Commercial
Chemicals Division/3M

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

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EPA
INFO. CONTROL BRANCH
1982 MAR 24 PM 3:36

CS 88 (21)
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March 22, 1982

3M

8EHQ-0382-0373/374S Supplement
88-810000364

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 ✓
0681TB0212	Rabbit oral alcohol ✓
0681TB0398	Rabbit oral FC-143 ✓
0681TR0110	Rat oral FC-143 (NOT HERE)

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	B0212	2999	
0381-0394	B0398	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:

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

000002

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
90 Day Oral Monkey - 090	143
90 Day Oral Rat - 089	143
90 Day Oral Monkey - 088	3422
90 Day Oral Rat - 086	3422
90 Day Oral Monkey - 092	95
90 Day Oral Rat - 085	95

P(e) 394

P(e) [redacted]

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:rmk

Enclosure

CS SAS
3/24/82

24

International Research and Development Corporation

8EHQ-0382-0373/374 Supp.
88-8100364

SPONSOR: 3M Company

COMPOUND: FM-3422

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. Geil, D.V.M., Vice President and Director of Pathology
N. D. Jefferson, B.A., Acting Director of Small Animal Toxicology
F. A. Ruecker, D.V.M., M.S., Staff Pathologist

Date: November 10, 1978

137-086

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ANALYSIS OF FM-3422 IN SERUM AND LIVER OF RATS FROM I.R.D.C. STUDY

DOSE	SURVIVAL		PPM FM-3422 IN SERUM		PPM R_F IN SERUM		UG FM-3422 IN LIVER		UG R_F IN LIVER	
PPM	M	F	M	F	M	F	M	F	M	F
0	5/5	5/5	0	0	0.6	-	<0.2	<0.2	10	5
100	5/5	5/5	<0.1	<0.1	100	120	14	7	12000	4800
300	5/5**	3/5	0.1	<0.3	285*	335	10	6	25000	12000
1000	0/5	0/5	-	-	-	-	-	-	-	-

* $C_8F_{17}SO_3^-$ IDENTIFIED BY F NMR

** 22% WEIGHT DECREASE. LIVER CHANGES AT 300 PPM & ABOVE.

D-B-0-6

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I. SYNOPSIS

FM-3422 was fed in the diet at levels of 30, 100, 300, 1,000, 3,000 and 10,000 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 consumptions were recorded weekly. Hematological, biochemical and urinalysis studies were conducted during the pretest period and at 1 and 3 months of study.

All rats at the 1,000-, 3,000- and 10,000-ppm dosage levels died between days 9 and 29 of the study. Most of these rats exhibited one or more of the following signs of overt toxicity including: emaciation, altered posture, convulsions, reduced motor activity and/or increased sensitivity. Time-to-onset of signs of toxicity and length of life span decreased as the dosage level was increased.

At the 30-ppm dosage level, neither changes in appearance nor deaths were noted for either male or female rats. Females had a slightly depressed weight gain when compared to the controls. With the following exceptions, laboratory test values were within the expected range: at 1 month, γ-glutamyl transpeptidase (γ-GTP) levels were slightly elevated for two males; at 3 months, γ-GTP values were elevated for three males. One male rat with elevated γ-GTP (1 month) had a normal γ-GTP value at 3 months but slightly elevated plasma glutamic pyruvic and oxalacetic transaminase (PGPT and PGOT) values. Two male rats at the 30-ppm dosage level also showed slightly elevated blood urea nitrogen (BUN) values at 1 month of study.

At the 100-ppm dosage level, only one rat (a female) manifested any behavioral or appearance change (excessive salivation, week 4). A reduction in body weight gain occurred with both sexes. Food consumption was depressed for the females. Laboratory values were within the expected range.

At the 300-ppm dosage level, an increased incidence of possibly compound-related signs was noted. Two females died after the collection of blood for clinicopathology. One male and one female had an accumulation of material around the eye(s) and/or nose, and one female had a dilated pupil. Group mean body weight and food consumption levels were significantly lower ($p<0.01$) for both males and females. At 3 months of study, erythrocyte counts, hemoglobin and hematocrit values for males and females at the 300-ppm dosage level were slightly lower when compared to the control values (statistically significant). Biochemical values showed some changes for alkaline phosphatase, PGPT, PGOT, Y-GTP and for BUN values for one or more rats of each sex at each sampling period.

Compound-related gross liver lesions consisting of enlargement, accentuated lobulations, brown discoloration and gray/yellow/white areas of discoloration were observed in the 300-ppm group and to a lesser extent in the 100-, 1,000- and 3,000-ppm groups. Brown kidney discoloration was observed in the 300-ppm group, and stomach hyperemia/congestion and red/brown foci/hemorrhages were noted in some rats from the 1,000-, 3,000- and 10,000-ppm groups. A statistically significant liver weight increase in 100- and 300-ppm rats was considered compound-related. Compound-related, microscopic, liver lesions consisting of hepatocellular hypertrophy and hyperplasia, hepatocellular vacuolation, hepatocellular necrosis and hepatocellular and Kupffer cell accumulation of brown pigment were observed to varying degrees in all treated groups. Compound-related, microscopic, kidney lesions consisting of tubular nephrosis, tubular proteinaceous casts, and intracellular accumulations of brown pigment and reddish brown hyaline droplets in tubular epithelial cells were observed in some rats from the 300-ppm group.

II. COMPOUND

The compound was received from 3M Company, Saint Paul, Minnesota on October 28, 1977 as indicated below:

Label	Description
FM-03422 41-2700-3422-0 Lot 784/ Net-35 DR-1	off-white to tan waxy substance

III. CLINICAL STUDIES

A. METHOD:

1. General Procedure:

Thirty-five male (240-306 g) and 35 female (180-226 g) 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 on 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 4, 1977 with Groups I-VI (control, 100-, 300-, 1,000-, 3,000- and 10,000-ppm dosage levels). Group VII (30 ppm) was initiated on November 25, 1977 in accordance with an approved protocol modification. Surviving rats from Groups I-VI were sacrificed on February 2, 1978. The study was terminated by sacrifice of the Group VII rats on February 23, 1978.

2. Compound Administration:

The compound was mixed weekly with ground Purina® Laboratory Chow® (i.e., ground basal diet) to provide dosage levels of 30, 100, 300, 1,000, 3,000 and 10,000 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. Diet samples (100 grams each) were taken immediately after preparation of each diet 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: an appropriate amount of FM-3422 was dissolved in 15 ml of ACS grade acetone. The resulting

solution was thoroughly mixed with 500 g of Purina® Laboratory Chow® in a Hobart blender to produce the premix. To provide the proper dosage levels, appropriate quantities of the premix were mixed with the ground basal diet using a twin-shell blender equipped with an intensifier bar. The diets were prepared weekly.

3. Observations:

The rats were observed twice daily for overt signs of toxicity and for mortality. Detailed observations normally were recorded weekly; daily observations were recorded whenever a specific abnormal condition existed. Individual body weights and food consumptions 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 overnight urine samples were obtained for analysis from all surviving rats. Food and water were withheld during 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 activity (PGOT)[5], plasma alkaline phosphatase activity[5], γ-glutamyl transpeptidase (γ-GTP)[6], creatinine phosphokinase[7] and calcium[8]. Alkaline phosphatase values from the pretest (baseline) period were not measured because of interference by the anticoagulant.

c. Urinalysis:

Urinalysis included description of color and appearance; measurement of volume, pH[9] and specific gravity; qualitative tests for

protein[9], glucose[9], ketones[9], bilirubin[9] and occult blood[9]; 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 weight (at 13 weeks), food consumption (weeks 1-13), hematological, biochemical and urinalysis parameters at 1 and 3 months, and absolute and relative terminal 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:

At the 30- and 100-ppm dosage levels, one male (30 ppm) had an accumulation of red material around the right eye from week 11 to week 13; one female (100 ppm) exhibited excessive salivation and rales during week 4. No other rats at these dosage levels manifested any signs of toxicity.

At 300-ppm dosage level, an increased frequency of possibly compound-related signs were noted. Two females died shortly after the collection of blood (one at 1 month; one at 3 months) without any signs of overt toxicity having been noted. One male had an accumulation of red material around the left eye and nose. One female rat had an accumulation of red material around the right eye and another female had a dilated right pupil (weeks 5 and 6).

All rats at the 1,000-, 3,000- and 10,000-ppm dosage levels died prior to the scheduled sacrifice. Most deaths occurred following manifestation of one or more of the following signs of overt toxicity: emaciation, altered posture (hunched back), reduced motor activity, convulsions following handling, increased sensitivity to outside stimuli, accumulation of red material (right eye and mouth or nose), accumulation of yellow material (anogenital region), dilation of the right pupil and excessive salivation and rales. The incidence of these findings was as follows:

Observation	No. of Rats/Observation/Dosage Level					
	Control	30 ppm	100 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Emaciation					6	10	10
Alt. posture					4	8	10
Red. motor act.					1	1	7
Convulsions					3	5	
Inc. sensitivity					2		
Red material (eyes)	1	1		3		1	
Red material (nose, mouth)				1	2	2	1
Yellow material					2		2
Pupillary dilation				1			
Excessive salivation			1				

Survival after 3 months of compound consumption was as follows:

Treatment Group	No. Surviving/No. Initiated	
	MALE	FEMALE
Control	5/5	5/5
30 ppm	5/5	5/5
100 ppm	5/5	5/5
300 ppm	5/5	3/5
1,000 ppm	0/5	0/5
3,000 ppm	0/5	0/5
10,000 ppm	0/5	0/5

2. Body Weights (Tables 1-2):

Changes in body weight were similar for male rats at the 30-ppm and 100-ppm dosage levels when compared with control male rats. Female rats at the 30-ppm dosage level showed slightly lower gains in body weights and female rats at the 100-ppm dosage level showed moderately lower gains in body weight when compared with control female rats. Male and female rats at the 300-ppm dosage level showed markedly lower gains in body weight. The group mean body weights at 13 weeks of study were significantly lower for the females ($p<0.05$) at the 100-ppm dosage level and for male and female rats ($p<0.01$) at the 300-ppm dosage level when compared with control rats. Rats at the 1,000-, 3,000- and 10,000-ppm dosage level showed moderate to marked losses of body weight prior to death.

The group mean body weights (and the percent difference from the control group) at 13 weeks of study were as follows:

Treatment Group	Group Mean Body Weights, g (% difference from Control) MALE	FEMALE
Control	501	286
30 ppm	491 (- 2.0)	269 (- 5.9)
100 ppm	482 (- 3.8)	248 (-13.3)
300 ppm	392 (-21.8)	227 (-20.6)

3. Food Consumption (Tables 3-4):

Group mean average for food consumption consistently declined as the dosage level was increased. Mean differences for the 300 ppm group (males and females) when compared with the control were statistically significant at $p<0.01$.

Treatment Group	Average Food Consumption (grams/rat/day) MALE	FEMALE
Control	27.6	19.9
30 ppm	27.2	19.3
100 ppm	27.4	18.6
300 ppm	24.2	15.1

4. Laboratory Tests (Tables 5-16):

a. Hematology:

Hematological values for rats at the 30- and 100-ppm dosage levels at 1 and 3 months of study and for rats at the 300-ppm dosage level at 1 month of study were within the expected range. The 3-month erythrocyte counts, hemoglobin and hematocrit values for male and female rats at the 300-ppm dosage level were slightly lower (statistically significant) when compared with the control values. All other hematologic values, at 3 months of study, for rats at the 300-ppm dosage level were within the expected range.

b. Biochemistry:

With the exceptions of the following findings, all biochemical values were within the expected range.

At the 30-ppm dosage level (1-month sampling period), two male rats had slightly elevated γ-GTP levels (9 units/ml/rat). At the 3-month sampling, one of these γ-GTP levels was still elevated and two other males had elevated γ-GTP levels (10 units/ml/rat). The group mean γ-GTP level for male rats at the 30-ppm dosage level was significantly lower ($p<0.05$) when compared with the control group mean. One male rat (3-month sampling) had a slightly elevated PGPT (154 units/ml) and PGOT (299 units/ml); this rat had had a slightly elevated γ-GTP level at 1 month which had declined by 3 months. Two male rats at the 30-ppm dosage level also showed slightly elevated blood urea nitrogen values at 1 month of study.

