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

Experiment No.:	0681TR0362
Conducted At:	Safety Evaluation Laboratory Riker Laboratories, Inc. St. Paul, Minnesota
Dosing Period:	September 28, 1981 through October 8, 1981
Study Director:	E. G. Gortner

E. G. Gortner 7/19/82
E. G. Gortner Date
Senior Research Technologist
Animal Teratology Reproduction

Eldon G Lamprecht 7-16-82
E. G. Lamprecht, DVM, PhD Date
Research Veterinary Pathologist

Marvin T Case 7/19/82
M. T. Case, DVM, PhD Date
Manager, Pathology-Toxicology
Safety Evaluation Laboratory

William C McCormick 7.20.82
W. C. McCormick, MS Date
Toxicologist
Toxicology Services

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SUMMARY

In a previous rat teratology study of a fluorochemical alcohol, FM-3422, gestation day 20 gross lens findings on uncoded freehand sectioned fetuses were interpreted as compound and dose related abnormalities. The objective of this current study was to compare the histological appearance of fetal rat lenses that had been freehand sectioned to the histological appearance of lenses which had not been freehand sectioned. Groups of thirty time-mated Crl:CD®(SD)BR and CDF®(F-344)/CrlBR rats were orally dosed at 0 and 25 mg/kg/day with T-2999CoC, a comparable alcohol, on days 6 through 15 of gestation. Four fetuses from each of 20 litters per group were selected. The identities of the fetuses were concealed from the investigators by coding. Gross lens observations were made on freehand sections of two fetuses per litter. Microscopic lens observations were made on all four coded fetuses following microtome sectioning and tissue staining.

T-2999CoC caused maternal toxicity (low body weight gain) in the pregnant dams of both rat strains. The compound did not affect either the gross or microscopic lens appearance of fetuses from treated dams. The compound-related occurrence of lens findings reported in the previous rat teratology study could not be repeated when the fetuses were coded before freehand sectioning and gross evaluation.

The gross finding of a lens cleft and the microscopic finding of a space at the lens vesicle vestige are artifacts created independently by freehand and microtome sectioning. They represent a separation between the embryonal nucleus lens cells and the lens epithelium.

The gross finding of a lens dark streak is a normal observation of the embryonal nucleus. The embryonal nucleus is an area of normal lens cell degeneration located in the central region of the lens. Lens cell degeneration was present microscopically in every lens sectioned across the central region of the lens.

Introduction

A Toxic Substances Control Act (TSCA) Section 8(e) notice was filed by the 3M Company based on rat teratology study results on FM-3422[a]. Partly responsible for filing the 8(e) notice was the observation of a fetal lens finding which was found only in the treatment groups. The observation was reported as a teratogenic change because it was found only in compound treated dose groups and had a dose-related incidence of occurance.

T-2999CoC (FM-3924) was selected for use in the current study because it is more representative of present production than is FM-3422. Further, T-2999CoC potentially presents greater risk than FM-3422 because of the somewhat higher content of more soluble lower homologues.

The protocol for the current study was originally designed with two objectives. The first objective was to report the effect of T-2999CoC treatment and the strain of rat on the incidence of the lens change. The second objective was to compare the incidence of lens changes of fetuses not exposed in utero to dosing and animal handling procedures used in teratology studies with the incidence of lens changes in the CD® control group. The animals were dosed and the dams were terminated. During this time, the laboratory gained insight into normal and abnormal lens development. As the result of discussions with scientists from industry, government and academia, the lens change originally reported in previous studies was thought to be an artifact of freehand sectioning, and the protocol was amended to test this hypothesis.

The objective of this study was changed to compare the histological appearance of gestation day 20 fetal rat lenses that have been freehand sectioned and then processed for microscopy to the microscopic appearance of gestation day 20 fetal rat lenses which have not been freehand sectioned before being processed for microscopy.

The study was sponsored by 3M Commercial Chemical Division, St. Paul, Minnesota and was conducted by the Safety Evaluation Laboratory, Riker Laboratories Inc., St. Paul, Minnesota. The two compound administration groups were dosed between September 28, 1981 and October 8, 1981. The protocol and list of principal participants can be found in Appendices I and II respectively.

All portions of the study were conducted according to the Good Laboratory Practice (GLP) regulations[b] and Safety Evaluation Laboratory Standard Operating Procedures. Appendix III contains the 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.

[a] FM-3422 is at least 96% $C_8F_{17}SO_2N(C_2H_5)CH_2OH$ (Riker Experiment No. 0680TR0010) and T-2999CoC (FM-3924) is about 88% $C_8F_{17}SO_2N(C_2H_5)CH_2OH$; in the remaining 11%, the perfluoroalkyl group contains three to seven carbon atoms.

[b] Federal Register, Vol. 43, No. 237, December 22, 1978 pp 60013-60025.

Materials and Methods

Ninety Crl:CD®(SD)BR rats[c] (CD®) and sixty CDF®(F-344)/CrlBR rats[d] (CDF®) were assigned cages according to a computer-generated random numbers table. They were sexually mature time-mated females weighing between 163-229g and 127-163g respectively (Appendix IV). The rats were housed individually in suspended, wire mesh, stainless steel cages in a temperature and humidity controlled room. They were identified by ear tags with that identification number indicated on the outside of the cage. The CD® non-treated group was identified only by a number on the outside of the cage. Feed[e] and water were available ad libitum. The lights were on a 12 hour light/dark cycle.

