Oral Teratology Study of FC-95 in Rats

Experiment No.:	0680TR0008
Conducted At:	Safety Evaluation Laboratory Riker Laboratories, Inc. St. Paul, Minnesota
Dosing Period:	July 14, 1980 through July 24, 1980
Study Director:	E. G. Gortner

E. A. Gortner 12/17/80

E. G. Gortner Date
Senior Research Technologist
Animal Reproduction-Teratology
Study Director

Elden G Lamprecht 12/17/80

E. G. Lamprecht Date
Research Veterinary Pathologist

Marvin T Case 12/18/80

M. T. Case, DVM, PhD Date
Manager, Pathology-Toxicology
Safety Evaluation Laboratory

Exhibit
1247
State of Minnesota v. 3M Co.,
Court File No. 27-CV-10-28862

Summary

Oral administration of FC-95 at doses of 10, 5 and 1 mg/kg/day to pregnant Sprague-Dawley rats during days 6 through 15 of gestation (period of organogenesis) resulted in fetuses with teratogenic changes in the lens of the eye. The teratogenic effect was a developmental eye abnormality which appeared to be an arrest in development of the primary lens fibers forming the embryonal lens nucleus, followed by secondary aberrations of the secondary lens fibers of the fetal nucleus. The lens abnormality occurred in all FC-95 dose groups, but the proportion of fetuses with the lens changes was significantly higher than the control group only in the 10 mg/kg/day group.

FC-95 administration was maternally toxic only to the 10 mg/kg/day group. At gestation days 12 through 20 their mean maternal body weights were significantly lower than the controls. FC-95 was not maternally toxic to the 5 and 1 mg/kg/day groups.

FC-95 was not embryotoxic and did not affect the ovaries or reproductive tract contents of the dams. The compound did not produce an increase in the number or proportion of abnormal fetal skeleton aberrations.

Introduction

This teratology study[a] of FC-95 in rats was conducted to evaluate the embryotoxic and teratogenic effects of orally administered FC-95. The study was sponsored by 3M Commerical Chemical Division, St. Paul, Minnesota and was conducted by the Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota. The compound administration period was from July 14 through July 24, 1980. 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 Spraque-Dawley derived rats were obtained from Charles River Breeding Laboratory and assigned cages according to a computer-generated random numbers table. The rats were then divided into four groups of 22 animals weighing 175 to 261 grams. The rats were housed individually in hanging stainless steel cages with wire mesh floors and fronts in a temperature and humidity controlled room. Food[b] and water were available ad libitum. The lights were on a 12 hour light/dark cycle.

The animals were observed daily from day 3 through day 20 of gestation for abnormal clinical signs. Body weights were recorded on days 3, 6, 9, 12, 15 and 20 of gestation and the rats dosed accordingly using a constant dose volume of 5 ml/kg of body weight. The four groups were dosed with FC-95 (Lot 640) suspended daily in corn oil at 0, 10, 5 or 1 mg/kg/day. FC-95 was administered daily by oral intubation with a syringe equipped with a ball-tipped intubation needle to the rats on days 6 through 15 of gestation (day 0 indicated by sperm-positive vaginal smear). FC-95 analytical characterization (see Appendix IV) was provided by 3M Commercial Chemical Division, St. Paul, Minnesota.

All animals were sacrificed on day 20 by cervical dislocation and the ovaries and uterus, including its contents, were examined immediately to determine the following: number of corpora lutea, number of viable fetuses, number of resorption sites, pup weights and sex, and any gross fetal abnormalities. Approximately one-third of the fetuses were fixed in Bouin's solution for subsequent free-hand sectioning by the Wilson technique to determine visceral abnormalities. The remaining fetuses were preserved in alcohol for clearing and staining of the skeleton with alizarin red to detect skeletal abnormalities. Selected free-hand sections were processed for histological evaluation.

[a] Riker Experiment No. 0680TR0008
[b] Purina Laboratory Chow, Ralston Purina Company, St. Louis, MO

Results

FC-95 administered during the period of organogenesis was toxic to the high dose group (10 mg/kg/day) maternal rats. The mean body weights of all dose groups were similar at gestation days three through nine (Table 1, Appendix V). At gestation days 12 through 20 the high dose group rats weighed significantly less than controls (0 mg/kg/day). The mean maternal body weights of mid (5 mg/kg/day) and low (1 mg/kg/day) dose groups were not different from the controls throughout the study. Even though FC-95 was maternally toxic at the high dose level, no compound-related clinical signs were observed in any of the dose groups.

FC-95 was not embryotoxic and did not affect the ovaries or reproductive tract contents of the dams. The mean number of male, female, total and dead fetuses; the mean number of resorption sites, implantation sites, corpora lutea and mean fetus weights of the three FC-95 dose groups were not significantly different from the controls (Table 2, Appendix VI). The high dose group did have a lower mean number of viable male, female and total fetuses than the other three groups which resulted from a lower number of embryos at the start of the study. Contributing pieces of evidence to the lower number of high dose embryos are the low mean number of implantation sites, corpora lutea, resorption sites and the absence of dead fetuses.