At 100-ppm dosage level all biochemical values were within the expected range and showed no changes that could be attributed to the compound.

At the 300-ppm dosage level (1 month) plasma alkaline phosphatase levels were elevated for all females (statistically significant at $p<0.01$ when the group mean was compared with the control

group mean). The group range for these values was 210 to 291 units/ml. One of these females also had an elevated PGOT (480 units/ml) value and an elevated PGPT (360 units/ml) value. Two male rats (at 1 month) had slightly elevated Y-GTP values (13 and 16 units/ml). At 3 months, three males and one female had elevated plasma alkaline phosphatase levels (227-281 units/ml). The group mean difference for these males was statistically significant when compared with the control mean. One male rat had a slightly elevated PGPT reading of 204 units/ml at 3 months. At 1 month of study, two female rats at the 300-ppm dosage level showed a slight and moderate increase in BUN. At 3 months of study, the BUN values for male rats at the 300-ppm dosage level generally were slightly higher than those of control male rats. The group mean blood urea nitrogen values for male rats at the 300-ppm dosage level was significantly higher ($p<0.01$) than the control male rats at 3 months of study.

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 rats were sacrificed by CO_2 inhalation and necropsied. The spleen, liver, kidneys and brain were weighed and representative tissues were collected in buffered neutral 10% formalin.* The adrenals, thyroid/parathyroid and pituitary were weighed after fixation. Liver samples, obtained from all of the rats at terminal sacrifice, were frozen and subsequently shipped to the sponsor.

Rats which died during the study period were necropsied as above with the exception that organ weights were not taken.

2. Histopathology:

Microscopic examination of fixed hematoxylin-eosin stained paraffin sections was performed for all rats in the control and 30-, 100-, 300-ppm dosage levels. The following tissues were examined:

- adrenals
- aorta
- bone
- brain (with segment of cervical cord attached)
- eyes
- heart (with coronary vessels)
- duodenum
- ileum
- jejunum
- colon
- kidneys
- liver
- lung
- thymus
- mammary gland
- nerve (with muscle)
- spleen
- pancreas
- prostate/uterus
- bone marrow (sternum)
- salivary gland
- spinal cord (lumbar)
- pituitary
- stomach
- testes/ovaries
- thyroid
- parathyroid
- urinary bladder
- mesenteric lymph node

and any other tissue with abnormalities.

In addition, livers from rats in the 1,000-, 3,000- and 10,000-ppm dosage levels were processed and microscopically examined.

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*Eyes fixed in Russell's fixative.

B. RESULTS:

1. Gross Pathology (Table 17) and Organ Weights (Tables 18-19):

Compound-induced, gross, liver lesions were observed in all 300-ppm rats which survived to terminal sacrifice. The lesions occurred singly or in combination and consisted of liver enlargement, accentuated lobulations, diffuse brown discoloration and gray/yellow/white areas of discoloration. Male rats appeared to be more severely affected. Similar, but less severe, liver lesions were observed in some rats from the 100-, 1,000- and 3,000-ppm groups. Additional compound-related gross lesions were observed in the stomachs (hyperemia/congestion, red/brown foci/hemorrhage) of some rats in the 1,000-, 3,000- and 10,000-ppm groups and kidneys (brown discoloration) of some rats from the 300-ppm group.

Statistically significant increases in absolute and relative liver weights in the 100- and 300-ppm groups were considered compound related with male rats more severely affected:

Organ	Dosage Level (ppm)	Sex	Weight	Change	p<
Liver	100	M	absolute, relative	increase	0.01, 0.01
	300	M	absolute, relative	increase	0.01, 0.01
		F	relative	increase	0.05

Other statistically significant organ weight variations observed in the 100- and 300-ppm groups were not accompanied by morphologic change and the biological significance of the variations is not known:

Organ	Dosage Level (ppm)	Sex	Weight	Change	p<
Kidneys	100	M	relative	increase	0.05
Brain	300	M	relative	increase	0.05
		F	relative	increase	0.05
Thyroid/Parathyroid	300	M	relative	increase	0.01
Pituitary	300	M	relative	increase	0.05

2. Histopathology (Tables 20-21):

Compound-induced microscopic alterations were observed in the livers from all treated groups and consisted of a very slight to marked enlargement (hypertrophy) of hepatocytes in the 30-, 100-, 300- and 1,000-ppm groups and increased numbers of hepatocytes (hyperplasia) in the 3,000- and 10,000-ppm groups. These alterations were centrilobular to panlobular in distribution with more liver involved as the dosage level increased.

A very slight-to-moderate increase in cytoplasmic vacuoles was observed in the livers of the 100- and 300-ppm groups. The vacuoles contained lipid (as verified by staining with oil red O) and were situated in a centrilobular to midzonal location.

Focal to multifocal coagulative hepatocellular necrosis of very slight-to-marked severity was observed in the 300-, 1,000-, 3,000- and 10,000-ppm groups. The lesion appeared to be primarily centrilobular to midzonal in location and there was a minimal amount of associated inflammatory infiltrate.

Increased intracytoplasmic accumulations of brown pigments in hepatocytes and sinusoidal macrophages (Kupffer cells) were observed primarily in livers from the 300-ppm group. Selected examples were stained for iron content using the Gomori's iron stain. Pigment in the Kupffer cells stained positive for iron while hepatocellular pigment was negative for iron.

Kidney lesions were observed in most rats of the 300-ppm group and consisted of a very slight-to-marked tubular nephrosis with associated proteinaceous cast formation and intracellular accumulation of brown pigment and reddish-brown hyaline droplets (protein) in tubular epithelial cells. The brown pigment was positive for iron as verified by the Gomori's iron stain. Mineralization of luminal tubular debris was observed in some rats. Whether the lesions developed as a result of a direct toxic effect of the compound on the renal parenchyma or indirectly

as a result of compound-effect on the liver with subsequent overload of the kidneys with bile and/or products of red blood cell breakdown could not be determined from histological examination.

Lesions observed in other tissues were not considered compound-related but were interpreted as spontaneous in origin.

References

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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. Sigma GGTP Procedure Bulletin #545. Sigma Chemical Co., St. Louis, Missouri.

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

8. Photovolt PV4, 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.

CS
SDS
3/24/82

International Research and Development Corporation

SPONSOR: 3M Company

COMPOUND: FM-3422

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
T. M. Saulog, D.V.M., M.S., Staff Pathologist

Date: January 16, 1979

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I. SYNOPSIS

FM-3422 was administered to rhesus monkeys by gavage at dosage levels of 1, 3, 10 or 30 mg/kg/day. A control group received the vehicle, propylene glycol. Two male and two female monkeys were initiated at each dosage level and also in the control group. The monkeys were observed twice daily for general physical appearance, behavior and pharmacotoxic signs. Body weights were recorded weekly. Hematological, biochemical and urinalysis studies were conducted once in the control period and at 1 and 3 months of study.

One monkey treated with 30 mg/kg/day died during the last week of the study.

On and after the second day of study, all the monkeys, including the control group showed anorexia, slight to marked decrease in activity and ataxia. The ataxia disappeared from all the monkeys directly after the amount of propylene glycol used was reduced from 5 to 2 ml/kg on study day 3. Occasional emesis and diarrhea (sometimes severe) were observed. Several weeks after the initiation of the study black stool, mucus and bloody mucus in the stool were observed in most of the monkeys from each group. For two monkeys receiving 30 mg/kg/day the above mentioned signs of toxicity persisted for almost all the treatment period. One monkey at the 30 mg/kg/day dosage level died during the last week of the study.

Most of the monkeys, including those in the control group lost body weight early in the study. At 12 weeks of study all the monkeys were at or near the original body weight except for the groups receiving 10 and 30 mg/kg/day. At the end of the study the differences in the mean body weights of the treated and control groups were not statistically significant.

Microscopically, the adrenals from one male and two female rhesus monkeys at the 30 mg/kg/day dosage level had compound-related slight to severe lipid depletion. The pancreas from the two female monkeys at the 30 mg/kg/day dosage level had compound-related moderate atrophy of exocrine cells. None of the tissues from the other monkeys had lesions that would be considered compound-related.

Statistically significant variations in sex group mean weights of liver and brain 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 28, 1977 as indicated below:

Label	Description
FM-3422 41-2700-3422-0 Lot 784/Net-35 DR-1	off-white to tan waxy substance

III. CLINICAL STUDIES

A. METHOD:

1. General Procedure:

Ten male (weighing from 3.00 to 4.35 kg) and 10 female (weighing from 3.05 to 4.20 kg) rhesus monkeys were initiated into this study. The monkeys were purchased from Primate Imports Corporation, Port Washington, New York on October 25, 1977. 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 (14 weeks), the monkeys were tattooed on the inner surface of the thigh and intrapalpebral tuberculin tests were conducted. Tuberculin tests also were conducted at bimonthly intervals during the treatment period. Complete physical examinations were conducted by the staff veterinarian prior to initiation of the compound administration and only monkeys in good health were selected for use.

This study was initiated on February 9, 1978. Terminal sacrifices were conducted on May 10, 1978.

2. Compound Administration:

At the end of the conditioning period the monkeys were divided into 5 groups on a random basis, so that the average body weights were similar:

Group	Number of Monkeys		Dosage Level
	Male	Female	
I	2	2	Control
II	2	2	1 mg/kg/day
III	2	2	3 mg/kg/day
IV	2	2	10 mg/kg/day
V	2	2	30 mg/kg/day

The test compound was dissolved in propylene glycol by heating to approximately 30° C. The solution was administered by gavage. For study day 1 and 2 all of the treated monkeys received 5 ml/kg of the solution. On and after study day 3 the amount of propylene glycol was reduced to 2 ml/kg. The control group received 5- and then 2-ml/kg of propylene glycol on the same regimen as the treated groups.

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. Food consumption was estimated.

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 (APTT)[7]. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated.

b. Biochemistry:

Biochemical studies included: blood glucose[8], blood urea nitrogen[8], serum alkaline phosphatase[8], serum glutamic oxaloacetic transaminase (SGOT)[8], serum glutamic pyruvic transaminase (SGPT)[8],

cholesterol[9], total protein[9], albumin[8], sodium[10], potassium[10], chloride[9], inorganic phosphate[9], γ-glutamyl transpeptidase[11], creatinine phosphokinase[12]. Sorbitol dehydrogenase was not determined because reliable data could not be obtained with the present instrumentation.

c. Urinalyses:

Urinalyses included: measurement of volume, pH[13] and specific gravity; description of color and appearance; qualitative tests for protein[13], glucose[13], ketones[13], occult blood[13]; and microscopic examination of the sediment.

d. Statistical Analysis:

Analysis of terminal body weights and organ weights (absolute and relative) and clinical laboratory tests were performed using analysis of variance (one-way classification), Bartlett's test for homogeneity of variances and the appropriate t-test (for equal and unequal variances) as described by Steel and Torrie using Dunnett's multiple comparison table to judge significance of differences.

B. RESULTS:

1. General Behavior, Appearance and Survival:

Monkey 7490 receiving 30 mg/kg/day died during the last week of the study. The death was preceded by a marked loss of body weight (1.25 kg) with a marked decrease in activity and ataxia.

Except for one monkey receiving 3 mg/kg/day, ataxia was observed for all the monkeys in the control and treated groups beginning at day 2. For most of the monkeys the ataxia was slight but for a few the ataxia was moderate or marked. With the reduction in the amount of propylene glycol use the ataxia disappeared usually before the end of the first week.

In addition to the ataxia the monkeys in the control and treated groups had anorexia, slight to moderate decreased activity appearing on study day 2. Occasional emesis also was observed.

For the monkeys in the control group, diarrhea was occasionally observed but for Monkey 7470, the diarrhea was chronic at the end of the study. After the second week of study, bloody mucus in the stool and black stool were noted. Except for the chronic diarrhea, the observations for the monkeys receiving 1 mg/kg/day were the same as that of the controls.