The rat was chosen because previous teratology studies on FM-3422 were conducted in this species. The 25 mg/kg/day dose level was selected because it gave a positive response to the lens finding in the previous teratology study.

The T-2999CoC composition, purity and stability were determined by the 3M Commercial Chemical group (Appendix VII). The test article was suspended in corn oil[f] daily. The test article solutions and the corn oil control article were administered by oral intubation to rats on days 6 through 15 of gestation[g] according to the following experimental design for dosing:

Rat Strain and Dose Group	T-2999CoC Dose Level	Group Size
CD® corn oil control article	0 mg/kg/day	30
CDF® corn oil control article	0 mg/kg/day	30
CD® T-2999CoC in corn oil	25 mg/kg/day	30
CDF® T-2999CoC in corn oil	25 mg/kg/day	30
CD® Non-treated group	rats not dosed	30

[c]Supplied by Charles River Breeding Laboratories, Inc., North Wilmington, MA.
[d]Supplied by Charles River Breeding Laboratories, Inc., Stoneridge, NY.
[e]Purina Laboratory Chow, Ralston Purina Company, St. Louis, MO.
[f]Supplied by Welch, Holmes and Clark, Harrison, NJ. - NF Quality.
[g]Day sperm detected is day 0 of gestation.

The T-2999CoC treated and control group animals were observed daily from day 3 through day 20 of gestation for abnormal clinical signs. The dams were weighed on days 3, 6, 9, 12, 15 and 20 of pregnancy. They were dosed according to the most recent body weight using a constant dose volume of 5 ml/kg of body weight. The CD® non-treated group was observed only for a live/dead check from days 3 through 20 of gestation.

All dams were terminated by cervical dislocation on day 20 of gestation. All of their fetuses were fixed in Bouin's solution. At least 23 dams in each group had four or more fetuses. Four fetuses from each of 20 litters from the CD® and CDF® T-2999CoC and corn oil control article groups were selected. The identities of the fetuses were concealed from the investigators by coding. Heads of two of the coded fetuses from each of the 20 litters were sliced in frontal-section through the center of the eye bulges and through the widest portion of the head. Freehand section gross lens observations, made with the aid of a 10-40X dissecting microscope, were recorded. The head sections bisecting the eye bulges were processed for histological observations after paraffin embedding the cut eye bulge surface down (Tables 2, 3, 4 and Tissue Processing Procedure #2 of Table 5). Heads of the remaining two coded fetuses from each of the 20 litters were sliced behind the eye bulges. The eye bulge-containing specimens were processed for histological observation by paraffin embedding the brain surface down. The specimens bisecting the eye bulge by freehand slicing were microtome sectioned to provide a histological evaluation of the same surface seen under the dissecting microscope. The specimens sliced behind the eye bulges were microtome sectioned to provide a histological evaluation of the center of the lens (Tissue Processing Procedure #1 of Table 5). The embedded specimens were sectioned at 5-6 microns and stained with hematoxylin and eosin. Histology observations were recorded. Fetuses from the CD® non-treated group were not evaluated.

The following is an experimental design outline for tissue processing and reporting fetal lens observations:

Rat Strain	T-2999CoC Dose Level mg/kg/day	2 Fetuses of 20 Litters Freehand Sectioned	2 Fetuses of 20 Litters Microtome Sectioned	No. Lenses Evaluated
CD®	0		X	80
	0	X	X	80
	25		X	80
	25	X	X	80
CDF®	0		X	68[h]
	0	X	X	66[h,i]
	25		X	80
	25	X	X	80

[h]Data from fetuses of dams N1R7521, 7523 and 7524 were not evaluated (Appendix I).
[i]Data from one fetus of dam N1R7533 was lost in transcription.

The individual lens, rather than the fetus, was the experimental unit. The results of the fetus as a possible experimental unit were also reported and compared with the results of the individual lens experimental unit. The chi-square test was used to statistically compare the incidence data generated by gross and microscope observations and to evaluate the affect of T-2999CoC on fetal lenses. The Dunnett's T test was used to evaluate body weight data. Statistical comparisons were made within each respective rat strain. The level of statistical significance was $p < 0.05$.

Results

The administration of 25 mg/kg/day of T-2999CoC to pregnant dams was maternally toxic in both strains of rats. Their mean body weights were significantly lower than that of controls on gestation days nine until termination for the CD® strain and on gestation days 9, 12, and 15 in the CDF® strain (Table 1).

T-2999CoC administration to pregnant dams did not affect the gross appearance of the pre-term fetal lens. Gross findings of a lens cleft at the anterocentral region and a dark streak in the center of the lens were reported in this study (Table 2). The percent of lenses with clefts or dark streak findings were not significantly different between the control and treated groups for the CD® strain. In the case of the CDF® 25 mg/kg/day group, the dark streak was visualized significantly more often than in the CDF® control group.

The individual lens was a more representative experimental unit than the individual fetus for reporting gross lens observations. Greater than 74 percent of the time, the lens cleft and streak findings were reported unilaterally in individual fetuses within each study group (Table 2).