FC-95 did not cause compound-related abnormal gross fetal findings (Table 3), nor did FC-95 treatment produce an increase in the number or proportion of abnormal fetal skeletal aberrations. Fetal skeleton results of the three compound treated groups were not significantly different from the control group (Table 4). The incidence and proportions of sternebrae nonossified and associated changes of sternebrae assymetrical, sternebrae bipartite and one sternebrae missing were unusually high in all dose groups of this study including the control group.

FC-95 was teratogenic in the rat at all dose levels administered in this study. The teratogenic effect was a developmental eye abnormality which appeared to be an arrest in development of the primary lens fibers forming the embryonal lens nucleus, followed by secondary aberrations of the secondary lens fibers of the fetal nucleus. All eye abnormalities were localized to the area of the embryonal lens nucleus although a variety of morphological appearances were present within that location. The range of morphological appearances as observed under the dissecting microscope varied from a slight discoloration of the lens near the anterior margin to a dark colored oval area, often containing a cleft, extending from beneath the lens epithelium to half-way through the lens posteriorly. Histologically the discolorations were due to presence of lens vesicle remnants surrounding the abnormal embryonal lens nucleus. One of the most severly affected eyes had most of the embryonal lens nucleus replaced by sinus spaces containing red blood cells. Also contributing to the discolorations were primary lens fibers which appeared to have not elongated. These lens fibers were tortuous and lacked nuclei in a normal lens bow of nuclei. Secondary lens fiber development progressed normally except immediately surrounding the abnormal embryonal nucleus. Secondary aberrations of secondary lens fibers included the bending of the fibers around the abnormal oval area, the subsequent formation of prominant anterior and posterior Y sutures of the converging fibers and lens vesicle remnants surrounding the embryonal nucleus.

The lens abnormality occurred in all dose groups except the control group. The proportion of fetuses with the lens abnormality in one or both lenses was significantly higher in the high dose group than the control (Table 5). The lens abnormality recorded for one control fetus under the dissecting microscope was an artifact when evaluated by transmission light microscopy. A no-effect dose level for the teratogenic lens abnormality was not established in this study.

Discussion

Optimal visual functional requirements are met during embryonic development by the differentiation of highly specialized populations of cells from undifferentiated precoursers and by the coordinated morphogenesis of the resulting tissues. Both processes are controlled to a remarkable extent by interactions which occur among emerging tissues. Each tissue of the eye is brought to its final state of differentiation, its cell population size and its definitive geometry, not only by intrinsic processes, but also by extrinsic influences exerted by neighboring tissues[1].

The embryonal origin of the lens is undifferentiated ectoderm. The tip of the optic vesicle, presumably the neural retina, plays the final role in inducing lens from overlying ectoderm and in aligning the lens precisely with the rest of the eye. Alternative or sequential action of tissues derived from endoderm (foregut) and mesoderm (heart) on the same target tissue decreases the probability that lens formation would be aborted by accidents during the early phases of induction. While the nature of the inductive influence remains unknown, there are indications that substances may be transferred from the presumptive neural retina to the overlying ectoderm during induction. A prolonged period of inductive interaction not only increases the probabililty that lens induction will occur successfully in the face of interference, but provides a mechanism for continuously adjusting the size, shape, position and orientation of the lens to that of the retina[2].

During the early stages of the inductive process, the ectodermal cells immediately overlying the tip of the optic vesicle elongate prependicularly to the body surface to form a thickened disc (lens placode). The change in cell shape is accomplished without change in cell volume. The number of cells, however, continues to increase during this period. Toward the end of lens placode formation, acidophilic fibrils appear in the apices of the lens placode cells. At about this time, the placode invaginates to form the lens cup. This invagination is independent of the concomitant invagination of the underlying optic vesicle, and is probably due to forces operating within the lens ectoderm. As the lens cup deepens, its opening (lens pore) becomes progressively constricted until its lips meet and fuse, cutting off the lens vesicle internally and re-establishing continuity in the overlying ectoderm. Closure of the lens pore is attended by, and possibly accomplished by, a local and temporaty restricted wave of cell death. Following closure of the lens pore, the cells at the back of the lens vesicle continue to elongate, under the influence of the neural retina, to form the lens fibers. As the fibers grow the cavity of the lens vesicle is obliterated. The lens cells toward the ectoderm, which do not elongate further, form the lens epithelium[2].

The cuboidal lens epithelial cells which face the cornea continue to grow after the lens vesicle forms. As the cells rotate through the equator region, they take their places on the surface of the growing fiber mass. These cells differentiate into secondary lens fibers at the equator and elongate rapidly toward the poles of the lens where they meet with other fibers in planes of junction called sutures. As secondary fibers grow their nuclei become positioned at about the center of the fibers and form a convex lens bow outward. Since the newer fibers are always deposited superficially, the oldest fibers in the lens come to lie centrally and are referred to collectively as the lens nucleus. With time the lens cell nuclei in this region become pycnotic and finally disappear. The cell fibers, however, are not broken down and removed but remain in place. Thus the size and shape of the lens are controlled by factors which control the number, size and shape of lens cells[2].