At the 3 mg/kg/day dosage level Monkey 7487 was observed to have black stool and mucus in the stool during weeks 11 and 13. Frequently, severe diarrhea was noted for Monkey 7474 during week 12. For the monkeys at the 10 mg/kg/day dosage level, occasional diarrhea was observed.

Of the monkeys receiving 30 mg/kg/day, 7499 had bloody mucus in the stool, emesis and severe diarrhea from the third day of the study. Severe diarrhea was observed for Monkeys 7472 and 7490. In addition for Monkey 7490 mucus in the stool, bloody mucus and blood in the stool also was noted. For Monkeys 7499 and 7490 the signs of toxicity persisted for most of the treatment period. Monkey 7490 that died in the last week of the study showed marked decrease in activity and marked ataxia on the day prior to death.

2. Body Weights (Table 1):

During the first month of study, all the monkeys lost weight including those in the control group. The percentage change in mean body weight, at monthly intervals were:

Study Weeks	Group, dose in mg/kg/day				
	0	1	3	10	30
0- 4	-3.94	-0.29	-0.29	-7.04	-7.32
4- 8	+1.16	+3.25	+4.57	+1.82	-3.04
8-12	+3.76	+2.87	+1.09	+0.60	-3.98

For the group receiving 30 mg/kg/day the low mean body weight resulted mainly from the 1.25 kg loss in body weight for Monkey 7490,

which died. However, there were no statistically significant differences between the body weights of the treated and control monkeys.

3. Laboratory Tests (Tables 2-10):

After the first month of treatment no unusual changes were observed for the blood and urine analyses. For Monkey 7491, receiving 10 mg/kg/day, there was a slight decrease in hematocrit, hemoglobin concentration and erythrocyte count at 1 month of study as compared to the pretreatment period. This decrease was not found at 3 months of study.

For most of the monkeys at 3 months of study, the blood glucose values were in the lower range of expected values. Also at 3 months of study, low cholesterol values (56 and 67 mg/100 ml) were found for two monkeys receiving 30 mg/kg/day, and for one monkey receiving 10 mg/kg/day (84 mg/100 ml). For Monkey 7477 which had a cholesterol value of 67 mg/100 ml, a low potassium value (3.5 meq/liter) was found also.

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.

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

and any other tissue(s) with lesions.

*Phencyclidine HCl - BioCeutic Laboratories, Inc., St. Joseph, Missouri.

B. RESULTS:

1. Gross Pathology (Table 11) and Organ Weights (Table 12):

No gross lesions considered compound-related were seen in monkeys sacrificed after 90 days of study.

Statistically significant variations in sex group mean weights of liver and brain occurred at the 10 and 30 mg/kg/day dosage levels as follows:

Organ	Dosage Level (mg/kg/day)	Sex	Weight	Change	p<
Liver	10	M	relative	increase	0.05
	30	M	absolute, relative	increase, increase	0.05, 0.01
Brain	10	M	absolute	decrease	0.01
	30	M	absolute	decrease	0.05

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

2. Histopathology (Table 13):

One male and two female monkeys at the 30 mg/kg/day dosage level, sacrificed after 90 days of study, had slight to severe lipid depletion in the adrenal glands. The two female monkeys at the 30 mg/kg/day dosage level had moderate atrophy of pancreatic exocrine cells. The lesion was characterized by decreased cell size with loss of zymogen granules. One of the two pancreas also had several eosinophilic intranuclear inclusions in exocrine cells. These microscopic findings were considered compound-related.

No microscopic changes which were considered compound-related were seen in the adrenal glands of male or female monkeys at the 1, 3 or 10 mg/kg/day levels. No microscopic changes which were considered compound-related were seen in the pancreas of male monkeys at the 30 mg/kg/day level or in male or female monkeys at the 1, 3 or 10 mg/kg/day levels. No microscopic lesions in tissues other than the adrenal glands and pancreas of monkeys at the 30 mg/kg/day levels were considered compound-related.

References

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12. Creatinine phosphokinase - Sigma Procedure Bulletin 520.

FM-3422: Ninety Day Subacute Rhesus Monkey Toxicity Study.

TABLE 1. Individual and Group Mean Body Weights, Kilograms.

Group, Monkey Number	Sex	Control		Week of Study												
		-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Control:																
7470	M	4.40	4.35	4.40	4.35	4.30	4.20	4.00	3.90	3.80	3.80	3.80	3.75	3.80	4.05	3.55*
7479	M	3.10	3.00	3.25	3.15	3.15	3.05	3.15	3.20	3.25	3.30	3.20	3.20	3.25	3.35	3.10*
7493	F	3.75	3.80	3.70	3.70	3.60	3.55	3.60	3.60	3.70	3.70	3.60	3.80	3.60	3.85	3.55*
7495	F	3.00	3.05	3.00	3.00	2.85	2.85	2.90	3.00	3.05	3.00	2.90	2.95	3.00	3.05	2.75*
Mean		3.56	3.55	3.59	3.55	3.48	3.41	3.41	3.43	3.45	3.45	3.38	3.43	3.41	3.58	3.24
1 mg/kg/day:																
7467	M	3.85	4.00	3.75	3.95	3.75	3.85	3.85	3.95	3.95	4.00	3.95	4.00	4.05	4.10	3.85*
7473	M	3.25	3.20	3.30	3.25	3.10	3.10	3.10	3.25	3.20	3.15	3.10	3.10	3.25	3.60	2.90*
7489	F	3.05	3.05	2.95	3.00	2.90	3.00	3.05	3.10	3.20	3.15	3.15	3.25	3.20	3.15	3.10*
7492	F	4.15	4.00	3.85	3.75	3.60	3.55	3.55	3.55	3.65	3.65	3.60	3.50	3.60	3.50	3.45*
Mean		3.58	3.56	3.46	3.49	3.34	3.38	3.39	3.46	3.50	3.49	3.45	3.46	3.53	3.59	3.33
3 mg/kg/day:																
7469	M	4.10	4.00	4.05	4.00	3.95	4.00	4.05	4.00	4.10	4.10	4.00	4.15	4.15	4.20	4.10*
7474	M	3.45	3.20	3.45	3.55	3.55	3.55	3.60	3.75	3.75	3.75	3.70	3.80	4.00	3.70	3.55*
7487	F	3.60	3.75	3.45	3.45	3.30	3.30	3.40	3.50	3.55	3.50	3.45	3.55	3.55	3.60	3.10*
7497	F	3.05	3.10	3.20	3.30	3.30	3.15	3.20	3.20	3.30	3.30	3.10	3.20	3.10	3.30	3.05*
Mean		3.55	3.51	3.54	3.58	3.53	3.50	3.56	3.61	3.68	3.66	3.56	3.68	3.70	3.70	3.45

*Terminal weight.

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Special Lens Oral Teratology Study of T-2999CoC in Rats

Experiment No.:	0680TR0020
Conducted At:	Safety Evaluation Laboratory
	St. Paul, Minnesota
Dosing Period:	February 9, 1981 through March 26, 1981
Study Director:	

12-22-81
Date

12/22/81
Date

Study Director

12/22/81
Date

000005

Summary

This special rat teratology study was conducted specifically to evaluate rat lenses exposed to T-2999CoC during the period of organogenesis. The objectives of the study were to determine a no-effect dose level of the lens abnormality reported in a previous teratology study and to describe the appearance of the lens abnormality in weanling rats. Groups of time mated female rats were orally dosed with T-2999CoC at 0, 25, 2.5 or 0.25 mg/kg/day on days 6 through 15 of gestation. Approximately one-half of the females were killed on day 20 of gestation while the rest of the females were allowed to litter and raise their pups to weaning.

Since this study was conducted, the lens findings have been found not to be abnormal. The lens findings observed are interpreted as a freehand sectioning artifact of a normal area of lens fiber degeneration. The lens artifact was observed in fetuses of all dose groups, including the control group. The 25 mg/kg/day dose group incidence of the artifact was significantly higher than the control group incidence. The histological appearance of the lens findings were the same in all dose groups including the control group. The differences among the dose group incidences were due to the manner and frequency in which the artifact was created and the limitations inherent in its visualization. T-2999CoC in utero exposed rat fetuses did not have compound-related lens changes.

Weanling rat pups which had been exposed in utero to T-2999CoC did not have lens nucleus abnormalities. The finding of no lens nucleus abnormalities in weanlings supports the conclusion that the lens findings in the rat fetuses were an artifact and not a teratogenic change.

The fetal lens findings observed under the dissecting microscope were artifact. T-2999CoC in utero exposed fetuses and weanlings did not have compound-related lens changes.

Introduction

A purified grade of T-2999CoC[a] was labeled a teratogen of the lens because apparent lens abnormalities were observed at the 75, 37.5 and 25 mg/kg/day dose levels in a rat teratology study (Experiment Number 0680TR0010). The objectives of this T-2999CoC study[b] were to determine a no-effect dose level of the lens abnormality and to describe the appearance of the lens abnormalility in weanling rats. The study was sponsored by the 3M Commercial Chemical Division, St. Paul, Minnesota and was conducted by Laboratory, St. Paul, Minnesota. Six sets of compound administration groups were dosed between February 9 and March 26, 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

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 135 to 242 grams, were then divided into four groups of 72 animals each. The study was conducted in six sets and the body weight data were interpreted in two replicates. 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 20 of gestation for abnormal clinical signs. Body weights were recorded on days 3, 6, 9, 12, 15 and 20 of gestation. The four groups were dosed with T-2999CoC suspended daily in corn oil at 0, 25, 2.5 and 0.25 mg/kg/day. The suspensions were administered daily using a constant dose volume of 5 mg/kg 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-2999CoC analysis was provided by 3M Commercial Chemical Division, St. Paul, Minnesota (Appendix IV).

Approximately one-half of the females were killed on gestational day 20. All of their fetuses were fixed in Bouin's fixative and the heads were freehand sectioned by the Wilson technique. The presence of lens findings were determined using the dissecting microscope. Fetal specimens with visible lens findings were imbedded in paraffin, sectioned at 5-6 microns, stained with hematoxylin and eosin and examined histologically. The remaining females were

[a] (technical grade)
[b] Study Number 0680TR0020)
[c] Purina Laboratory Chow, Ralston Purina Co., St. Louis, MO

0 0 9 4

allowed to litter in plastic shoe box cages bedded with ground corn cobs. On day 21 of lactation the eyes of the weanlings were examined by slit lamp biomicroscopy or indirect ophthalmoscopy. The weanling rats were then killed and their eyes were fixed in 10% buffered formalin, imbedded in paraffin, sectioned at 5-6 microns, stained with hematoxylin and eosin and evaluated histologically.

Results and Discussion

The first objective of this special lens teratology study was to determine a no-effect dose level of the lens abnormality reported in a previous teratology study. Since this study was conducted, the lens findings have been found not to be abnormal. The lens findings observed under the dissecting microscope are interpreted as a freehand sectioning artifact of a normal area of lens fiber degeneration. The fetal rat lens finding was incorrectly interpreted as a teratogenic change in Experiment Number 0680TR0010 and, therefore, the first objective of this study was not valid.

The 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.

Histologically, 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 sectioning accentuating the area of normal primary lens fiber degeneration.

The differences in the appearance of the lens artifact in individual fetuses and differences among the dose group incidences were largely due to the manner and frequency in which the artifact was created and the limitations inherent in its visualization. Statistically significant differences occurred among the incidences of fetuses observed to have the lens artifact. The 25 mg/kg/day dose group incidence was significantly higher than the 0 mg/kg/day dose group incidence (Table 1). The artifact was the same in all dose groups regardless of the morphological appearance described under the dissecting microscope and regardless of the statistical comparisons among the group incidences of the artifact. T-2999CoC in utero exposed fetuses did not have compound-related changes in their lenses.