The range of agreement between the gross lens findings and the microscopic findings was highly variable. In general, there was a within strain trend for a high proportion of microscopic confirmations of a dark streak and for a low proportion of confirmations of the lens cleft (Table 3). In utero exposure to T-2999CoC did not affect the ratios of confirmation of gross with microscopic findings in either strain of rat.

When the number of gross lens findings confirmed microscopically were compared with the total number of microscopic observations, low ratios of agreement resulted (Table 4). Two factors were largely responsible for the low ratios of agreement for the embryonal nucleus finding; the large numbers of microscopic observations of that structure (Table 4) and the small numbers of gross observations of the dark streak (Table 3). The same two factors were responsible for the low ratios of agreement for the lens vesicle space finding with one exception. Ten gross lens clefts and eight microscopic lens vesicle spaces were observed in the CDF® 25 mg/kg/day dose group. However, only two of the gross observations were confirmed microscopically.

The gross evaluation of freehand sectioned fetal lenses has limitations as a technique for assessing lens morphology. In the CD® fetuses, over half of the lenses were sectioned across the embryonal nucleus (Table 5) and were therefore at risk of having the gross observation of a dark streak. Only a small portion of the lenses at risk had a dark streak on gross examination while a majority of the lenses were reported as having no visible change (Table 2). A majority of lenses reported to be not sectioned based on gross observations had microscopic findings of sectioned lenses (Appendix V).

Lenses noted on the gross examination to have a dark streak were not different in appearance microscopically from the lenses sectioned through the embryonal nucleus. The dark streak was not an abnormality. Rather, its presence was indicative of the embryonal nucleus being occasionally visualized when the lens was freehand sectioned through the central region. The gross lens cleft and microscopic lens vesicle space are artifacts generated by either freehand or microtome sectioning. The sectioning artifacts appear to be generated independently by both methods of sectioning.

Both freehand sectioning and microtome sectioning were very effective in transecting the fetal lenses. Between 85 and 100 percent of the lenses were transected in this study (Table 5). The remainder were lost in tissue processing, shattered beyond interpretation or had foreign material debris rendering them not readable. There was a trend for the combined freehand and microtome sectioned lenses to be lost, shattered or contain foreign material more often than lenses only microtome sectioned. To a limited degree the method of tissue processing did influence the incidence of microscopic lens observations.

Identifying the region of sectioning within the lens is possible based microscopically on morphological differences between the lens regions. One distinguishing characteristic of every gestation day 20 fetal lens sectioned through the central region was the presence of the embryonal nucleus; an area of lens cell degenerative change. The lens vesicle is an open space created by a separation of the embryonal nucleus lens cells from the subcapsular epithelium at the lens vesicle vestige. T-2999CoC administration to pregnant dams did not affect the microscopic appearance of pre-term lenses of their fetuses (Table 5).

Discussion

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 probability 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.[1]

During the early stages of the inductive process, the ectodermal cells immediately overlying the tip of the optic vesicle elongate perpendicularly to the body surface to form a thickened disc called the 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 lens pore opening 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 temporary 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[1].

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 embryonal nucleus. With time the lens cell nuclei in this region become pyknotic 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[1].

The microscopic appearance of the gestation day 20 fetal rat lens is dependent on the region sectioned. When sectioned at the equator region, the lens is characterized by a regularity of curved fibers extending between the cuboidal anterior lens epithelium and the posterior lens capsule. The lens bow is a prominant line of viable cell nuclei originating at the lens equator and coursing through the acentric region toward the anteriocentral portion of the lens. The acentric region lies between the equatorial region and the region containing the embryonal nucleus. When sectioned through the acentric region, the lens is characterized by a regularity of curved fibers also extending between the cuboidal anterior lens epithelium and the posterior lens capsule. Although there is posterior pole suture development at this point in development, it is not conspicuous. Suture development is not present at the anterior pole because of the anterior placement of the embryonal nucleus and because secondary lens cells of the fetal nucleus have not converged. The lens bow of the acentric region is located more anteriorly and appears less densely populated than at the equator because of lens cell elongation.

Sections through the central region contain the more mature cells of the embryonal nucleus. The lens bow in the embryonal nucleus progressively disappears as the nuclei lose viability. The lens cells are in various stages of degeneration within the embryonal nucleus. A vestige of the lens vesicle covers a majority of the anterior embryonal nucleus surface. The lens vesicle vestige serves as the line of apposition between the embryonal nucleus and the lens epithelial cells.

The embryonal nucleus of gestation day 20 fetal lenses is an area of normal lens cell degeneration. Lens cell degeneration was present in every lens sectioned across the central region. Some morphological components of the normal degenerative changes are as follows: loss of order of formerly linearly arranged lens fibers, smaller lens fibers, granular-appearing cytoplasm and nuclear pyknosis. Minor variations in the progression of lens cell degeneration are possibly due to differences in fetal age and the proximity of the section to the center or to the periphery of the embryonal nucleus. The embryonal nucleus was occasionally visualized and reported on gross observation to be a dark streak in the lens.