The teratogenic effect of FC-95 probably occurred during the portion of organogenesis between differentiation of lens tissue from ectoderm and the formation of secondary lens fibers surrounding the embryonal lens nucleus. The exact time of the teratogenic insult and the morphogenesis of the abnormality were not determined in the study. The developmental lens abnormality appears to be unique because it has not been described as a compound-related abnormality[3]. A similar-appearing structural lens abnormality has been reported to occur spontaneously in rat fetuses, but with a very low incidence of 1.2%[4]. The abnormality resembles the Fraser developmental lens abnormality of a mutant mouse strain which results from degenerative primary lens cells[5].

References

1. Coulombre AJ, Coulombre JL: Abnormal Organogenesis of the Eye, in Wilson J,, Fraser FC (eds): Handbook of Teratology:2 Mechanisms and Pathogenesis. New York, Plenum Press, 1977, pp 329-341.

2. Coulombre AJ: The Eye, in DeHaan RL, Ursprung H (eds): Organogenesis. New York, Holt Rinehart and Winston, 1965, pp 227-232.

3. Mann I: Development Abnormalities of the Eye, 2nd ed. Philadelphia, JB Lippincott Co., 1957.

4. Weisse I, Niggeschulze A, Stotzer H: Spontaneous congenital cataracts in rats, mice and rabbits. Archiv Fuer Toxikologie 32: pp 199-207, 1974.

5. Hamai Y, Kuwabara T: Early cytologic changes of Fraser cataract. An electron microscopic study. Investigative Ophthalmology 14 (7): pp 517-527, 1975.

Table 1

Oral Teratology Study of FC-95 in Rats
Mean Maternal Body Weights with Standard Deviations

Dose Group		Gestation Day 3	6	9	12	15	20
0 mg/kg/day	MEAN	200	223	247	272	305	380
	STAN. DEV	16.7	17.6	20.9	20.5	24.4	33.8
10 mg/kg/day	MEAN	199	223	243	257[a]	277[a]	343[a]
	STAN. DEV	11.8	13.8	18.2	16.2	18.6	34.6
5 mg/kg/day	MEAN	205	228	249	268	294	373
	STAN. DEV	20.0	16.4	12.6	13.2	17.8	23.8
1 mg/kg/day	MEAN	205	226	252	272	303	379
	STAN. DEV	18.8	19.1	19.7	19.5	24.6	31.8

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

Table 2

Oral Teratology Study of FC-95 in Rats
Mean Litter Data and Pup Weights with
Standard Deviations[a]

Dose Group	No. of Animals	VIABLE FETUSES M	F	TOTAL	DEAD FETUSES	RESORPTION SITES	IMPLANTATION SITES	CORPORA LUTEA	MEAN WT. FETUS(G)
0 mg/kg/day	20	5.2	4.9	10.0	0.0	0.7	10.8	11.2	4.3
		1.7	2.1	2.3	0.0	0.9	2.5	2.7	0.4
10 mg/kg/day	17	3.8	3.9	7.7	0.0	0.4	8.1	9.2	4.3
		2.8	2.6	4.3	0.0	0.6	4.3	3.1	0.3
5 mg/kg/day	17	5.0	5.5	10.5	0.0	0.7	11.2	11.1	4.2
		1.9	2.0	2.2	0.0	1.0	2.2	2.0	0.3
1 mg/kg/day	19	4.7	5.4	10.1	0.1	0.4	10.6	10.9	4.2
		1.7	2.1	2.8	0.2	0.8	2.7	2.6	0.4

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

Table 3

Oral Teratology Study of FC-95 in Rats
Number of Fetuses with Gross Findings[a]

Finding	0 mg/kg/day	10 mg/kg/day	5 mg/kg/day	1 mg/kg/day
No. of fetuses examined	201	131	178	192
Umbilical hernia	1	---	1	---
Runted	---	1	---	1
Total Normal Fetuses	200	130	177	191
Total Abnormal Fetuses	1	1	1	1

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

Table 4

Oral Teratology Study of FC-95 in Rats
Number and Percent of Fetuses with Skeleton Findings[a]

