalities and collected in 10% neutral buffered formalin (eyes in Russell's fixative). Liver samples, obtained from all of the rats at terminal sacrifice, were frozen and subsequently shipped to the sponsor.

Two female rats which died prior to termination were also necropsied and tissues collected as above.

2. Histopathology:

Microscopic examination of formalin-fixed, hematoxylin and eosin stained, paraffin sections was performed for all rats in control, 100-, 300-, and 1,000-ppm groups. The following tissues were examined histologically.

brain with cervical cord	aorta	pancreas
lumbar spinal cord	spleen	liver
peripheral nerve	mesenteric lymph node	kidneys
eyes	thymus	urinary bladder
pituitary	bone with marrow (sternum)	testes
thyroid with parathyroid	salivary gland	ovaries
adrenals	small intestines (duodenum, jejunum, ileum)	prostate
lung	colon	uterus
heart with coronary vessels		skin (mammary gland)
		any tissue(s) with gross lesions

In addition, the livers from rats from the 10- and 30-ppm dosage level were also microscopically examined.

B. RESULTS:

1. Gross Pathology (Table 15) and Organ Weights (Tables 16-17):

Gross necropsy observations in liver, such as enlargement, and varying degrees of discoloration on the surface were present among male rats at the 1,000-ppm level and were considered compound related. No such observation was present among the females at 1,000 ppm or in males or females from lower dietary-level groups.

Statistically significant variations in sex-group mean weights of several organs occurred between the control and experimental groups. These variations were of unknown biological significance with the exception of the increase in liver weight noted in males at the 300- and 1,000-ppm dosage levels. This variation in liver weights was accompanied by morphologic alterations. One female rat at 1,000-ppm, also had morphologic alterations noted in the liver.

Organ	Group	Sex	Weight	Change	P
Liver	30 ppm	M	absolute, relative	increase	0.01, 0.05
	300 ppm	M	absolute, relative	increase	0.01, 0.01
	1,000 ppm	M	absolute, relative	increase	0.01, 0.01
		F	absolute, relative	increase	0.05, 0.05
Kidney	10 ppm	F	relative	increase	0.05
	30 ppm	M	absolute	increase	0.05
	100 ppm	M	relative	increase	0.05
	300 ppm	M	relative	increase	0.05
	1,000 ppm	M	relative	increase	0.01
Brain	1,000 ppm	M	relative	increase	0.01

2. Histopathology (Table 18):

Compound-related liver lesions occurred in almost all male test rats at 100, 300 and 1,000 ppm and one female at 1,000 ppm. The lesion consisted of focal to multifocal, very slight to slight cytoplasmic enlargement (hypertrophy) of hepatocytes in centrilobular to midzonal regions of the affected liver lobules. These were accompanied in some instances by increased amount of yellowish-brown pigment resembling lipofuscin in cytoplasm of hepatocytes and occasionally in sinusoidal lining cells. The incidence and relative severity of the above lesions were more pronounced among male rats at the 1,000-ppm dietary level.

References

1. Coulter Hemoglobinometer, Coulter Electronics, 590 W. 20th Street, Hialeah, Florida.

2. Microhematocrit, John B. Miale, 3rd Ed., 1967, The C. V. Mosby Company, p. 1154.

3. Coulter Particle Size Counter, Model Zβ, Coulter Electronics, 590 W. 20th Street, Hialeah, Florida.

4. Gradwhol's Clinical Laboratory Methods and Diagnosis, Frankel and Reitman, Editors 6th Ed., 1963, The C. V. Mosby Company, p. 1132.

5. Technicon Auto Analyzer 6/60 Micro Methodology.

6. Glutamyl Peptidase - Sigma GGTP Procedure Bulletin #545, Sigma Chemical Co., St. Louis, MO.

7. Micro Auto Analyzer II, 6/60 Micro Methodology.

8. Photovolt P94, Photovolt Corporation.

9. Multistix (Ames Reagent Strips).

10. Steel, R. G. D. and Torrie, J. H. (1960), Principles and Procedures of Statistics, McGraw-Hill, New York, N. Y.

11. Dunnett, C. W., New Tables for Multiple Comparisons with a Control, Biometrics, Sept. 1964.

Flomax® Hydrochloride [illegible]: Ninety Day Subchronic Oral Toxicity Study.