Spurious microscopic observations of fetal lens findings, which are not hereditary or are not caused by in utero compound exposure, have been reported in rats. Varying stages of lens cell degeneration have been detected in the term Sprague-Dawley rat lens.[2] These findings were interpreted to be compatible with stages of normal lens degeneration previously reported as being congenital or were typical responses to various natural and experimental influences. The incidence reported in gestation day 19 and 20 fetuses was less than 4 percent. None of the findings were considered deleterious to the developing rat fetus or lens. A 3.6 percent incidence of fetuses with congenital degenerative lens findings was reported in gestation day 18 Chbb:THOM (SPF) rat fetuses.[4] These findings included individual lens cells or small groups of lens cells with swelling, disintegration and formation of vacuoles in their cytoplasm. Since lens cell degeneration is a normal development change which initially occurs in the embryonal nucleus, the lens findings of these two reports could have been normal observations made on lenses sectioned through the embryonal nucleus. An increase in the incidence could result from making observations on older gestation age fetuses and with an increase in frequency of sectioning through the embryonal nucleus. Both reports labeled their findings as cataracts without seeing a lens opacity in pups following parturition. Technically the term cataract is applied to an obvious opacity or an opacity that can be seen by focal illumination with a slit lamp.[3]

The gross finding of a lens cleft and the microscopic finding of a space at the lens vesicle vestige are artifacts created by freehand and microtome sectioning. The spaces were observed microscopically only in sections which also contained the embryonal nucleus. The absence of attachment of the embryonal nucleus cells to the lens epithelium plus the difference in consistency between the embryonal nucleus and secondary lens cells predispose the lens vesicle vestige to separation once transected. Low ratios of confirmation and agreement of gross with microscopic occurrance of the separations suggest that the artifact is highly subject to tissue processing as well as being independently created by freehand and microtome sectioning.

It is possible to freehand and microtome section through fetal lenses with an equally high level of proficiency. Gross observations of freehand sectioned fetuses however are unsatisfactory for evaluating the lens when compared with microscopic observations. Gross observations are limited in not being able to define both the region in which lenses are sectioned and the morphological characteristics responsible for the lens findings reported.

The experimental design for evaluation of fetal lenses in this study differed in the following three ways from the previous teratology study of the FM-3422 compound: 1) all fetuses were coded before freehand sectioning for gross and microscopic observation, 2) all fetuses provided for in the protocol were evaluated microscopically, and 3) the lens, rather than the fetus, was the experimental unit.[1] Compound related gross lens findings were reported in the 25 mg/kg/day dose group in the previous teratology study when the fetuses were read uncoded. These compound-related occurrences were not repeated when the fetuses were coded before freehand sectioning and gross evaluation.

[1]Riker Experiment Number 0680TR0010.

T-2999CoC administration to pregnant dams during the period of organogenesis did not affect either the gross or microscopic lens appearance of their pre-term fetuses. This conclusion is consistant with the absence of lens abnormalities observed in lactation day 21 pups similarly exposed to T-2999CoC at the same dose level.[k]

[k]Riker Experiment Number 0680TR0020.

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WP:rm/26n

TABLE 1

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

Mean Maternal Body Weights(g) With Standard Deviations Listed By Gestation Day in Two Strains of Rats and Two Dose Levels of T-2999CoC.

Rat Strain	Dose (mg/kg/day)	Number Of Dams	Gestation Day 3	6	9	12	15	20
CD®	0	24	202 ±16	226 ±16	247 ±18	277 ±19	303 ±21	378 ±30
	25	28	197 ±14	221 ±15	237[a] ±16	258[a] ±19	280[a] ±21	351[a] ±25
CDF®	0	26	142 ±6	155 ±8	161 ±7	171 ±9	177 ±16	209 ±20
	25	27	140 ±7	154 ±7	156[a] ±7	156[a] ±9	163[a] ±12	201 ±17

[a]The treatment group weights were significantly lower than the control group weights within each strain of rat (Dunnett's T Test $p < 0.05$).

WP:rm/26m5

TABLE 2

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

Number and Percent[a] of Freehand Section Gross Fetal Lens Findings For Individual Lens[b] and Fetus[c] Experimental Units In Two Strains of Rats and Two Dose Levels of T-2999CoC.

Rat Strain	Dose (mg/kg/day)	FREEHAND SECTION GROSS FINDINGS[d] (Lens Experimental Unit) Lens Cleft[f]	Dark Streak[g]	No Visible Change	Lens Not Sectioned
CD®	0 mg	15 (19)	4 (5)	60 (75)	5 (6)
CD®	25	20 (25)	9 (11)	42 (53)[h]	15 (19)[h]
CDF®	0[e]	6 (9)	5 (8)	40 (61)	15 (23)
CDF®	25	10 (13)	17 (21)[h]	43 (54)	14 (18)
		(Fetus Experimental Unit)		Fetuses With Dark Streak or Lens Cleft Findings	
CD®	0	11 (28)	3 (8)	11 (28)	
CD®	25	17 (43)	8 (20)	19 (48)	
CDF®	0[e]	5 (15)	5 (15)	8 (24)	
CDF®	25	8 (20)	14 (35)	18 (45)	

[a] Percent in parentheses ( ).

[b] n = 80 lenses.

[c] n = 40 fetuses.

[d] It is possible to have one or both gross findings (lens cleft or dark streak) in one or both lenses of an individual fetus.

[e] n = 66 lenses and n = 33 fetuses.