Skeleton Finding	0 mg/kg/day	10 mg/kg/day	5 mg/kg/day	1 mg/kg/day
Fontanelle not closed	10 (7)	10 (11)	7 (6)	5 (4)
Frontal nonossified	4 (3)	1 (1)	---	1 (1)
Parietal nonossified	2 (1)	1 (1)	1 (1)	1 (1)
Interparietal nonossified	3 (2)	---	---	1 (1)
Occipital nonossified	1 (1)	1 (1)	---	---
Sternebrae nonossified	114 (81)	77 (85)	100 (81)	107 (81)
Sternebrae asymmetrical	53 (38)	23 (25)	36 (29)	39 (29)
Sternebrae bipartite	7 (5)	4 (4)	5 (4)	6 (5)
One sternebrae missing	30 (21)	13 (14)	26 (21)	26 (20)
Two sternebrae missing	10 (7)	2 (2)	4 (3)	6 (5)
13 ribs	5 (4)	2 (2)	2 (2)	6 (5)
13 ribs spurred	7 (5)	7 (8)	8 (7)	4 (3)
Wavy ribs	1 (1)	2 (2)	---	1 (1)
Protrusion on ribs	6 (4)	9 (10)	3 (2)	8 (6)
One body of the vertebrae bipartite	32 (23)	21 (23)	25 (20)	32 (24)
Two bodies of the vertebrae bipartite	18 (13)	7 (8)	11 (9)	9 (7)
Three bodies of the vertebrae bipartite	4 (3)	1 (1)	1 (1)	3 (4)
Four bodies of the vertebrae bipartite	---	---	---	1 (1)
Total No. Normal Fetuses	7 (5)	3 (3)	10 (8)	10 (8)
Total No. Abnormal Fetuses	133 (95)	88 (97)	113 (92)	123 (92)
Total No. of Fetuses Examined	140	91	123	133

[a] Treatment groups were not significantly different from the control (Chi-square $p < 0.05$)
( ) = percent of total examined

Table 5

Oral Teratology Study of FC-95 in Rats
Number and Percent of Fetuses with Internal Findings

Internal Finding	0 mg/kg/day	10 mg/kg/day	5 mg/kg/day	1 mg/kg/day
Eye abnormality	1[a] (2)	14[b] (35)[c]	4[b] (7)	2[b] (3)
Thoracic cavity full of blood	---	1 (3)	---	---
Enlarged atria	1 (2)	---	---	---
Enlarged renal pelvis area in the kidney	3 (5)	---	---	3 (5)
Abdominal cavity full of blood	4 (7)	2 (5)	5 (9)	2 (3)
Total No. Normal Fetuses	52 (85)	23 (57)	47 (85)	53 (90)
Total No. Abnormal Fetuses	9 (15)	17 (43)	8 (15)	6 (10)
Total No. of Fetuses Examined	61	40	55	59

[a] Eye abnormality was an artifact and was not considered for statistical evaluations
[b] Eye abnormalities were developmental lens abnormalities with secondary lens aberrations
[c] Significantly higher than the control (Chi-square $p < 0.05$)
( ) = percent of total examined

Appendix I

Oral Teratology Study of FC-95 in Rats
Protocol

Objective

A teratology study will be used to evaluate the embryotoxic and teratogenic effects of orally administered FC-95 to pregnant rats during the period of organogenesis. The procedure complies with the general recommendations of the FDA issued in January, 1966 ("Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use"). The study will be conducted according to the 1978 Good Laboratory Practice regulations and Safety Evaluation Laboratory's Standard Operating Procedures.

Sponsor

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

Testing Facility

Safety Evaluation Laboratory, Riker Laboratories, Inc., St. Paul, Minnesota.

Study Director

E. G. Gortner

Start of Dosing

Mid July, 1980.

Test System

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

Test System Identification

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

Appendix I (Continued)

Randomization

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

Control Article

Corn oil.

Test Article

FC-95.

Analytical Specifications

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

Dosage Levels and Experiment Design

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

Dose Group	Dose Level	Group Size
High	10 mg/kg/day	22 ♀
Mid	5 mg/kg/day	22 ♀
Low	1 mg/kg/day	22 ♀
Control	0 mg/kg/day	22 ♀

The oral route of administration will be used because of metabolism studies showed radiolabeled FC-95 was well absorbed. No dietary contaminants are known to interfere with the test article.

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

The females will be killed on day 20 and the ovaries, uterus and its contents will be examined to determine: number of corpora lutea, number of fetuses (live and dead), number of resorption sites, number of implantation sites, pup weight and gross abnormalities. Approximately one-third of the pups will be fixed in Bouin's solution for subsequent free-hand sectioning by the Wilson technique to determine any visceral abnormalities using a dissecting

Appendix I (Concluded)

microscope. The remaining approximately two-thirds of the pups will be fixed in ethyl alcohol for subsequent skeletal examination after clearing and staining with alizarin red.

Data Analysis and Final Report

The proposed statistical methods to be used for analysis of the data are: Dunnett's t test for dam and pup weights, number of fetuses, number of resorption sites, number of implantation sites and number of corpora lutea; Chi square for percent abnormalities. The proposed date for the final report is 2-3 months after detailed pup examinations have been completed (approximately fourth quarter, 1980).