TABLE 1. Group Mean Body Weights, Organ Weights, Organ/Body Weight Ratios and Survival.

Week of Study	Control Mean Body Wt.	Control Weight Ranges	Control Survival	30 mg/kg Mean Body Wt.	30 mg/kg Weight Ranges	30 mg/kg Survival	8 mg/kg Mean Body Wt.	8 mg/kg Weight Ranges	8 mg/kg Survival	800 mg/kg Mean Body Wt.	800 mg/kg Weight Ranges	800 mg/kg Survival	80 mg/kg Mean Body Wt.	80 mg/kg Weight Ranges	80 mg/kg Survival	1,600 mg/kg Mean Body Wt.	1,600 mg/kg Weight Ranges	1,600 mg/kg Survival
MALES:																		
-1	238	226-251	5/5	245	231-254	5/5	241	229-250	5/5	244	222-256	5/5	240	224-244	5/5	238	225-245	5/5
0	277	268-292	5/5	278	256-283	5/5	282	262-292	5/5	274	236-292	5/5	279	260-295	5/5	280	252-290	5/5
1	302	285-321	5/5	309	294-338	5/5	315	306-343	5/5	307	285-338	5/5	307	296-318	5/5	295	276-309	5/5
2	342	318-372	5/5	345	316-373	5/5	350	339-380	5/5	340	319-368	5/5	346	325-375	5/5	352	328-380	5/5
3	378	360-404	5/5	380	338-408	5/5	385	364-410	5/5	368	336-384	5/5	376	348-394	5/5	382	345-379	5/5
4	398	369-430	5/5	396	352-444	5/5	402	368-445	5/5	389	336-408	5/5	390	356-394	5/5	398	362-390	5/5
5	406	368-429	5/5	430	368-478	5/5	419	393-465	5/5	387	339-429	5/5	394	358-380	5/5	397	358-398	5/5
6	426	386-470	5/5	436	386-464	5/5	448	418-476	5/5	411	364-461	5/5	396	365-438	5/5	412	374-438	5/5
7	427	383-470	5/5	437	384-488	5/5	459	414-486	5/5	410	362-445	5/5	397	365-418	5/5	392	365-384	5/5
8	440	412-481	5/5	445	373-510	5/5	454	432-508	5/5	442	380-483	5/5	406	377-434	5/5	390	377-415	5/5
9	442	429-440	5/5	447	386-520	5/5	486	458-530	5/5	438	390-481	5/5	408	380-450	5/5	398	384-434	5/5
10	477	428-501	5/5	477	375-527	5/5	502	443-539	5/5	444	395-500	5/5	427	397-475	5/5	408	397-432	5/5
11	479	437-510	5/5	493	392-542	5/5	502	476-556	5/5	438	417-515	5/5	445	402-492	5/5	424	397-432	5/5
12	485	442-512	5/5	501	408-544	5/5	518	479-552	5/5	481	428-518	5/5	448	419-495	5/5	429	398-456	5/5
13	466	428-495	5/5	478	396-523	5/5	500	442-548	5/5	457	401-500	5/5	431	402-478	5/5	427	397-448	5/5
FEMALES:																		
-1	163	156-174	5/5	163	158-171	5/5	160	151-172	5/5	166	156-179	5/5	164	156-172	5/5	161	154-174	5/5
0	174	166-187	5/5	174	168-185	5/5	177	167-186	5/5	180	172-191	5/5	182	174-194	5/5	179	173-191	5/5
1	202	193-215	5/5	205	198-220	5/5	212	203-229	5/5	212	198-234	5/5	209	200-216	5/5	205	194-224	5/5
2	218	195-228	5/5	217	203-230	5/5	221	208-239	5/5	228	198-245	5/5	216	207-224	5/5	211	198-230	5/5
3	234	216-246	5/5	226	220-242	5/5	236	229-248	5/5	244	216-260	5/5	232	224-248	5/5	225	218-234	5/5
4	236	220-244	5/5	232	218-260	5/5	241	229-248	5/5	251	240-270	4/5	238	218-248	4/5	228	218-236	5/5
5	242	226-265	5/5	251	224-274	5/5	256	241-268	5/5	260	236-275	4/5	247	240-272	4/5	235	218-258	5/5
6	248	228-270	5/5	258	244-284	5/5	264	248-288	5/5	281	248-294	4/5	252	232-264	4/5	252	232-280	5/5
7	265	239-282	5/5	264	232-291	5/5	263	242-282	5/5	271	255-299	4/5	256	238-282	4/5	261	234-284	5/5
8	262	236-291	5/5	263	241-300	5/5	274	254-295	5/5	285	248-301	4/5	262	234-288	4/5	261	241-290	5/5
9	268	234-296	5/5	268	246-319	5/5	282	246-306	5/5	293	238-320	4/5	264	236-288	4/5	268	232-294	5/5
10	274	233-298	5/5	272	253-311	5/5	291	246-324	5/5	305	238-340	4/5	268	263-331	4/5	262	248-298	5/5
11	275	248-311	5/5	278	258-327	5/5	290	232-299	5/5	305	246-336	4/5	275	238-290	4/5	280	242-300	5/5
12	277	246-300	5/5	279	253-326	5/5	298	248-310	5/5	306	250-336	4/5	278	234-295	4/5	276	234-300	5/5
13	275	234-291	5/5	268	232-308	5/5	288	251-305	5/5	287	250-308	4/5	271	254-292	4/5	275	232-296	5/5