[f] A lens cleft is a space at the anterocentral region of the fetal lens.

[g] A dark streak is a dark colored area in the central region of the lens.

[h] The treatment group was significantly different from the control group within the experimental unit and within the strain of rat (chi-square with Yates correction $p < 0.05$).

WP:rm/26m2

TABLE 3

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

Ratios[a] and Percent[b] of Freehand Section Gross Lens Findings Confirmed Microscopically For Individual Lens[c] and Fetus[d] Experimental Units in Two Strains of Rats and Two Dose Levels of T-2999CoC.

Rat Strain	Dose (mg/kg/day)	Freehand Section Gross Findings[fg]			
		Lens Cleft[h]		Dark Streak[i]	
		Individual Lens	Individual Fetus	Individual Lens	Individual Fetus
CD®	0	8/15 (53)	6/11 (55)	4/4 (100)	3/3 (100)
	25	9/20 (45)	9/17 (53)	6/9 (67)	6/8 (75)
CDF®	0[e]	2/6 (33)	2/5 (40)	2/5 (40)	2/5 (40)
	25	2/10 (20)	2/8 (25)	11/17 (65)	11/14 (79)

[a] Ratios are the number of gross observation findings recorded following freehand sectioning and confirmed microscopically, divided by the number of gross observation findings.

[b] Percent in parentheses ( ).

[c] n = 80 lenses.

[d] n = 40 fetuses.

[e] n = 66 lenses and n = 33 fetuses.

[f] It is possible to have one or both gross findings (lens cleft or dark streak) in one or both lenses of an individual fetus. A fetus with one or two similar findings is counted only once.

[g] The treatment group ratios of gross findings confirmed microscopically were not significantly different from the control group ratios within each strain of rat (chi-square with Yates correction $p < 0.05$).

[h] A lens cleft is an observed space in the anterocentral region of the fetal lens. It is confirmed microscopically by the presence of a space between the subcapsular epithelium and the lens cells of the embryonal nucleus. Microscopically the space is a separation at the lens vesicle vestige.

[i] A dark streak is an observed dark colored area in the central region of the lens. It is confirmed microscopically by the presence of the embryonal nucleus.

WP:rm/26ml

TABLE 4

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

Ratios[a] of and Percent[b] Agreement of Gross Individual Lens Findings[c] With Microscopic Lens Findings in Two Strains of Rats and Two Dose Levels of T-2999CoC.

Rat Strain	Dose (mg/kg/day)	Individual Lens Microscopic Findings[e]	
		Lens Vesicle Space[f]	Embryonal Nucleus[g]
CD®	0	8/27 (30)	4/40 (10)
	25	9/27 (30)	6/44 (14)
CDF®	0[d]	2/10 (20)	2/20 (10)
	25	2/8 (25)	11/28 (39)

[a] Ratios are the number of agreements between gross findings recorded following freehand sectioning and microscopic findings divided by the number of microscopic findings.

[b] Percent in parentheses ( ).

[c] n = 80 lenses.

[d] n = 66 lenses.

[e] The treatment group ratios of agreement between gross and microscopic lens findings were not significantly different from the control group ratios within each strain of rat (chi-square with Yates correction $p < 0.05$).

[f] A lens vesicle space is an open space created by a separation of the embryonal nucleus lens cells from the subcapsular epithelium at the lens vesicle vestige. The ratio is the number of agreements between the gross finding of a lens cleft and the microscopic finding of a lens vesicle space divided by the number of lens vesicle spaces observed.

[g] An embryonal nucleus is an area of normal degenerative change located in the center of the lens. The ratio is the number of agreements between the gross finding of a dark streak and the microscopic finding of an embryonal nucleus divided by the number of embryonal nuclei observed.

TABLE 5

Special Lens Oral Teratology Studyof T-2999CoC
in Two Strains of Rats

Number and Percent[a] of Fetal Lenses[b] With Microscopic Findings Listed by Lens Region in Two Strains of Rats, Two Levels of T-2999CoC and Two Tissue Processing Procedures With an Index of Lens Sectioning Proficiency.

			Microscopic Findings And Lens Region Sectioned						
			Central Region						
Rat Strain	Dose (mg/kg/day)	Tissue[d] Processing Procedure	Lens Vesicle[e] Space	Embryonal[f] Nucleus	Acentric Region	Equatorial Region	Lens Missing	Lens Shattered[g] or Foreign Material	Index of Lens[h] Sectioning Proficiency
CD●	0	1	18 (23)	33 (41)	45 (56)	2 (3)	0 (0)	2 (3)	(100)
		2	27 (34)	40 (50)	32 (40)	4 (5)	4 (5)	5 (6)	(95)
CD●	25	1	23 (29)	39 (49)	33 (41)[i]	4 (5)	2 (3)	6 (8)	(95)
		2	27 (34)	44 (55)	26 (33)[i]	6 (8)	2 (3)	12 (15)[i]	(95)
CDF●	0	1[c]	9 (13)	20 (29)	44 (65)	2 (3)	0 (0)	10 (15)	(100)
		2[c]	10 (15)	20 (29)	37 (54)	6 (9)	2 (3)	15 (22)	(93)
CDF●	25	1	4 (5)	27 (34)	45 (56)[i]	5 (6)	2 (3)	11 (14)	(96)
		2	8 (10)	28 (35)	31 (39)[i]	9 (11)	6 (8)	18 (23)	(85)

[a] Percent in parentheses ( ).
[b] n = 80 lenses.
[c] n = 68 lenses.
[d] Tissue Processing Procedure #1 tissues were processed for microscopy. The lenses were not freehand sectioned. Tissue Processing Procedure #2 lenses were freehand sectioned before being processed for microscopy.
[e] A lens vesicle space is an open space created by a separation of the embryonal nucleus lens cells from the

Appendix I

TITLE: Protocol for Special Lens Oral Teratology Study of T-2999CoC[a] in Two Strains of Rats
(Riker Experiment Number 0681TR0362).