Appendix II

Oral Teratology Study of FC-95 in Rats
List of Principal Participating Personnel

NAME	FUNCTION
Edwin G. Gortner	Study Director
Elden G. Lamprecht	Veterinary Pathologist
Cathy E. Ludemann	Coordinator-Histology
Gary C. Pecore	Supervisor-Animal Care
Loren O. Wiseth	Technician

STATEMENT OF QUALITY ASSURANCE

STUDY NUMBER: 06B0TR0008

TITLE: Oral Teratology Study of FC-95 in Rats

Audits and/or inspections were performed by the Riker Quality Assurance Unit for the above titled study, and reported to the study director and to management as follows:

Date Performed	Date Reported
18 July 1980	21 July 1980
28 July 1980	28 July 1980
15 December 1980	17 December 1980
17 December 1980	17 December 1980

J. E. Orterstrom
Laboratory Quality Assurance
Riker Laboratories, Inc.

December 17, 1980
Date

APPENDIX IV

Test and/or Control Article Characterization

for

FC-95, Lot 640

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 May 8, 1980.

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 Tox Testing If Necessary

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

D-Rick 5/21/80

Sponsor Date

Form 19793 PWO

Appendix V

Oral Teratology Study of FC-95 in Rats
Individual Body Weights (g)

Dose Group and Rat No.	Study Day 3	6	9	12	15	20
0 MG/KG/DAY						
N0R 12996	186	212	235	254	287	357
N0R 12997	224	261	239	306	345	424
N0R 12998	216	238	240	277	315	397
N0R 12999	212	232	271	274	302	373
N0R 13000	224	250	245	307	335	435
N0R 13016	182	207	284	255	284	342
N0R 13018	175	201	259	246	277	354
N0R 13019	193	219	237	277	309	378
N0R 13020	194	221	236	277	319	400
N0R 13036	205	228	222	284	322	408
N0R 13040	186	208	233	261	293	381
N0R 13041	195	219	285	258	289	355
N0R 13043	220	239	253	295	340	426
N0R 13044	207	228	284	273	296	359
N0R 13060	230	248	235	310	349	442
N0R 13061	195	212	213	259	297	366
N0R 13062	210	229	222	272	302	362
N0R 13063	185	208	247	257	289	342
N0R 13064	188	211	250	256	289	368
N0R 13080	179	194	258	238	256	321
MEAN	200	223	247	272	305	380
STAN. DEV	16.7	17.6	20.9	20.5	24.4	33.8
NON PREGNANT ANIMALS						
N0R 13017	186	198	243	217	234	253
N0R 13042	188	209	260	247	255	272

Appendix V (Continued)

Oral Teratology Study of FC-95 in Rats
Individual Body Weights (g)

Dose Group and Rat No.	Study Day 3	6	9	12	15	20
10 MG/KG/DAY						
00R 13001	189	222	239	253	281	347
00R 13002	190	217	230	256	283	356
00R 13003	192	222	224	265	290	381
00R 13004	193	212	218	233	255	319
00R 13005	201	225	260	261	285	369
00R 13021	227	257	247	278	293	360
00R 13022	212	244	243	288	311	402
00R 13023	180	206	258	227	245	285
00R 13025	208	237	251	268	297	382
00R 13037	187	214	229	250	274	357
00R 13045	195	216	228	259	289	361
00R 13048	186	205	226	236	248	311
00R 13065	204	223	274	263	279	304
00R 13066	207	226	275	263	262	358
00R 13067	210	234	222	268	278	322
00R 13069	203	228	262	262	283	338
00R 13081	194	209	238	237	251	281
MEAN	199	223	243	257	277	343
STAN. DEV	11.8	13.8	18.2	16.2	18.6	34.6
NON PREGNANT ANIMALS						
00R 13024	195	217	233	230	242	252
00R 13046	187	209	242	228	232	231
00R 13047	184	201	244	221	233	235
00R 13049	213	237	243	250	251	266
00R 13068	216	232	236	239	250	261

Appendix V (Continued)

Oral Teratology Study of FC-95 in Rats
Individual Body Weights (g)

Dose Group and Rat No.	Study Day 3	6	9	12	15	20
5 MG/KG/DAY						
P0F 13005	192	218	233	258	272	340
P0F 13007	226	249	272	264	304	388
P0F 13008	197	225	262	262	288	394
P0F 13009	188	212	274	254	283	361
P0F 13010	194	220	245	263	282	343
P0F 13027	212	232	251	269	288	369
P0F 13028	215	235	228	274	294	383
P0F 13029	199	229	241	272	288	366
P0F 13030	176	210	260	270	294	379
P0F 13038	196	219	235	263	288	366
P0F 13050	188	204	239	246	265	333
P0F 13051	222	243	256	283	323	407
P0F 13053	235	248	242	291	325	408
P0F 13054	197	224	238	259	279	349
P0F 13070	254	266	245	297	327	410
P0F 13071	200	223	250	274	304	378
P0F 13072	188	211	260	256	292	363
MEAN	205	228	249	268	294	373
STAN. DEV	20.0	16.4	12.6	13.2	17.8	23.8
NON PREGNANT ANIMALS						
P0F 13026	217	235	294	252	252	261
P0F 13052	218	237	252	248	254	262
P0F 13073	206	231	250	250	244	259
P0F 13074	207	234	244	257	272	287
P0F 13082	195	214	240	225	232	240