The second objective of this special lens teratology study was to describe the appearance of the lens abnormality in weanling rats which had been exposed in utero to T-2999CoC. Again, since the fetal lens finding was an artifact, this objective also was not valid. All eyes of the weanling rats were observed by either slit lamp biomicroscopy or indirect ophthalmoscopy. Neither a lens cleft nor a fetal lens nuclear opacity were observed in any of the lenses of any of the weanlings in the control or compound-treated offspring. One high dose weanling did have a lens opacity, but the opacity did not involve the fetal nucleus (Table 2). The crystalline appearance of the centers of the weanling lenses in all dose groups made histologically sectioned and stained specimens unsuitable for evaluation of individual lens fibers. The finding of no lens nucleus abnormalities in weanlings supports the conclusion that the lens finding in the rat fetuses were an artifact and not a teratogenic change.

T-2999CoC was maternally toxic to the pregnant dams of the 25 mg/kg/day dose level in both study replicates. The compound caused the toxicity of reduced mean body weight gain during the dosing interval. The mean weight gained between days 9 and 12 of gestation for the 25 mg/kg/day dose group was significantly lower than the weight gained by the 0 mg/kg/day dose group during that same interval (Table 3).

No compound-related clinical signs were observed in any of the three compound treated groups. Six intubation deaths occurred during the study (Appendix V). One 25 mg/kg/day dose group dam died during parturition.

Table 1

Special Lens Oral Teratology Study of T-2999CoC in Rats
Results of Dissecting Microscopic Observations of
Gestational Day 20 Fetal Lens

Dose Group	Dams	Fetuses Examined	Dams Having Fetuses With Lens Findings N	Dams Having Fetuses With Lens Findings %	Fetuses with Lens Findings N	Fetuses with Lens Findings %
0 mg/g/day	28	292	13	46	41	14
25 mg/g/day	22	225	18	82[a]	55	24[a]
2.5 mg/g/day	27	271	18	67	59	22[a]
0.25 mg/g/day	26	279	14	54	41	16

[a]Significantly higher than the control (Chi-square with Yates correction $p < 0.05$)

Table 2

Special Lens Oral Teratology Study of T-2999CoC in Rats
Results of Ocular Examination Findings of Pups at Day 21 of Lactation

Dose Group	No. of Dams	Total Number of Pups	No Abnorm. Found	Abnorm. Found	Findings
0 mg/kg/day	29	291	288	3	Corneal Opacity Bilateral corneal ulcer with opacity Left eye grossly enlarged - 2 X normal size, lens may be absent
25 mg/kg/day	30	263	262	1	Opaque area of lens - 1 to 3 o'clock
2.5 mg/kg/day	28	266	263	3	Questionable cloudy area of cornea
0.25 mg/kg/day	26	211	209	2	Corneal opacity

8 0 9 8

Table 3

Special Lens Oral Teratology Study of T-7999CoC in Rats
Mean Body Weight Gain (g) of Pregnant Rats
Between Weighings with Standard Deviations

First Replicate

	DAY	6	9	12	15	20
0 mg/kg/day	MEAN	29	24	21	26	70
	STAN. DEV	10.9	7.9	8.0	15.5	29.1
25 mg/kg/day	MEAN	31	21	10[a]	27	70
	STAN. DEV	16.9	11.5	13.9	16.1	24.6
2.5 mg/kg/day	MEAN	33	23	14	30	65
	STAN. DEV	10.0	4.5	13.3	13.7	30.2
0.5 mg/kg/day	MEAN	31	22	15	31	64
	STAN. DEV	9.4	7.4	12.3	17.9	29.9

Second Replicate

	DAY	6	9	12	15	20
0 mg/kg/day	MEAN	30	21	30	25	70
	STAN. DEV	5.1	6.6	6.3	6.9	22.4
25 mg/kg/day	MEAN	29	19	22[a]	23	71
	STAN. DEV	11.8	8.3	6.7	7.1	15.3
2.5 mg/kg/day	MEAN	29	21	26[a]	28	76
	STAN. DEV	5.8	5.7	7.0	7.1	12.4
0.25 mg/kg/day	MEAN	32	20	28	23	68
	STAN. DEV	7.8	6.0	6.7	7.1	26.9

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

Appendix I

TITLE: Protocol for a Special Lens Oral Teratology Study of T-2999CoC[a] in

OBJECTIVE: A purified grade of T-2999CoC was teratogenic to the eye and caused developmental lens abnormalities at 75, 37.5 and 25 mg/kg/day in a rat teratology study (Experiment Number 0680TR0010). The objectives of the study are to determine a no-effect dose level of the lens abnormality and to describe appearance of the lens abnormality in the weanling rats. Th study will be conducted according to the 1978 Good Laborator Practice Regulations and the Safety Evaluation Laboratory's Standard Operating Procedures.

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

TESTING FACILITY:
St. Paul, Minnesota.

STUDY DIRECTOR:

START OF DOSING: February, 1981.

TEST SYSTEM: One hundred and ninety-two sexually mature, time mated Sprague-Dawley derived female rats from Charles River Breedi Laboratories will be housed in hanging stainless steel cages with wire mesh floors and fronts in a temperature and humidi controlled room. This strain of rat will be used because it used in the previous teratology study and time mated females 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-2999CoC[a].

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

[a] (technical grade

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

Dose Group	Dose Level	Group Size
High	25 mg/kg/day	48 ♀
Mid	2.5 mg/kg/day	48 ♀
Low	0.25 mg/kg/day	48 ♀
Control	0 mg/kg/day	48 ♀

The oral route of administration will be used because teratogenicity resulted by this route in the previous teratology study. No dietary contaminants are known to interfere with the test article.

The animals will be observed daily from day 3 through 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 will be dosed accordingly using a constant dose volume of 5 ml/kg of body weight.

Approximately one-half of the females will be killed on day 20 of gestation. All of their pups will be fixed in Bouin's solution and the heads will be free-hand sectioned by the Wilson technique. The presence of eye abnormalities will be determined using the dissecting microscope. Select eye sections can be sent to histopath for microscopid examination. The remaining females will be allowed to litter. On day 21 of lactation the eyes of neonates will be examined to determine the presence of lens abnormalities.

DATA ANALYSIS AND FINAL REPORT: The proposed statistical method to be used for analysis of the data is the Chi-square for percent of abnormalities. The proposed date for the final report is 2-3 months after day 21 of lactation and the pup eye exam has been completed (approximately second quarter, 1981).

Amendment to the Protocol for a Special Lens
Oral Teratology Study of T-2999CoC in Rats

An additional number of rats will be added to this study. The 96 new animals will be divided among the dose groups as follows:

Dose Group	Dose Level	No. of Animals
High	25 mg/kg/day	24
Mid	2.5 mg/kg/day	24
Low	0.25 mg/kg/day	24
Control	0 mg/kg/day	24

The reason for this amendment is a reduced number of pregnant animals in the first two groups of animals.

Appendix II

List of Principal Participating Personnel

NAME	FUNCTION

STATEMENT OF QUALITY ASSURANCE

STUDY NUMBER: 0680TR0020

TITLE: SPECIAL LENS ORAL TERATOLOGY STUDY OF T 2999CoC 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
12 February 1981	12 February 1981
23 February 1981	24 February 1981
18 March 1981	23 March 1981
18 March 1981	24 March 1981
31 March 1981	6 April 1981
25 & 27 April 1981	29 April 1981
21 December 1981	21 December 1981

Compliance Audit
Laboratories, Inc.

12/22/81
Date

APPENDIX IV

Test and/or Control Article Characterization

for

T-2999CoC

1. The identity strength, uniformity, composition, purity or other pertinent characterizations of the test and/or control substances have been determined and documented as of 8 Jan 81 *

2. The method of synthesis or origin of the test and control substances, including their amount and the method of bioassay (if applicable) is documented.

yes X no ____

3. The stability of the test and/or control substances have been determined or will be determined as of Completion of the Study.

The above information and documentation are located in the sponsor's records.

Sponsor — Jan 16, 1981 Date

*CCD Analytical Req. No. 16474 October 28, 1980

Appendix V

Special Lens Oral Teratology Study of T-2999CoC in Rats

Individual Body Weights (g) with Mean Body Weights and Standard Deviations for Pregnant Animals

Replicate 1

DAY		3	6	9	12	15	20
0 MG/KG/DAY							
N1R	1130	185	220	247	275	298	341
N1R	1132	202	221	250	277	308	369
N1R	1133	194	220	246	272	304	382
N1R	1134	230	251	264	291	317	377
N1R	1150	195	215	230	227	232	235
N1R	1151	170	212	242	262	290	337
N1R	1152	208	231	248	262	273	282
N1R	1153	206	226	242	268	295	376
N1R	1154	187	217	240	254	260	276
N1R	1179	192	218	244	269	303	386
N1R	1180	221	249	267	293	327	416
N1R	1181	184	211	226	247	283	345
N1R	1182	224	246	266	289	324	407
N1R	1198	206	226	249	256	261	271
N1R	1199	205	229	255	284	317	392
N1R	1200	200	228	255	278	318	395
N1R	1201	207	225	265	284	318	407
N1R	1202	193	211	226	253	284	359
N1R	1218	197	216	243	264	302	393
N1R	1414	175	226	252	282	309	407
N1R	1415	183	219	233	256	228	339
N1R	1416	201	240	274	298	330	422
N1R	1417	172	205	229	252	262	331
N1R	1433	174	224	245	251	307	403
N1R	1434	174	204	225	249	273	345
N1R	1435	157	160	205	222	262	357
N1R	1436	192	232	268	288	316	413
N1R	1453	178	223	248	275	301	397
N1R	1454	173	211	228	235	266	345
MEAN		193	221	245	266	292	362
STAN. DEV		17.4	16.7	15.9	19.6	27.5	47.9
NON PREGNANT ANIMALS							
N1R	1131	181	208	227	244	240	248
N1R	1170	194	223	240	259	263	273
N1R	1171	195	208	230	245	249	270
N1R	1178	209	235	248	0[a]	0	0
N1R	1219	191	219	242	250	252	266
N1R	1413	184	219	233	232	241	279
N1R	1437	184	225	249	228	251	258

[a] Animal died

CS 4?8
3/26/82

25

International Research and Development Corporation

SPONSOR: 3M Company

COMPOUND: Fluorad® Fluorochemical Surfactant FC-95

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
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J. S. Mehring, Ph.D., Director of Large Animal Toxicology

Date: December 18, 1978

137-092

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I. SYNOPSIS

Fluorad® Fluorochemical Surfactant FC-95 was administered by gavage to rhesus monkeys at dosage levels of 0, (distilled water only) 0.5, 1.5 and 4.5 mg/kg/day for 90 days. Two male and two female monkeys were initiated at each dosage level and also in the control group. The monkeys were observed twice daily for general physical appearance, behavior and pharmacotoxic signs. Body weights were recorded weekly. Hematological and biochemical studies and urinalysis were conducted once in the control period and at the end of the first and third month of the study.

The monkeys treated at the 4.5-mg/kg/day dosage level died or were sacrificed *in extremis* between week 5 and 7 of the study. These monkeys exhibited signs of toxicity in the gastrointestinal tract (anorexia, emesis, black stool and dehydration) from the first or second day of study. All the high-dose monkeys had decreased activity and before death showed marked to severe rigidity, convulsions, generalized body trembling, prostration and loss of body weight. The mean body weight decreased from 3.44 kg at the beginning of the study to 2.70 kg at week 5 of study. At 1 month of study all monkeys at the 4.5-mg/kg/day dosage level had decreased serum cholesterol values and serum alkaline phosphatase activity.

All monkeys at the 1.5-mg/kg/day dosage level survived to the end of the study. The monkeys exhibited slightly decreased activity from the first week of the study which occasionally became moderate to marked. Monkeys at the 1.5-mg/kg/day dosage level occasionally had black stools, diarrhea, mucous in the stool and bloody stool and exhibited dehydration or general body trembling at the end of study. The monkeys from this group had a slight decrease in mean body weight.

In the laboratory tests, there was a decrease in the alkaline phosphatase activity and the concentration of inorganic phosphate in the serum at 3 months of study.