OBJECTIVE: Changes were detected in day 20 fetal lenses of Sprague-Dawley rats in teratology studies run on T-2999CoC. The Sprague-Dawley strain of rat may be predisposed to develop the lens change. In addition, the threshold for development of the lens change may be lowered by handling procedures used in conducting teratology studies. One objective of this study is to compare the incidence of the lens change in fetuses of both control and T-2999CoC treated Sprague-Dawley and Fisher strain dams. The second objective is to compare the incidence of lens changes of fetuses not exposed in utero to dosing and handling procedures used in teratology studies (Sprague-Dawley non-treated group) with the incidence of lens changes in the Sprague-Dawley control group. 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: Fourth quarter, 1981

TEST SYSTEM: Ninety sexually mature, time mated Sprague-Dawley derived female rats and sixty Fisher 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. These strains of rats will be used because time mated females are readily available and they will provide a comparison between two strains. 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. The Sprague-Dawley non-treated group will be identified only by a number 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] FM-3924

Appendix I (Continued)

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

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

Strain	Dose Level	Group Size
Sprague-Dawley	25 mg/kg/day	30
Fisher	25 mg/kg/day	30
Sprague-Dawley	0 mg/kg/day	30
Fisher	0 mg/kg/day	30
Sprague-Dawley	No treatment	30

The oral route of administration will be used because it is the route of exposure in previous studies in which lens changes were observed. No dietary contaminants are known to interfere with the test article.

The T-2999CoC treated and control group 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 Sprague-Dawley non-treated group will only be observed for a live/dead check from days 3 through 20 of gestation. 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 could be sent to histopath for microscopic examination.

DATA ANALYSIS AND FINAL REPORT: The proposed statistical method to be used for analysis of the data is the Fisher exact probability and Chi-square for percent of abnormalities. The proposed date for the final report is 2-3 months after the pup eye exams have been completed (approximately fourth quarter, 1981).

Appendix I (Continued)

Amendment for Special Lens Oral Teratology Study of T-2999CoC in Two Strains of Rats

The subject of this study, a fetal lens change, was thought to be a strain-related phenomenon which was possibly precipitated by stress of handling procedures used in teratology studies. Since the original protocol issued, the laboratory has gained insight into the development of the lens change. The change is now thought to be an artifact of freehand sectioning by the Wilson Technique. This amends the protocol consistant with studying the lens change as an artifact.

OBJECTIVE: (Amendment replaces the Objective section of the procotol.) Changes were detected in gestational day 20 fetal lenses of Sprague-Dawley rats in teratology studies run on T-2999CoC. The objective of this study is to report the comparison of the histological appearance of gestational day 20 fetal rat lenses that have been freehand sectioned by the Wilson technique and then processed for histology with the histological appearance of gestational day 20 fetal rat lenses which have not been freehand sectioned before being processed for histology. The comparison will be done on Sprague-Dawley and Fisher rat strains.

DOSAGE LEVELS AND EXPERIMENTAL DESIGN: (Amendment replaces the last three sentences.) All of the fetuses will be fixed in Bouin's solution. Four fetuses from each of 20 litters from the Sprague-Dawley and Fisher T-2999CoC and control vehicle treated groups will be coded before being processed for dissecting microscopic and histological evaluations. Two of the coded fetuses from each of the litters under consideration will have their heads freehand sectioned by the Wilson Technique. The freehand section lens observations will be recorded. The head sections bisecting the eyes will then be processed for histological observation after embedding with the brain side down. The remaining two coded fetuses from each of the litters under consideration will have their heads freehand sectioned behind the eyes. The sections will be processed for histological observations by paraffin embedding with the brain side down. The embedded specimens will be sectioned at 5-6 microns and stained with hematoxylin and eosin. Histology observations will be recorded.

DATA ANALYSIS AND FINAL REPORT: (Amendment replaces this section of the protocol.) The experimental unit is the individual lens. The statistical test for incidence data will be the Chi-square test. The proposed date for the final report is the first quarter, 1982.

Appendix I (Concluded)

Protocol Deviations

CDF® animals NIR7521, 7522, 7523 and 7524 were mistakenly dosed with T-2999CoC at 25 mg/kg/day on gestation day 13. Fetuses of animals NIR7521, 7523 and 7524 were selected for lens evaluation. Since they were control group fetuses and they had been exposed to T-2999CoC in utero, their data was excluded from analysis. The fetuses affected by this protocol deviation are listed by dam as follows:

NIR7521	225	226	227	228
NIR7523	89	90	91	92
NIR7524	85	86	87	88

This deviation left 68 lenses subject to microscopic evaluation in the CDF® 0 mg/kg/day group. Table 5 reflects the absence of these twelve fetuses. Tables 2, 3 and 4 also reflect their absence plus the absence of fetus 94 from the CDF® 0 mg/kg/day group whose gross observation data were lost in transcription.