Appendix V (Concluded)

Oral Teratology Study of FC-95 in Rats
Individual Body Weights (g)

Dose Group and Rat No.	Study Day 3	6	9	12	15	20
1 MG/KG/DAY						
00R 13011	198	224	250	261	288	367
00R 13012	217	235	248	282	310	386
00R 13013	183	204	230	267	300	379
00R 13014	198	221	224	272	301	376
00R 13015	200	228	253	284	326	413
00R 13031	214	258	241	300	332	411
00R 13032	195	220	246	255	276	322
00R 13033	204	210	244	285	320	407
00R 13034	193	226	254	262	286	355
00R 13035	185	201	236	251	271	352
00R 13039	225	252	302	301	334	416
00R 13055	201	220	232	261	303	379
00R 13056	204	223	259	263	286	371
00R 13057	196	211	236	250	268	333
00R 13059	201	224	264	270	301	375
00R 13075	185	206	283	257	291	362
00R 13077	198	215	263	262	296	363
00R 13078	208	226	243	262	297	374
00R 13082	261	279	278	323	368	459
MEAN	205	226	252	272	303	379
STAN. DEV	18.8	19.1	19.7	19.5	24.6	31.8
NON PREGNANT ANIMALS						
00R 13058	183	205	247	226	238	253
00R 13076	192	213	236	254	278	270
00R 13079	196	218	228	244	255	266

Appendix VI

Oral Teratology Study of FC-95 in Rats
Individual Litter Data with Pup Weights

Dose Group and Rat No.	VIABLE FETUSES M	F	TOTAL	DEAD FETUSES	RESORPTION SITES	IMPLANTATION SITES	CORPRA LUTEA	MEAN FETUS AVG	WT (G) M	F
0 mg/kg/day										
N0R 12996	5	4	9	0	1	10	9	4.9	5.1	4.7
N0R 12997	4	9	13	0	1	14	16	3.6	3.8	3.5
N0R 12998	7	4	11	0	0	11	12	4.3	4.3	4.2
N0R 12999	7	4	11	0	2	13	13	4.0	4.1	3.9
N0R 13000	7	7	14	0	0	14	17	4.1	4.2	4.0
N0R 13016	4	5	9	0	1	10	9	3.7	3.3	4.0
N0R 13017	NOT PREGNANT									
N0R 13018	7	3	10	0	1	11	11	4.5	4.5	4.3
N0R 13019	6	1	7	0	0	7	6	5.1	5.1	4.9
N0R 13020	4	8	12	0	0	12	12	4.7	4.8	4.7
N0R 13036	5	5	10	0	1	11	8	4.1	4.3	3.9
N0R 13040	6	7	13	0	0	13	12	4.4	4.5	4.2
N0R 13041	6	3	9	0	1	10	12	4.2	4.3	4.1
N0R 13042	NOT PREGNANT									
N0R 13043	4	6	10	0	3	13	15	4.2	4.4	4.1
N0R 13044	5	2	7	0	0	7	11	3.9	3.8	3.9
N0R 13060	8	5	13	0	1	14	12	4.1	4.1	3.9
N0R 13061	4	6	10	0	2	12	11	4.2	4.3	4.1
N0R 13062	3	4	7	0	0	7	9	4.1	4.4	3.8
N0R 13063	1	5	6	0	0	6	9	4.3	4.3	4.3
N0R 13064	5	7	12	0	0	12	12	4.2	4.2	4.2
N0R 13080	6	2	8	0	1	9	9	4.4	4.4	4.2
MEAN	5.2	4.9	10.0	0.0	0.7	10.8	11.2	4.3		
STAN. DEV.	1.7	2.1	2.3	0.0	0.9	2.5	2.7	0.4		

Appendix VI (Continued)