All monkeys at the 0.5-mg/kg/day dosage level survived to the end of the study. Monkeys at this dosage level exhibited an occasional soft stool, diarrhea, anorexia and emesis. Slightly decreased activity was noted in three monkeys at this dosage level. At 3 months of study a slight decrease in the serum alkaline phosphatase activity was noted.

No gross or microscopic pathological lesions which were considered compound-related were seen in tissues other than the adrenals, pancreas, and submandibular salivary glands of male and female rhesus monkeys at the 4.5-mg/kg/day dosage level. Microscopically, the adrenals from male and female monkeys at the 4.5-mg/kg/day dosage level had compound-related marked diffuse lipid depletion; the pancreas from male and female monkeys at the 4.5-mg/kg/day dosage level had compound-related moderate diffuse atrophy of exocrine cells; the submandibular salivary glands from male and female monkeys had compound-related moderate diffuse atrophy of the serous alveolar cells.

No statistically significant variations in sex group mean weights of organs occurred between the control and experimental groups.

II. COMPOUND

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

Label	Description
Fluorad® Fluorochemical Surfactant FC-95 3-M Stock No. 98-0207-0103-7 Lot 640 Net wt. 5 lbs. 2,2 kg.	white powder

III. CLINICAL STUDIES

A. METHOD:

1. General Procedure:

Eight male (weighing from 2.55 to 3.55 kg) and eight female (weighing from 2.70 to 3.75 kg) rhesus monkeys were initiated in this study. The monkeys were purchased from Primate Imports, Port Washington, New York. 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 also were conducted twice during the treatment period. Complete physical examinations were conducted by the staff veterinarian prior to initiation of compound administration. Only monkeys in good health were selected.

This study was initiated on February 16, 1978. Terminal sacrifices were conducted on May 17, 1978.

2. Compound Administration:

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

Dosage Level (mg/kg/day)	Number of Monkeys	
	Male	Female
0 (Control)	2	2
0.5	2	2
1.5	2	2
4.5	2	2

The monkeys in the control group were previously employed as the control group in an aborted 90 day study in monkeys on FC-95 (study 137-087). The test compound suspended in distilled water, was administered 7 days a week by gavage. All doses were given in the same volume of water. This volume of distilled water was given to the 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, behavior and pharmacotoxic signs. Individual body weights were recorded weekly. General physical examinations were conducted in the control period and monthly during the study. Food consumption was estimated.

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] and activated partial thromboplastin time[7] (APTT). Mean corpuscular hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin concentration were calculated.

b. Biochemistry:

Biochemical studies included: the determinations of fasting blood glucose[8], blood urea nitrogen[8], the activities of serum alkaline phosphatase[8], serum glutamic oxalacetic

transaminase[8], and serum glutamic pyruvic transaminase[8], and the concentrations of cholesterol[9], total protein[9], albumin[8], sodium[10], potassium[10], chloride[9], inorganic phosphate[9], as well as the activities of γ-glutamyl transpeptidase[11] and creatinine phosphokinase[12].

c. Urinalysis:

Urinalysis included: measurement of volume, pH[13] and specific gravity; description of color and appearance; qualitative tests for protein[13], glucose[13], ketones[13], occult blood[13] and microscopic examination of the sediment.

d. Statistical Analysis:

All statistical analyses compared the treatment groups with the control group, by sex.

Body weights (week 13), percent change in body weights between control period and 1, 2 and 3 months (sexes combined), hematological, biochemical and urinalysis parameters (months 1 and 3) and absolute and relative organ weights (terminal sacrifice) 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[14] using Dunnett's[15] multiple comparison tables to judge significance of differences.

B. RESULTS:

1. General Behavior, Appearance and Survival:

There was no mortality in the control, 0.5- and 1.5-mg/kg/day dosage levels. Three monkeys at the 4.5-mg/kg/day dosage level died between week 5 and 6 of the study. The fourth monkey from this group was sacrificed in extremis in week 7 of the study.

a. Control:

The monkeys from this group showed an occasional soft stool or diarrhea (slight to severe). For one day Monkey 7358 had

bloody diarrhea, anorexia and slight ataxia and for 3 days Monkey 7368 had anorexia. Emesis was noted rarely.

b. 0.5 mg/kg/day:

Monkeys 7466 and 7504 exhibited occasional diarrhea or soft stools. For Monkeys 7463 and 7483 the soft stools and diarrhea were frequent with occasional appearance of mucus in the stools. On one occasion bloody mucus in the stool and in the refuse pan was observed for Monkey 7483. Occasional anorexia and emesis were noted for Monkeys 7463, 7466 and 7504. Similarly, a slight intermittent decrease in activity was noted for Monkeys 7463, 7483 and 7504.

c. 1.5 mg/kg/day:

All monkeys at this dosage level exhibited a slight decrease in activity during the first week of study. For Monkey 7501 the decreased activity frequently was noted throughout the study and by week 13 had become moderate. Although no emesis, soft stools and diarrhea were noted for this monkey, slight general body trembling and mucus in the stool was observed in week 12, as well as slight dehydration in weeks 12 and 13. For Monkey 7501 and 7500 anorexia became persistent during weeks 12 and 13. For Monkey 7500 diarrhea and soft stool appeared along with mucus in the stool at week 2, black stool at week 8 and slight dehydration in week 12. This monkey exhibited "bruising" around the right eye followed by "bruising" around both eyes at week 12.

For Monkey 7486 occasional soft stool and diarrhea were observed along with anorexia which became persistent in week 10. During weeks 10 and 11 slight dehydration was also noted. Monkey 7462 had occasional soft stool, emesis for 1 day each in weeks 1 and 3 and the decreased activity was observed in week 1 only.

d. 4.5 mg/kg/day:

At 4.5 mg/kg/day the monkeys showed apparent signs of toxicity in the gastrointestinal tract from the first to the second day of the study (anorexia and partial anorexia, emesis, black stool and/or dehydration from slight to moderate). All had decreased activity from slight to severe. Before death they showed marked to severe rigidity, convulsions, moderate generalized body trembling and finally prostration. Monkey 7485 had petechial hemorrhages on the skin of the inguinal area, thorax and face and Monkey 7484 had pale gums after week 3 of the study.

2. Body Weights (Table 1):

Changes in the body weights were similar for monkeys in the control and at the 0.5-mg/kg/day dosage level. The male monkeys in the 1.5-mg/kg/day dosage level had a decrease in their body weight from 3.15 kg at the beginning of the study to 2.93 kg at the end of the study and from 3.22 kg to 2.75 kg for the female monkeys. The change in body weight for this group of monkeys was not statistically significant from the control group.

The monkeys at the 4.5-mg/kg/day dosage level showed a loss of the body weight after 2 weeks of the study. Their average body weight decreased from 3.44 kg at the beginning of the study to 3.23 kg (week 2) - 3.13 kg (week 3) - 2.97 kg (week 4) - 2.70 kg (week 5) for both sexes. At week 6 and 7 of the study they died. There was no statistical evaluation of the body weights for monkeys at the 4.5-mg/kg/day dosage level, because they were not alive at week 13, when the statistics were done.

3. Laboratory Tests (Tables 2-13):

a. One Month of the Study:

At 1 month of study there were no pathological changes in values for monkeys from the control and 0.5-mg/kg/day dosage level.

At the 1.5-mg/kg/day dosage level only Monkey 7501 had a slight decrease in the erythrocytes which was recovered in the third month of study.

Both the male and female monkeys at the 4.5-mg/kg/day dosage level had low values for serum cholesterol which were statistically significance.

Monkey 7484 had a decreased alkaline phosphatase activity (312 unit/l), serum potassium (3.6 meq/liter) and chloride concentration (102 meq/liter) and Monkey 7503 also had low serum chloride concentration (102 meq/liter).

Although the S.G.O.T. activity of male Monkeys 7484 and 7485 was in the highest expected normal range (115 units) the differences between these values and that of the control were not statistically significant.

The only unusual changes in the urinalysis were the appearance of glucose from Monkey 7500 (1.5 mg/kg/day) and from Monkeys 7485 and 7503 (4.5 mg/kg/day). However, these monkeys had no pathological increase of the blood glucose.

b. Three Months of the Study:

There was a statistically significant decrease in the serum alkaline phosphatase activity for the male monkeys at the 0.5-mg/kg/day dosage level and for two Monkeys, 7500 and 7501, at the 1.5-mg/kg/day dosage level. These latter monkeys also had a decreased values of serum potassium concentrations. Monkey 7501 at 1.5-mg/kg/day had very low serum cholesterol and Monkey 7500 at 1.5-mg/kg/day had a slight decrease in the inorganic phosphate (4.2 mg/100 ml) concentration.

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 animals in the control and treatment groups. The following tissues were examined;

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

and any other tissue(s) with lesions

*Phencyclidine HCl - BioCeutic Laboratories, Inc., St. Joseph, Missouri.

B. RESULTS:

1. Gross Pathology (Table 14) and Organ Weights (Table 15):

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

No statistically significent variations in sex group mean weights of organs occurred between the control and experimental groups.

2. Histopathology (Table 16):

All male and female monkeys at the 4.5 mg/kg/day dosage level had marked diffuse lipid depletion in the adrenals. One male and two females at the 4.5 mg/kg/day dosage level had moderate diffuse atrophy of the pancreatic exocrine cells. The lesion consisted of decreased cell size and loss of zymogen granules. Two male and one female monkeys at the 4.5 mg/kg/day dosage level had moderate diffuse atrophy of the serous alveolar cells characterized by decreased cell size and loss of cytoplasmic granules. These microscopic findings were considered compound-related.

No microscopic changes which were considered compound-related were seen in the adrenals, pancreas or submandibular salivary glands in male and female monkeys at the 0.5 and 1.5 mg/kg/day dosage levels. No microscopic lesions in tissues other than the adrenals, pancreas and submandibular salivary glands of male and female monkeys at the 4.5 mg/kg/day dosage level were considered compound-related.

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This report from the International Research and Development Corporation concerning fluorochemical surfactant FC-95 has been removed from "Confidential" and placed in "Non-Confidential" status as of March 18, 1982.

W. Pearlson 3/24/82

W. H. Pearlson
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this report is not confidential per attached matter signed by Dr. Pearlson

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I. SYNOPSIS

Fluorad® Fluorochemical Surfactant FC-95 was fed in the diet at levels of 30, 100, 300, 1,000 and 3,000-ppm to Charles River CD rats for 3 months. Five male and 5 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 consumptions were recorded weekly. Hematological, biochemical and urinalysis studies were conducted during the pretest period and at 1 and 3 months of study.

At dosage levels of 300, 1,000 and 3,000 ppm, all rats died prior to scheduled termination of the study. Overt signs of toxicity including: emaciation, convulsions, altered posture, red material (right eye and/or mouth), yellow material (anogenital region), increased sensitivity to external stimuli and reduced motor activity were evident prior to most deaths. Time of death was directly related to the dosage level with the earliest deaths seen in the 3,000-ppm treatment group.

At the 30-ppm dosage level, neither male nor female rats showed any compound related changes in appearance or behavior. Mean body weights were slightly lower for the rats at the 30-ppm dosage level when compared to the controls. At the 30-ppm dosage level, one female rat showed a slightly elevated glucose level and one male rat showed a slightly elevated alkaline phosphatase valve at 1 month of study. At 3 months of study, one male rat at the 30-ppm dosage level showed slight to moderate elevations in glucose, blood urea nitrogen and γ-glutamyl transpeptidase activity.

At the 100-ppm dosage level at 3 months, body weight means when compared to the controls were lower for males and for females. Food consumption means when compared to the controls were lower (statistically significant) for males and females. At the 100-ppm

dosage level, slight increases in creatinine phosphokinase (CPK) and alkaline phosphatase activity, slight to moderate increases in glucose and blood urea nitrogen, and slight to marked increases in plasma glutamic oxalacetic and pyruvic transaminase activities (PGOT and PGPT) were seen at 1 month of study. At 3 months of study, slight to moderate decreases in hemoglobin, hematocrit and erythrocyte counts were seen for male and female rats and slight to moderate increases in PGOT and PGPT activities were seen for two of the three surviving female rats at the 100-ppm dosage level.