Gross and microscopic lens observations were not made on CD® non-treated group fetuses.

Appendix II

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

List of Principal Participating Personnel

Name	Function
Edwin G. Gortner	Study Director
Elden G. Lamprecht	Veterinary Pathologist
Wm. C. McCormick	Toxicology Services Representative
Gary C. Pecore	Supervisor of Animal Care
Inara Porietis	Histopathology Technologist
Loren O. Wiseth	Technician

APPENDIX III

STATEMENT OF QUALITY ASSURANCE

STUDY NUMBER: 0681TR0362

TITLE: Special Lens Oral Teratology Study of T-2999CoC in Two Strains of 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
October 7, 1982	October 14, 1981
October 12, 1981	October 21, 1981
March 22, 1982	March 29, 1982
June 4,7,8, 1982	June 17, 1982
June 28-29, 1982	July 15, 1982
July 15, 1982	July 16, 1982

Carl E. Van Buskirk
Compliance Audit
Riker Laboratories, Inc.

July 16, 1982
Date

Appendix IV

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

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

CD®

	DAY	3	6	9	12	15	20
0 MG/KG/DAY							
N1R	7802	194	218	234	268	298	379
N1R	7803	181	203	216	247	273	337
N1R	7804	199	221	239	271	303	380
N1R	7805	196	223	237	274	300	369
N1R	7816	175	200	218	248	272	349
N1R	7818	201	229	255	288	327	422
N1R	7819	201	219	237	264	292	361
N1R	7820	178	198	221	243	255	326
N1R	7831	229	258	284	318	345	451
N1R	7832	206	234	257	289	304	385
N1R	7833	215	241	264	293	321	396
N1R	7834	176	207	232	266	290	363
N1R	7835	198	222	238	265	300	383
N1R	7846	204	221	240	266	290	335
N1R	7847	189	216	240	271	302	373
N1R	7848	218	247	270	299	316	347
N1R	7849	211	231	255	281	299	377
N1R	7850	220	246	270	302	321	395
N1R	7861	212	244	264	303	333	414
N1R	7862	219	246	262	289	317	400
N1R	7863	209	237	260	288	320	415
N1R	7864	182	209	225	254	279	363
N1R	7879	202	222	243	272	299	371
N1R	7880	225	239	261	292	317	391
MEAN		202	226	247	277	303	378
STAN. DEV		15.7	16.3	18.2	19.4	20.9	29.6

NON PREGNANT ANIMALS

N1R	7801	171	201	214	210	224	237
N1R	7817	212	241	256	278	270	286
N1R	7865	203	215	224	236	238	253
N1R	7876	200	229	253	272	262	284
N1R	7877	210	230	243	259	271	291
N1R	7878	209	233	242	245	252	258

Appendix IV (Continued)

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

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

CD®

	DAY	3	6	9	12	15	20
25 MG/KG/DAY							
01R	7806	210	232	254	280	309	390
01R	7807	188	213	226	252	279	344
01R	7808	184	212	224	248	266	336
01R	7809	193	216	227	249	258	323
01R	7810	211	239	264	283	307	388
01R	7822	210	230	251	277	308	382
01R	7823	180	209	219	240	263	334
01R	7824	163	181	197	217	237	297
01R	7825	185	208	232	261	280	361
01R	7836	187	208	232	243	254	318
01R	7837	215	243	261	294	326	389
01R	7838	186	210	224	251	275	337
01R	7839	187	208	222	249	269	329
01R	7840	192	207	216	233	255	316
01R	7851	205	234	250	262	279	353
01R	7852	191	212	236	252	274	346
01R	7853	216	234	259	281	306	382
01R	7854	193	213	230	252	275	343
01R	7855	198	223	241	257	270	344
01R	7866	211	244	237	283	312	388
01R	7867	201	227	234	250	270	348
01R	7868	198	227	240	264	271	354
01R	7869	227	252	268	288	305	383
01R	7870	213	239	252	272	290	370
01R	7881	203	226	243	260	281	362
01R	7882	198	219	241	260	277	345
01R	7883	187	212	226	245	268	326
01R	7885	191	213	234	231	263	348
MEAN		197	221	237	258	280	351
STAN. DEV		13.7	15.3	16.2	18.6	21.0	25.3
NON PREGNANT ANIMALS							
01R	7821	197	220	237	236	252	259
01R	7884	207	232	248	244	245	253

Appendix IV (Continued)