*Significantly lower than the control group at $p < 0.05$.

000016

Fluoxetine Fluoxamoxetine Fluvoxamine - Human Day Substance Use - [illegible]

TABLE 3: Individual Weekly Dose Weights, Years

Group, Age Arm	Sex	Baseline		1	2	3	4	5	6	7	8	9	10	11	12	13
Control																
71332	M	244	276	316	340	340	374	418	440	438	414	470	434	392	324	344
71333	M	226	240	296	318	314	364	394	410	420	434	410	334	407	492	474
71334	M	213	283	314	280	400	430	414	431	447	432	470	392	338	342	428
71335	M	221	291	322	377	406	400	424	430	430	442	440	364	362	386	424
71336	M	224	244	232	218	240	340	364	378	384	412	426	418	377	332	420
71337	F	247	277	312	336	340	390	384	380	378	374	390	364	337	358	342
71338	F	234	267	296	324	326	314	354	364	337	318	370	380	374	374	364
71339	F	174	247	324	330	332	332	354	354	358	374	398	412	384	384	382
71340	F	164	173	210	233	246	344	358	372	392	384	384	334	386	334	332
71341	F	134	166	191	193	214	220	226	214	217	234	232	232	234	234	234
Placebo																
71342	M	256	273	313	317	366	380	390	414	420	447	434	413	484	445	433
71343	M	333	430	394	410	428	332	368	384	386	371	376	372	392	408	406
71344	M	251	383	414	371	404	406	434	462	432	499	500	502	512	522	494
71345	M	245	280	328	352	394	388	420	432	436	494	474	344	324	342	322
71346	M	239	268	320	334	394	398	434	466	468	410	410	422	364	344	414
71347	F	159	170	200	212	220	220	224	234	237	247	232	242	234	234	242
71348	F	171	133	207	227	244	234	260	258	252	271	278	274	272	282	290
71349	F	161	180	220	230	232	260	276	294	291	301	310	314	322	336	364
71350	F	158	167	202	207	234	226	243	254	232	251	246	232	242	240	237
71351	F	162	168	190	208	224	218	248	247	234	234	260	257	272	272	276
10 mg																
71352	M	247	297	316	362	408	380	442	424	446	321	339	334	338	334	324
71353	M	239	262	304	337	364	360	342	418	414	432	430	392	372	372	392
71354	M	257	280	343	380	410	402	380	476	431	399	314	333	344	332	343
71355	M	230	269	314	333	372	384	412	440	430	449	462	482	484	374	374
71356	M	229	261	333	364	370	380	405	432	412	460	470	484	474	357	344
71357	F	160	179	204	230	240	230	234	234	238	268	272	272	284	284	292
71358	F	171	171	230	234	290	260	278	284	284	294	300	324	292	324	314
71359	F	172	186	220	226	244	244	264	276	270	293	294	291	318	330	314
71360	F	151	163	201	213	230	216	233	242	236	242	250	248	336	334	314
71361	F	162	178	210	208	226	230	241	258	242	237	260	266	272	264	274