Compound-related gross changes such as emaciation, areas of discoloration involving the stomach and liver were observed among treated rats that died prior to sacrifice. Similar changes were also observed in the liver of a few rats sacrificed at termination of study from the 30- and 100-ppm groups.

Microscopically, compound-related lesions were observed among all test groups. Morphological changes consisting of centrilobular to midzonal cytoplasmic enlargement (hypertrophy) of hepatocytes were observed in the livers. Necrosis of liver cells was also present among these rats. The incidence and relative severity of the above lesions were more evident among male test rats.

In addition, especially among rats from the 300-, 1,000-, and 3,000-ppm dosage level that died prior to sacrifice, changes involving the primary (thymus, bone marrow) and secondary (spleen, mesenteric lymph nodes) lymphoid organs, stomach, intestines, muscle and skin, were observed and were all considered as compound related.

The other changes described in the tissues of these rats were lesions of naturally occurring diseases.

II. COMPOUND

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

Label	Description
Fluorad® Fluorochemical Surfactant FC-95 3M Stock No. 98-0207-0103-7 Lot 640 Net. Wt. 5 lbs 2.2 kg	white powder 6 x 5 lb tins

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III. CLINICAL STUDIES

A. METHOD:

1. General Procedure:

Thirty male (196 to 232 g each) and 30 female (165 to 206 g each) Charles River CD rats purchased as weanlings 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 ground Purina® Laboratory Chow® and water ad libitum. During the actual study, the rats were provided the appropriate test diet and water ad libitum.

This study was initiated November 2, 1977 and terminated by sacrifice of all remaining rats on January 31, 1978.

2. Compound Administration:

The compound was mixed weekly with ground Purina® Laboratory Chow® (i.e., ground basal diet) on a weight/weight basis to provide dosage levels of 30, 100, 300, 1,000 and 3,000 ppm for feeding. Five male and five female rats were used at each dosage level and in a control group. Control rats received the ground basal diet. Diet samples (100 each) were taken immediately after preparation of each diet 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: before weighing, the compound was ground using a mortar with pestle. The appropriate quantity of Flourad® Fluorochemical Surfactant FC-95 was mixed with 500 g of the ground basal diet in a Hobart blender to provide the premix. The proper quantity of premix was subsequently blended in a twin-shell blender, equipped with an intensifier bar, with the amount of ground Purina® Laboratory Chow® necessary to

provide the appropriate quantity by group of prepared diet. Control rats received the ground basal diet.

3. Observations:

The rats were observed twice daily for overt signs of toxicity and for mortality. Detailed observations were recorded weekly or daily when a specific condition existed. 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 overnight urine samples were obtained from all surviving rats for analysis. Food and water were withheld prior to the collection of samples.

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[5] (BUN), plasma glutamic pyruvic (PGPT) and plasma glutamic oxalacetic (PGOT) transaminase activities[5], plasma alkaline phosphatase activity[5], γ-glutamyl transpeptidase[6] (γ-GTP), creatinine phosphokinase (CPK)[7] and calcium[8]. Plasma alkaline phosphatase values were not obtained for the pretest (baseline) period because of interference from the anticoagulant.

c. Urinalysis:

Urinalysis included: measurement of volume, pH[9], and specific gravity; description of color and appearance; qualitative tests for protein[9], glucose[9], ketones[9], bilirubin[9], and occult blood[9]; and microscopic examination of 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 (1-13 weeks), hematological, biochemical and urinalysis parameters (1 and 3 months) and absolute and relative organ weights (terminal) 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 the significance of differences.

B. RESULTS:

1. General Behavior, Appearance and Survival:

Control rats exhibited no behavioral anomalies related to the testing procedure. Rats given the compound at 30 ppm of the diet exhibited no specific behavioral signs of toxicity and there was no mortality. Changes in general behavior and appearance for male rats at the 100-ppm dosage level included the following: increased sensitivity to external stimuli and red material around the mouth were observed for one rat; increased sensitivity to external stimuli, leans to left and convulsions following handling were noted for a second rat; and one rat was cold to the touch (one day after collection of blood) and died. No compound-related signs of overt toxicity were noted for any of the females at 100-ppm dosage level.

All rats in the 300-, 1,000- and 3,000-ppm dosage levels died prior to the scheduled sacrifice. Most exhibited one or more of the following signs of overt toxicity: emaciation, convulsions following handling, altered posture (hunched back), red material around right eye, yellow material around the anogenital region, increased sensitivity to external stimuli, reduced motor activity and moist red material around the mouth or nose. At 300 ppm, death for males occurred from day 13 through day 25 and for females from day 18 through day 28. At 1,000 ppm, death for males and females occurred from day 8 through day 14. At 3,000 ppm, death for males and females occurred from day 7 through day 8.

Observation	Number of Rats/Observation/Dosage Level			
	100 ppm	300 ppm	1,000 ppm	3,000 ppm
Emaciation		5	10	6
Convulsion	1	10	3	1
Altered position (hunched back)	1	1	7	6
Red material (right eye)	2	1		
Yellow material (anogenital region)		2		3
Increased sensitivity	2	2		
Reduced motor activity				4
Moist red material (mouth)	1	1	1	

Survival (prior to sacrifice) after 3 months of compound administration was as follows:

Dosage Level	No. Surviving/No. Initiated	
	Male	Female
Control	5/5	5/5
30 ppm	5/5	5/5
100 ppm	2/5	3/5
300 ppm	0/5	0/5
1,000 ppm	0/5	0/5
3,000 ppm	0/5	0/5

2. Body Weights (Tables 1-2):

Comparison of mean body weights of the 30-ppm and 100-ppm groups indicate a lower body weight gain at both dosage levels when compared to the controls. Moderate to marked losses of body weight were seen prior to death for rats at the 300-, 1,000- and 3,000-ppm dosage level.

Group mean body weights after 90 days of compound administration were as follows:

Dosage Level	Group Mean Body Weights, g			
	Male		Female	
Control	472	2.1	264	3.8
30 ppm	431 (- 8.7)	2.3	243 (- 8.0)	4.1
100 ppm	393 (-16.7)	2.5	221 (-16.3)	4.5

3. Food Consumption (Tables 3-4):

Group mean averages for food consumption declined consistently as the dosage level was increased.

Dosage Level	Average Food Consumption (grams/rat/day)			
	Male		Female	
Control	27.4		19.7	
30 ppm	26.0	1.8	19.0	2.3
100 ppm	23.9	6.0	15.8	7.1
300 ppm	12.8	18.4	9.3	15.6
1,000 ppm*	7.5	35.2	6.6	37.6
3,000 ppm*	5.0	72.0	4.2	75.6

*values for 1 week only

4. Laboratory Tests (Tables 5-8):

a. Hematology:

At the 30-ppm dosage level (1 and 3 months) and at the 100-ppm dosage level (1 month) hematologic values were within the expected range.

For the 100-ppm dosage level at 3 months, the two surviving male rats had lower erythrocyte, hemoglobin, hematocrit and leucocyte counts (differences when compared to controls were statistically significant, $p<0.01$). The three surviving female rats had slightly

lower hemoglobin and reticulocyte counts (statistically significant, $p<0.05$) as well as slightly lower erythrocyte, hematocrit and leucocyte counts. All other hematologic values of male and female rats at this dosage level were within the expected range.

b. Biochemistry:

One control rat showed moderately elevated PGOT and PGPT activity at 3 months of study.

At the 30-ppm dosage level, one female rat showed a slightly elevated glucose level and one male rat showed a slightly elevated alkaline phosphatase value at 1 month of study. At 3 months of study, one male rat at the 30-ppm dosage level showed slight to moderate elevations in glucose, blood urea nitrogen and γ-glutamyl transpeptidase activity.

At the 100-ppm dosage level, slight to moderate increases in glucose (3 males, 1 female) and blood urea nitrogen (5 males, 4 females) were seen at 1 month of study. Also noted at 1 month of study, for 3 males and 2 females at the 100-ppm dosage level, were slight to marked increases in PGOT and PGPT. The creatinine phosphokinase (CPK) levels generally were slightly increased for rats at the 100-ppm dosage level at 1 month of study. Alkaline phosphatase activity also was slightly elevated for a few rats at the 100-ppm dosage level at 1 month of study. The group mean glucose values for males, blood urea nitrogen values for males and females, and CPK and alkaline phosphatase values for females at the 100-ppm dosage level were significantly higher ($p<0.05$) than the control means at 1 month of study.

At 3 months of study, 2 of the 3 surviving female rats at the 100-ppm dosage level showed slight to moderate increases in PGOT and PGPT activity. One of these females also showed a slight elevation in CPK activity.

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 90 days of compound administration, five rats/sex in the control and 30-ppm groups, and two and three female rats in the 100-ppm groups were sacrificed with carbon dioxide and 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.

All rats that died prior to termination, were also necropsied, and tissues were collected as above.

2. Histopathology:

Microscopic examination of formalin-fixed, hematoxylin and eosin stained, paraffin sections was performed for all rats in the control, 30-, 100-, 300-, 1,000- and 3,000-ppm dosage levels. The following tissues were examined histologically:

brain with cervical cord	aorta	pancreas
lumbar spinal cord	spleen	liver
peripheral nerve	mesenteric lymph nodes	kidneys
eyes	thymus	urinary bladder
pituitary	bone with marrow (sternum)	testes
thyroid	salivary gland	ovaries
parathyriod	stomach	prostate
adrenals	small intestine (3 levels)	uterus
lung	colon	skin (mammary gland)
heart with coronary vessels		and all tissues with gross lesions

B. RESULTS:

1. Gross Pathology (Table 17) and Organ Weights (Tables 18-19):

Compound-related gross necropsy lesions and/or observations were present among rats in all experimental groups. The livers of these rats showed varying degrees of discoloration and/or enlargement, while in the stomach, foci of discoloration in glandular mucosa,

presumably hemorrhages were also observed. In addition to the above gross observation, emaciation was evident especially among rats that died prior to sacrifice (300-, 1,000- and 3,000-ppm levels).

2. Histopathology (Table 20):

Compound-related lesions were observed histologically in the following organs and tissues.

a. Liver: Slight to marked, focal necrosis of liver cells with no lobular distribution was seen among rats at the 100-ppm and higher treatment levels. The above lesion was accompanied by focal to multifocal, very slight to slight, cytoplasmic enlargement (hypertrophy) of hepatocytes in the centrilobular to midzonal regions of the affected liver lobules in all test groups, especially amone male rats. There was a slight increase in cytoplasmic vaculoation of hepatocytes in 30-ppm treated rats, but this finding was not evident at the higher dose levels; therefore, this lesion was considered insignificant.

b. Stomach: Mucosal hyperkeratosis and/or acanthosis was observed in the forestomach (nonglandular portion) while mucosal hemorrhages and/or erosions were observed in the glandular portion.

c. Bone Marrow: Hypocellularity was noted.

d. Thymus: Depletion in number ans size of lympoid follicles was noted.

e. Spleen: Decrease in size of the organ (atrophy) with a corresponding decrease in size and number of lymphoid follicles and cells was observed.

f. Mesenteric Lymph Nodes: Depletion in size and number of lymphoid follicles was noted.

g. Small Intestine (duodenum, jejunum, ileum): Decreased atrophy in the height and thickness of the villi were noted.

h. Skeletal Muscle: Atrophy was evident.

i. Skin: Epidermal hyperkeratosis and/or acanthosis was noted.

The findings in the stomach, lymphoid organs, intestine, muscle and skin, were prominent among all rats (300-, 1,000- and 3,000-ppm levels) that died prior to sacrifice.