Oral Teratology Study of FC-95 in Rats
Individual Litter Data with Pup Weights

Dose Group and Rat No.	VIABLE FETUSES M	F	TOTAL	DEAD FETUSES	RESORPTION SITES	IMPLANTATION SITES	CORPRA LUTEA	MEAN FETUS WT (GM) AVG	M	F
10 mg/kg/day										
00R 13001	4	6	10	0	1	11	10	4.5	4.6	4.5
00R 13002	3	6	9	0	2	11	11	3.9	4.1	3.8
00R 13003	4	7	11	0	0	11	12	4.2	4.4	4.1
00R 13004	7	2	9	0	0	9	9	4.2	4.2	4.0
00R 13005	5	7	12	0	0	12	12	4.3	4.3	4.2
00R 13021	1	3	4	0	0	4	7	4.4	4.4	4.4
00R 13022	11	2	13	0	0	13	14	4.2	4.2	3.8
00R 13023	2	0	2	0	0	2	5	4.8	4.8	0.0
00R 13024	NOT PREGNANT									
00R 13025	6	6	12	0	0	12	12	4.4	4.5	4.4
00R 13037	5	5	10	0	0	10	12	4.3	4.4	4.2
00R 13045	4	6	10	0	1	11	11	4.5	4.6	4.4
00R 13046	NOT PREGNANT									
00R 13047	NOT PREGNANT									
00R 13048	5	3	8	0	1	9	8	4.2	4.3	3.9
00R 13049	NOT PREGNANT									
00R 13065	0	1	1	0	0	1	6	4.3	0.0	4.3
00R 13066	4	8	12	0	0	12	11	4.0	4.2	3.9
00R 13067	1	1	2	0	1	3	8	4.6	4.8	4.5
00R 13068	NOT PREGNANT									
00R 13069	2	3	5	0	1	6	6	3.5	3.3	3.7
00R 13081	0	1	1	0	0	1	3	4.1	0.0	4.1
MEAN	3.8	3.9	7.7	0.0	0.4	8.1	9.2	4.3		
STAN. DEV.	2.8	2.6	4.3	0.0	0.6	4.3	3.1	0.3		

Appendix VI (Continued)

Oral Teratology Study of FC-95 in Rats
Individual Litter Data with Pup Weights

Dose Group and Rat No.	VIABLE FETUSES M	F	TOTAL	DEAD FETUSES	RESORPTION SITES	IMPLANTATION SITES	CORPRA LUTEA	MEAN FETUS WT (G) AVG	M	F
5 mg/kg/day										
P0R 13006	3	3	6	0	3	9	9	4.6	4.8	4.4
P0R 13007	9	3	12	0	0	12	12	4.2	4.4	4.0
P0R 13008	5	7	12	0	0	12	12	3.9	4.0	3.8
P0R 13009	5	4	9	0	0	9	8	3.8	4.1	3.5
P0R 13010	4	7	11	0	0	11	12	3.9	4.0	3.8
P0R 13026	NOT PREGNANT									
P0R 13027	8	3	11	0	2	13	10	4.0	4.1	3.8
P0R 13028	4	8	12	0	0	12	13	4.3	4.4	4.3
P0R 13029	4	3	7	0	0	7	10	4.8	5.2	4.3
P0R 13030	4	9	13	0	1	14	14	4.5	4.5	4.5
P0R 13038	5	5	10	0	0	10	10	4.4	4.7	4.2
P0R 13050	4	5	9	0	1	10	9	4.0	4.2	3.9
P0R 13051	4	7	11	0	2	13	12	4.3	4.4	4.2
P0R 13052	NOT PREGNANT									
P0R 13053	9	5	14	0	0	14	14	3.6	3.7	3.5
P0R 13054	4	6	10	0	0	10	11	4.2	4.3	4.1
P0R 13070	5	8	13	0	2	15	14	4.2	4.2	4.2
P0R 13071	3	6	9	0	1	10	9	4.4	4.7	4.3
P0R 13072	5	4	9	0	0	9	9	4.3	4.4	4.2
P0R 13073	NOT PREGNANT									
P0R 13074	NOT PREGNANT									
P0R 13082	NOT PREGNANT									
MEAN	5.0	5.5	10.5	0.0	0.7	11.2	11.1	4.2		
STAN. DEV.	1.9	2.0	2.2	0.0	1.0	2.2	2.0	0.3		

Appendix VI (Concluded)

Oral Teratology Study of FC-95 in Rats
Individual Litter Data with Pup Weights

Dose Group and Rat No.	VIABLE FETUSES M	F	TOTAL	DEAD FETUSES	RESORPTION SITES	IMPLANTATION SITES	CORPRA LUTEA	MEAN FETUS AVG	M	F
1 mg/kg/day										
00R 13011	7	3	10	0	0	10	8	4.4	4.5	4.4
00R 13012	5	6	11	0	0	11	12	4.1	4.1	4.0
00R 13013	3	6	9	0	0	9	11	4.4	4.4	4.3
00R 13014	5	7	12	0	0	12	13	3.4	3.8	3.2
00R 13015	4	6	10	0	0	10	9	3.8	3.8	3.8
00R 13031	7	6	13	0	0	13	14	4.0	3.9	4.1
00R 13032	1	1	2	0	0	2	4	3.8	4.3	3.3
00R 13033	4	9	13	0	0	13	14	4.5	4.6	4.5
00R 13034	2	4	6	0	3	9	8	5.0	5.1	4.9
00R 13035	5	5	10	0	1	11	11	4.6	4.7	4.4
00R 13039	6	6	12	0	0	12	12	4.3	4.4	4.2
00R 13055	7	4	11	0	1	12	12	4.3	4.2	4.3
00R 13056	5	6	11	1	1	13	11	4.1	4.3	4.0
00R 13057	4	5	9	0	1	10	12	3.9	3.8	3.9
00R 13058	NOT PREGNANT									
00R 13059	6	4	10	0	0	10	11	4.1	4.3	3.8
00R 13075	6	4	10	0	0	10	11	4.2	4.2	4.1
00R 13076	NOT PREGNANT									
00R 13077	3	5	8	0	1	9	9	4.4	4.4	4.4
00R 13078	5	5	10	0	0	10	10	4.6	4.9	4.4
00R 13079	NOT PREGNANT									
00R 13083	4	11	15	0	0	15	15	4.1	4.2	4.0
MEAN	4.7	5.4	10.1	0.1	0.4	10.6	10.9	4.2		
STAN. DEV.	1.7	2.1	2.8	0.2	0.8	2.7	2.6	0.4		