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ANALYSIS OF $C_8F_{17}SO_3^-$ IN SERUM AND LIVER OF RATS FROM I.R.D.C. STUDY*

DOSE	SURVIVAL		PPM $C_8F_{17}SO_3^-$ IN SERUM		PPM $C_8F_{17}SO_3^-$ IN LIVER	
PPM	M	F	M	F	M	F
0	5/5	5/5	-	<0.5	-	-
30	5/5	5/5	250	300	102	151
100	2/5	3/5	550	450	215	240
300	0/5	0/5	-	-	-	-
1000	0/5	0/5	-	-	-	-
3000	0/5	0/5	-	-	-	-

*FC-05

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An Epidemiologic Mortality Study of Employees at the Chemolite Plant

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An Epidemiologic Mortality Study of Employees at

the Chemolite Plant

Introduction

A retrospective cohort mortality study of employees at the Chemolite Plant in Cottage Grove, Minnesota was conducted. All employees who had worked at least six months at the Plant were included in the study. The basic objectives of the study were twofold:

1. To determine whether the mortality experience of employees at the Chemolite Plant was significantly different from that expected in a population group of this demographic composition.
2. To determine whether the mortality experience of employees in the Chemical Division of the Chemolite Plant was significantly different from that expected.

Methods

All employee records for all divisions of the Chemolite Plant are stored in a central location (Personnel Office). The personnel recordkeeping system has been designed so that complete records have been maintained on all employees who ever worked at the Chemolite Plant. These records are also maintained for employees who transfer within the 3M Corporation. Thus, in constructing the study cohort it was assumed that a complete roster could be assembled of all employees who ever worked at the Chemolite Plant since its opening in 1947.

A careful review of the personnel records indicated that a total of 4218 had been employed at Chemolite for some period of time since 1947. Of these, 3688 were employed for at least 6 months. This group comprised

the basic cohort for study.

Information was abstracted from personnel records. In many instances a number of records existed for a given employee and these records were stored in different areas in the personnel office. Nevertheless, all sources of information were ascertained and the appropriate data abstracted.

For each individual the following information was coded onto Fortran coding sheets:

Employee number

Name

Address

Telephone number

Social security number

Sex

Birthdate

Marital status

Date of first employment at Chemolite

Date of last employment at Chemolite

Period of employment in the Chemical Division

Status (current, retired, deceased, disabled, laidoff, transferred, terminated, reason not stated and returned to school)

Date of death (if noted on record)

Area transferred to (if noted on record)

For each of the 3688 employees, two computer cards were punched with the information mentioned above.

A computer tape listing the name, social security number and birthdate of

all but the current employees was developed and sent to the Social Security Administration (SSA) to ascertain those individuals who had died during the period of study (1947 - 1978).

A total of 180 deaths were ascertained from SSA records. For each death the state of last residence, the state of last employment, or the state from which the benefit was filed along with the date of death was listed. This information was used to obtain a copy of the death certificate for each decedent. Unfortunately, since the most vital piece of information - state in which the death occurred - was not provided by the SSA, acquisition for all death certificates was a formidable task.

For those deaths for which a death certificate was not available from the state of last residence, the state of last employment, or the state from which the benefit was filed, a search was made in Minnesota using the Minnesota Health Department's commulative microfiche death index 1962 - 1978 and the death records prior to 1962. Information (name, social security number, birthdate, date of death) on all deaths for which the death certificate was not acquired by any of the above mentioned methods was sent to the Wisconsin Health Department to determine if a death certificate was on file in their records.

Utilizing these procedures, 168 of the 180 (93.3%) death certificates were acquired. Information on the remaining twelve with a request for a copy of the death certificate was sent to every state health department except Minnesota, Wisconsin and New York. Minnesota and Wisconsin were excluded since their records had already been reviewed. New York was excluded since they require the borough in which the death occurred to

ascertain the death certificate. However, if any of the twelve death certificates remain outstanding after a search by all other state health departments, then an attempt will be made to access New York vital records.

The death certificates were coded by a professional nosologist according to the International Classification of Diseases (I.C.D.), Eighth Revision. All information in the cause of death section of the death certificate was coded. This included the underlying cause of death, immediate cause of death, conditions related to the immediate cause of death (referred to on the death certificate as "as a consequence of" or "due to"), and other significant conditions. These codes along with the individual's name and social security number (to facilitate linking these data to the main file) were transferred onto Fortran coding sheets and keypunched.

In addition to identifying the deaths, SSA reported that for 485 members of the study cohort no benefits were currently being paid. This meant that SSA could not emphatically state whether these individuals were dead or alive. However, published reports (see for example Wang, H. and MacMahon, B., J. Occup. Med., 21, 745, 1979) have stated that the ascertainment of mortality by SSA is virtually complete and that additional follow up on individuals for whom no information is available from SSA records is unnecessary. Nevertheless, in order to be absolutely certain that all deaths were ascertained, every effort was made through extensive tracing efforts to determine the current status of these 485 unaccounted for study members. The status of virtually all of these people was determined in this way.

Method of Analysis

The method of analysis is based on a comparison of the observed to expected death rates specific for cause, age, time, sex and race. The

method is described in detail in Monson, R., Analysis of relative survival and proportional mortality, Comp. Biomed. Res., 7, 325-332, 1974.

The output from this analysis provides the observed and expected number of deaths, the ratio of observed to expected (SMR), and the upper and lower 95% confidence limits.

The 95% confidence limits are defined as follows:

$$\text{Lower Limit} = LL = (O(1 - 1/9O - 1.96/3\sqrt{1/O})^3)/E$$

$$\text{Upper Limit} = UL = ((O+1)(1 - 1/9(O+1) + 1.96/3\sqrt{1/(O+1)})^3)/E$$

Where O = observed number of deaths

E = expected number of deaths

Results

Records on 4,218 employees were reviewed and abstracted (Table 1). Only those who worked at Chemolite for six or more months (3,688) were included in the mortality followup.

Of the 180 known deaths, 168 (93.3%) death certificates were obtained. There were 159 male deaths and 21 female deaths.

Table 2 presents the results for Chemolite Plant male employees. Overall, the number of deaths (149) was significantly less than expected (208.3). This "deficit" was primarily due to the lower than expected number of deaths from diseases of the circulatory system and all external causes of death. The observed-to-expected ratio for cancer deaths was 1.0, indicating no disparity between the number of deaths observed and the number expected. The only O/E ratios which exceeded 1.0 were for deaths due to asthma (2.8) and motor vehicle accidents (1.1). However, neither even approached statistical significance.

Table 1. Epidemiologic Mortality Study of Chemolite Plant
July, 1976 - December, 1979

Records Abstracted	4,218
Number Studied	3,688
Number of Deaths	180 (4.9%)
Death Certificates Analyzed	168

Table 2. Results of Cause-Specific Mortality Analysis
Males, Chemolite Plant (≥ 6 months)

Cause of Death	O	E	O/E	LL	UL
140 - Malignant Neoplasms	37	37.1	1.0	.7	1.3
000 - All Infective and Parasitic Diseases	1	2.5	.4	.0	2.3
Tuberculosis	1	1.3	.8	.0	4.5
240 - Allergic, Endocrine, Metab., Nutr. Diseases	3	3.4	.9	.2	2.6
Diabetes Mellitus	2	2.8	.7	.1	2.6
320 - All Diseases of Nervous Sys. & Sense Organs	2	2.4	.9	.1	3.1
390 - All Diseases of Circulatory System	64	83.0	.8	.6	<1.0
Chronic Rheumatic Heart Disease	1	2.6	.4	.0	2.2
Arteriosclerotic H.D., Incl. CHD	50	61.4	.8	.6	1.1
All Vascular Lesions of CNS	4	8.5	.5	.1	1.2
460 - All Respiratory Diseases	6	8.9	.7	.3	1.5
All Pneumonia	2	3.5	.6	.1	2.1
Emphysema	1	2.0	.5	.0	2.8
Asthma	1	.4	2.8	.0	15.8
520 - All Diseases of Digestive System	7	12.4	.6	.2	1.2
All Gastric and Duodenal Ulcer	1	1.3	.8	.0	4.4
Cirrhosis of Liver	4	7.6	.5	.1	1.4
800 - All External Causes of Death	29	49.0	.6	.4	.8
All Accidents	24	33.4	.7	.4	>1.0
Motor Vehicle Accidents	20	18.3	1.1	.6	1.6
Suicide	5	10.1	.5	.2	1.2
All Causes of Death	149	208.3	.7	.6	.8

Table 3. Results of Cause-Specific Mortality Analysis
Males. Chemolite Plant (≥ 6 months)
Cancer

Cause of Death	O	E	O/E	LL	UL
All Malignant Neoplasms	37	37.1	1.0	.7	1.3
Cancer of Digestive Organs & Peritoneum	9	9.0	1.0	.5	1.9
150 - Cancer of Esophagus	1	.8	1.3	.0	7.1
151 - Cancer of Stomach	1	1.6	.6	.0	3.5
153 - Cancer of Large Intestine	3	2.9	1.1	.2	3.1
154 - Cancer of Rectum	1	1.0	1.0	.0	5.6
157 - Cancer of Pancreas	2	1.9	1.1	.1	3.9
Cancer of Respiratory System	9	12.5	.7	.3	1.4
161 - Cancer of Larynx	1	.5	1.9	.0	10.7
162 - All Cancer of Lung (Prim. & Sec.)	8	11.8	.7	.3	1.3
172 - Cancer of Skin	1	1.1	.9	.0	5.0
185 - Cancer of Prostate	2	1.0	2.0	.2	7.3
186 - Cancer of Testis	1	.7	1.5	.0	8.3
189 - Cancer of Kidney	1	1.0	1.0	.0	5.6
191 - Cancer of Brain & Other CNS	2	1.9	1.1	.1	3.9
All Lymphopoietic Cancer	4	5.0	.8	.2	2.0
201 - Hodgkins Disease	2	1.1	1.9	.2	6.8
208 - Cancer of Lymphatic Tissue	2	.9	2.2	.3	8.0

158 Other Digestive 1
195 Ill Defined Sites 1
199 Malig. Neopl. Without Specif. of Site 6

In Table 3, the 37 cancer deaths are distributed by site. None of the results were significant.

Tables 4 and 5 present results for deaths among men who were employed for at least six months in the Chemical Division of the Chemolite Plant. Overall, the observed number of deaths (55) was less than expected (66.1). This was mainly the result of the lower than expected number of deaths from diseases of the circulatory system. The highest O/E ratio, 9.2, occurred for deaths due to asthma. However, this ratio, based on only a single death in the 21 year study period, was not statistically significant.

A total of 13 cancer deaths occurred among the Chemical Division male employees. This was approximately the number expected (11.2). Although the O/E ratio for a number of cancer sites exceeded one, none were statistically significant.

A distribution of the 19 observed deaths among female employees is presented in Table 6. No major departures from expected were noted in these results.

Discussion

The lower than expected mortality from all causes is often found in studies of occupational cohorts. This reflects "the healthy worker" effect. Generally, individuals who are disabled or chronically ill are excluded from certain work situations, particularly those requiring considerable physical activity. Thus, employee cohorts compared to the general population generally have more favorable mortality outcomes, particularly for younger employees. To compensate for this effect the data could be reviewed with an O/E ratio of less than 1, (perhaps .9 is appropriate) considered equivalent to no difference between observed and expected deaths. Reviewing the data with

Table 4. Results of Cause Specific Mortality Analysis
Males. Chemical Division (≥6 Months)

Cause of Death	O	E	O/E	LL	UL
140 - Malignant Neoplasms	13	11.2	1.2	.6	1.9
240 - Allergic, Endocrine, Metab., Nutr. Diseases	2	1.1	1.9	.2	6.8
Diabetes Mellitus	1	.9	1.1	.0	6.5
320 - All Diseases of Nervous Sys. & Sense Organs	1	.8	1.3	.0	7.0
390 - All Diseases of Circulatory System	16	24.3	.7	.4	1.1
Arteriosclerotic H.D. Incl. CHD	14	17.9	.8	.4	1.3
460 - All Respiratory Diseases	4	2.7	1.5	.4	3.9
All Pneumonia	2	1.1	1.9	.2	6.7
Asthma	1	.1	9.2	.1	51.1
520 - All Diseases of Digestive System	5	3.9	1.3	.4	3.0
All Gastric and Duodenal Ulcer	1	.4	2.6	.0	14.5
Cirrhosis of Liver	3	2.4	1.3	.3	3.7
800 - All External Causes of Death	14	18.1	.8	.4	1.3
All Accidents	12	12.5	1.0	.5	1.7
Motor Vehicle Accidents	9	7.0	1.3	.6	2.4
Suicide	2	3.6	.6	.1	2.0
All Causes of Death	55	66.1	.8	.6	1.1

Table 5. Results of Cause-Specific Mortality Analysis
Males. Chemical Division (≥6 Months)
Cancer

Cause of Death	O	E	O/E	LL	UL
140 - Malignant Neoplasms	13	11.2	1.2	.6	1.9
Cancer of Digestive Organs & Peritoneum	3	2.6	1.1	.2	3.3
Cancer of Esophagus	1	.2	4.4	.1	24.5
Cancer of Large Intestine	1	.9	1.2	.0	6.6
Cancer of Pancreas	1	.6	1.8	.0	10.2
Cancer of Respiratory System	4	3.7	1.1	.3	2.8
Cancer of Larynx	1	.2	6.7	.1	37.3
Cancer of Lung (Prim. & Sec.)	3	3.5	.9	.2	2.5
Cancer of Skin	1	.4	2.7	.0	15.1
Cancer of Prostate	1	.3	3.9	.1	21.8
Cancer of Testis	1	.3	4.0	.1	22.1

199 - Malig. Neopl. Without Specification of Site - 3

Table 6. Specific Causes of Death
Females. Chemolite Plant (36 months)

		O	E
009	Diarrheal disease	1	.4
162	Malig. neopl. of lung	1	1
172	Malig. neopl. of skin	1	.2
174	Malig. neopl. of breast	2	2.5
183	Malig. neopl. of ovary	1	.8
191	Malig. neopl. of brain	1	.4
402	Hypertensive H.D.	1	
410	Acute M.I.	1	
412	Chronic Ischemic H.D.	4	7.5
427	Symptomatic H.D.	2	
472	Influenza with other resp. manifestations	1	1
493	Asthma	1	.1
812	Car accident with other car	1	
819	Car acc. of unspecified nature	1	1.5
	Total	19	

a null hypothesis of .9 would not substantially alter the conclusions.

Deaths for U.S. white males and females were used to derive expected values. A more appropriate comparison population might have been deaths for the counties in which the employees resided, namely Dakota, Ramsey and Washington in Minnesota and Pierce and St. Croix in Wisconsin. There are two major problems with using data from these counties to derive expected values. First, age - sex - race and cause specific death rates are not available by county for periods back to the late 1940's. Second, if such rates could be derived from the basic data, the relatively small population bases would result in highly unstable rates. One strategy for considering geographic differences in mortality is to examine available data for the counties of interest in relation to the United States rates. For cancer, age adjusted death rates are available by county for the period 1950-69. These data were examined for the above mentioned counties and compared to the rates for the United States. For those cancer sites where the rate was based on an average of at least one death per year the only notable difference between the rates for the 5 counties and the U.S., was for lung cancer where the age-adjusted rate was appreciably lower in 4 of the 5 counties than in the U.S. as a whole (the rate for Ramsey county approximated the U.S. rate). The O/E ratio for lung cancer was less than 1.0. This is an underestimate of the SMR if calculated using county rates. However, re-calculating the SMR using county data to derive expected values would probably result in an SMR of about 1.5, which would not be statistically significant.

Although the results of this analysis revealed no significant findings, a number of SMR's, particularly for the cancers, did exceed one. Thus, continued surveillance for mortality is warrented.

As of the writing of this report (February 26, 1980) information on the 12 deceased study members whose death certificates were not available at the time the data was analyzed is as follows:

2 died in service, one in Vietnam and one in Korea

4 died from cardiovascular disease

2 died from cancer

1 died from injuries sustained in an airplane crash

3 cause of death unknown (2 of these deaths occurred among employees who worked at Chemolite for less than 6 months)

From the above distribution of deaths, it is obvious that the addition of these deaths to the analyses presented in this report does not alter the conclusions.

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Oral Teratology Study of T-2999CoC in Rabbits

Experiment No.: 0681TB0212

Conducted At: Safety Evaluation Laboratory

St. Paul, Minnesota

Dosing Period: May 10, 1981 through June 26, 1981

Study Director:

Summary

The oral administration of T-2999CoC at 0, 15, 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 teratogenic. Compound-related teratogenic effects were not seen on gross, internal or skeletal examinations of rabbit fetuses.

T-2999CoC was embryotoxic at the 15 mg/kg/day level. This embryotoxicity was seen as a high incidence of abortions or total resorptions, increased numbers of resorption sites and low numbers of viable fetuses. The 15 mg/kg/day fetuses experienced poor neonatal survival during a 24 hour incubation. The 5 and 1.5 mg/kg/day dose levels were free from the embryotoxicity and poor neonatal survival seen in the higher dose level.

T-2999CoC caused maternal toxicity of failure to gain net mean body weight between the initiation of dosing and the termination of the study only in the 15 mg/kg/day level. No compound-related clinical signs were unique to T-2999CoC treatment of pregnant rabbits.

Fetal rabbit lenses from all dose groups were evaluated histologically. T-2999CoC did not cause lens abnormalities in fetal rabbits exposed to the compound in utero.

Introduction

This teratology study[a] in rabbits was conducted to evaluate the embryotoxic and teratogenic effects of orally administered T-2999CoC[b]. The study was sponsored by 3M Commercial Chemical Division, St. Paul, Minnesota and was conducted by the Safety Evaluation Laboratory, , St. Paul, Minesota. Two sets of compound administration groups were dosed between May 10 and June 26, 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 1601 to 2917 grams, were than 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[c] and water were available ad libitum. The lights were on a 12 hour light/dark cycle. Each female rabbit was injected with 3 mg of pituitary luteinizing hormone via the ear vein before breeding. 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-2999CoC suspended daily in corn oil at 0, 15, 5 and 1.5 mg/kg/day. The suspensions 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-2999CoC characterization was provided by 3M Commercial Chemical Division, St. Paul, Minnesota and corn oil clearance was provided by Analytical Chemistry, , St. Paul, Minnesota (Appendix IV).

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 than 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 alcohol for clearing and staining of the skeleton with alizarin red to detect skeletal abnormalities. A total of 156 fetal rabbit eyes were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5-6 microns, stained with hematoxylin and eosin and evaluated histologically. This was done because a

[a] Experiment Number 0681TB0212
[b] (technical grade)
[c] Purina Rabbit Chow, Ralston Purina Co., St. Louis, MO

lens artifact in rat teratology studies was being evaluated concurrently with this study. The rabbit was used as a second species in case the lens finding was a true abnormality in the rat.

Results and Discussion

The oral administration of T-2999CoC to pregnant rabbits during the period of organogenesis caused maternal toxicity of low mean body weight gain. The high (15 mg/kg/day) dose does lost significantly more weight than the controls (0 mg/kg/day) between days 6 and 9 of the study (Table 1, Appendix V). They also failed to gain net body weight between the first day of dosing and the end of the study. The mid (5 mg/kg/day) and low (1.5 mg/kg/day) dose group mean maternal body weight gains and losses were never significantly different from the controls group. No compound-related clinical signs were unique to T-2999CoC treatment of rabbits.

Maternal deaths occurred during the study. No deaths could be attributed directly to compound-related toxicity, even though the compound caused maternal toxicity of low body weight gain.

A compound-related high incidence and percentage of abortions or total resorptions occurred only in the high dose group rabbits; however, the increased incidence was not statistically significant (Table 2). The incidence and percentage of abortions or total resorptions of the mid and low dose groups were not different from the control group.

T-2999CoC was embryotoxic at the high dose level. The T-2999CoC treated high dose does had a significantly lower number of viable fetuses and significantly higher number of resorption sites than the control group (Table 3, Appendix VI). The numbers of viable fetuses and resorption sites of the mid and low dose groups were not significantly different from the controls. The following litter data measurements were not affected by compound administration to the does: number of dead fetuses, number of implantation sites, number of corpora lutea and mean fetus weight.

The fetuses of T-2999CoC treated high dose does experienced poor neonatal survival. The high dose fetuses had a significantly higher mortality rate than control fetuses (Table 2) during a 24 hour incubation. Survival of mid and low dose fetuses was comparable to controls.

No major gross malformations were observed to be compound-related. Two fetuses from separate control dose group does had clubbed forepaws (Table 4, Appendix VII). Small and runted fetuses were seen in the control, mid and low dose groups, but not in the high dose group. No internal abnormalities were found.

The incidences of skeletal findings among fetuses of rabbits receiving T-2999CoC were generally not different from the findings for the control fetuses. The only exception was a significantly higher incidence of fetuses

with 13 ribs spurred in the low dose group than in the control group (Table 5, Appendix VIII). The oral administration of T-2999CoC to pregnant rabbits was not teratogenic at the dose levels tested.

Nine abnormal lenses were found on histological examination of hematoxylin and eosin stained fetal rabbit eyes. All nine abnormal lenses were from fetuses of control doe N1B1196. The remainder of the fetal lenses either had no significant changes, were not readable, or had autolysis. T-2999CoC did not cause lens abnormalities in fetal rabbits exposed to the compound in utero.

Table 1

Oral Teratology Study of T-2999CoC in Rabbits

Mean Body Weight Gain or Loss (g) of Pregnant Rabbits Between Weighings with Standard Deviations

	DAY	6	9	12	15	18	29
0 mg/kg/day	MEAN	83	-36	-9	8	30	234
	STAN. DEV	47.8	60.4	75.0	68.4	92.3	110.6
15 mg/kg/day	MEAN	56	-120[a]	-50	1	10	159
	STAN. DEV	31.5	67.7	63.4	73.5	72.4	137.8
5 mg/kg/day	MEAN	47	-48	-24	-23	5	244
	STAN. DEV	49.3	48.2	75.1	68.2	66.0	113.3
1.5 mg/kg/day	MEAN	63	-28	1	21	56	178
	STAN. DEV	25.9	77.0	55.7	60.1	62.1	59.1

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

0236

Table 2

Oral Teratology Study of T-2999CoC in Rabbits

Reproductive Performance and Fetal Survival

Dose Group	Number of Animals	Conception Rate No.	%	Incidence of Total Abortions or Resorptions	%	24 Hour Incubation Mortality Rate	%
0 mg/kg/day	18	15	83	2/15	13	16/81	20
15 mg/kg/day	18	16	89	7/16	44	14/28[a]	50
5 mg/kg/day	18	16	89	1/16	6	14/79	18
1.5 mg/kg/day	18	14	78	2/14	14	14/58	24

[a] Significantly higher than the control (Chi-square with Yates correction $p < 0.05$)

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Table 3

Oral Teratoloby Study of T-2999CoC in Rabbits

Mean Litter Data and Fetal Weights with Standard Deviations

GRP	NO. OF ANIMALS	VIABLE FETUSES M	F	TOTAL	DEAD FETUSES	RESORPTION SITES	IMPLANTATION SITES	CORPORA LUTEA	MEAN WT. FETUS(G)
0 mg/kg/day	12	4.0	2.7	6.7	0.1	0.2	7.1	7.4	38.0
STAN DEV		1.8	1.7	2.3	0.3	0.9	2.1	1.7	5.5
15 mg/kg/day	8	2.0[a]	1.5	3.5[a]	0.4	1.5[a]	5.4	6.5	34.8
STAN DEV		1.2	1.3	1.8	0.7	1.5	2.1	1.6	7.1
5 mg/kg/day	14	2.9	2.8	5.6	0.0	0.6	6.2	6.5	36.0
STAN DEV		1.3	1.3	1.8	0.0	0.8	2.2	1.5	5.0
1.5 mg/kg/day	10	3.2	2.6	5.8	0.0	0.5	6.3	6.7	36.9
STAN DEV		1.4	1.3	2.3	0.0	1.0	2.1	1.3	5.2

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

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