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Amendment to the Final Report of the Oral
Teratology Study of FC-95 in Rats

Experiment No.: 0680TR0008
Issued: 12/18/80

Please add the amended summary, the amended table 5, and the amendment to the results and discussion sections to the above report. The study conclusions were changed by this amendment to the report.

E E Gortner 7/26/82
E. G. Gortner Date
Senior Research Technologist
Animal Teratology Reproduction

Elden G Lamprecht 7-26-82
E. G. Lamprecht, DVM, PhD Date
Research Veterinary Pathologist

Maurice T Case 7/27/82
M. T. Case, DVM, PhD Date
Manager, Pathology-Toxicology
Safety Evaluation Laboratory

Amended Summary (p. 1) to the Oral Teratology Study of FC-95 in Rats
Experiment No. 0680TR0008

Oral administration of FC-95 at doses of 10, 5 and 1 mg/kg/day to pregnant Sprague-Dawley rats during days 6 through 15 of gestation (period of organogenesis) was not teratogenic.

FC-95 administration was maternally toxic only to the 10 mg/kg/day group. At gestation days 12 through 20 the maternal body weights of the high dose females were significantly lower than the controls. FC-95 was not maternally toxic to the 5 and 1 mg/kg/day groups.

FC-95 was not embryotoxic and did not affect the ovaries or reproductive tract contents of the dams. The compound did not produce an increase in the number or proportion of fetal skeleton variations.

Amendment to the Results and Discussion Sections (p. 3-5)
of the Oral Teratology Study of FC-95 in Rats

Experiment No. 0680TR0008

(This amendment addresses the last two paragraphs of the results section and the entire discussion section.)

FC-95 was labeled a teratogen of the lens because apparent lens abnormalities were observed at the 10, 5 and 1 mg/kg/day dose levels. Based on subsequent studies, particularly Riker Experiment No. 0681TR0362, the interpretations of these observations have been extensively modified. The lens findings observed under the dissecting microscope are now known to be either freehand sectioning artifacts or a normal area of lens cell degeneration. The fetal rat lens findings were incorrectly interpreted as a teratogenic change in this study.

The gross finding of a lens cleft was an artifact created by freehand sectioning. It represents a separation between the embryonal nucleus lens cells and the lens epithelium. The gross finding of a lens dark streak was a normal observation of the embryonal nucleus. The embryonal nucleus is an area of normal lens cell degeneration in the gestation day 20 fetus.

The gross appearance of the rat lens at day 20 of gestation is determined by the region of the lens which is transected by freehand sectioning. In a subsequent study (Riker Experiment No. 0681TR0362) the compound-related occurence of the lens findings could not be repeated when the fetuses were coded before freehand sectioning and gross evaluation. The range of gross lens observations and the differences among the dose group incidences were due to the manner and frequency in which the lens cleft artifact was created by freehand sectioning and the limitations inherent in visualizing the embryonal nucleus.

In summary, FC-95 <u>in utero</u> exposed fetuses did not have compound-related changes in their lenses.

Amended Table 5 (p. 10)

Oral Teratology Study of FC-95 in Rats
Number and Percent of Fetuses with Internal Findings

Internal Finding	0 mg/kg/day	10 mg/kg/day	5 mg/kg/day	1 mg/kg/day
Lens findings[a]	1 (2)	14 (35)[b]	4 (7)	2 (3)
Thoracic cavity full of blood	---	1 (3)	---	---
Enlarged atria	1 (2)	---	---	---
Enlarged renal pelvis	3 (5)	---	---	3 (5)
Abdominal cavity full of blood	4 (7)	2 (5)	5 (9)	2 (3)
No. of Fetuses Examined	61	40	55	59

[a] The lens findings observed under the dissecting microscope were either freehand sectioning artifacts or a normal area of lens cell degeneration

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

( ) = percent of total examined

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Amended Appendix VII

STATEMENT OF QUALITY ASSURANCE

STUDY NUMBER: Amendment to 0680TR0008

TITLE: Amendment to the Final Report of the Oral Teratology Study of FC-95 in Rats

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

Date Performed	Date Reported
July 16 and 19, 1982	July 21, 1982
July 22, 1982	July 23, 1982

Gab E Van Buskirk
Compliance Audit
Riker Laboratories, Inc.

Date July 23, 1982

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