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

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

CDF●

	DAY	3	6	9	12	15	20
0 MG/KG/DAY							
N1R	7521	131	148	152	164	181	223
N1R	7522	136	133	147	158	159	168
N1R	7523	152	170	172	184	196	224
N1R	7524	144	159	163	173	182	204
N1R	7525	136	149	155	162	146	186
N1R	7531	149	163	168	178	185	225
N1R	7532	135	145	151	159	171	201
N1R	7533	143	159	167	177	191	219
N1R	7534	133	148	158	170	183	214
N1R	7535	146	162	169	181	193	180
N1R	7541	144	157	165	179	164	214
N1R	7543	139	154	160	175	186	219
N1R	7544	145	161	166	185	196	239
N1R	7545	144	159	161	168	178	217
N1R	7551	138	150	159	169	182	226
N1R	7552	143	154	160	160	161	164
N1R	7553	140	153	157	172	184	226
N1R	7554	147	159	161	171	167	201
N1R	7555	144	157	162	171	179	203
N1R	7561	135	143	151	159	154	185
N1R	7562	140	153	155	149	138	183
N1R	7564	152	166	173	179	192	228
N1R	7565	146	158	162	172	178	215
N1R	7571	130	142	150	158	156	182
N1R	7572	148	161	166	174	185	226
N1R	7573	149	160	166	180	189	231
N1R	7574	136	153	160	172	183	217
N1R	7575	149	159	165	177	193	222
	MEAN	142	155	161	171	177	209
	STAN. DEV	6.2	8.0	6.7	9.0	15.6	20.3
NON PREGNANT ANIMALS							
N1R	7542	127	134	142	144	142	147
N1R	7563	135	149	153	153	150	149

Appendix IV (Concluded)

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

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

CDF®

	DAY	3	6	9	12	15	20
25 MG/KG/DAY							
01R	7526	147	161	160	159	156	200
01R	7527	127	143	141	130	130	142
01R	7528	142	159	166	171	177	210
01R	7529	135	151	150	156	160	202
01R	7530	138	151	153	155	165	200
01R	7536	141	155	157	166	170	213
01R	7537	140	152	157	156	168	208
01R	7538	138	151	157	159	168	211
01R	7539	149	163	163	163	170	210
01R	7540	139	155	162	166	180	213
01R	7547	129	144	148	151	161	192
01R	7548	137	149	152	155	161	196
01R	7549	134	148	149	150	162	197
01R	7550	131	146	151	148	160	203
01R	7558	142	156	158	156	161	195
01R	7559	163	177	178	170	182	233
01R	7560	142	154	153	155	166	214
01R	7566	137	151	156	155	156	201
01R	7567	146	158	160	159	161	206
01R	7568	146	159	164	142	155	185
01R	7569	137	150	152	152	174	201
01R	7570	143	153	149	157	165	209
01R	7576	151	163	166	164	172	213
01R	7577	137	147	152	162	174	215
01R	7578	138	153	153	153	151	186
01R	7579	134	147	152	155	165	205
01R	7580	143	153	157	140	137	174
MEAN		140	154	156	156	163	201
STAN. DEV		7.3	7.1	7.4	8.9	11.5	16.5
NON PREGNANT ANIMALS							
01R	7546	141	152	150	141	138	151
01R	7556	139	148	146	146	148	158
01R	7557	148	159	152	150	152	158

Appendix V
Special Lens Oral Teratology Study of
T-2999CoC in Two Strains of Rats

Gross and Microscopic Lens Observations[a] of Individual Fetuses
Freehand Sectioned Across the Lenses[b]

CD®	Gross Findings				Microscopic Findings					
0 mg/kg/day					Central Region					
Fetus Number	Dark Streak	Lens Cleft	NVC	Lens Not Sectioned	Lens Vesicle Space	Embryonal Nucleus	Acentric Region	Equatorial Region	Lens Missing	Lens Shattered or Foreign Material
3			R	L			R	L		
4			RL				RL			
29			RL		R	R	L			
30			L	R				L	R	
41			RL		L	RL				
44			RL		R	RL				
57		L	R		L	RL				
58			RL		L	L	R			L
151		L	R		L	L	R			L
152		L	R			L	R			
154			RL			RL				
155			RL				RL			
161			RL				RL			
163			RL		L	L		R		
169			RL		R	R	L			
171			RL		L	RL				
181			RL			RL				
184			RL		RL	RL				
186			RL		L	L			R	
188			RL				R		L	

[a] R = right lens
L = left lens

[b] Tissue processing Procedure #2 of Table 5

Appendix V (Continued)
Special Lens Oral Teratology Study of
T-2999CoC in Two Strains of Rats

Gross and Microscopic Lens Observations[a] of Individual Fetuses
Freehand Sectioned Across the Lenses[b]

CD®	Gross Findings				Microscopic Findings					
0 mg/kg/day					Central Region					
Fetus Number	Dark Streak	Lens Cleft	NVC	Lens Not Sectioned	Lens Vesicle Space	Embryonal Nucleus	Acentric Region	Equatorial Region	Lens Missing	Lens Shattered or Foreign Material
218		L		R	RL	RL				
219			RL		L	L	R			
235			RL		L	L	R			
236			RL			L		R		
259	R	R		L		R	L			
260			RL		RL	RL				
262			RL		L	L	R			
264	R	R		L		R	L			
277		R	L				RL			
278			RL				RL			R
281		RL					RL			
283			RL		RL	RL				
285			RL		L	L	R			
288		RL			R	R			L	
295			RL				RL			
296	RL	RL			RL	RL				RL
297			RL				RL			
300			RL				RL			
319		RL			RL	RL				
320			RL			R	L			

[a] R = right lens
L = left lens

[b] Tissue processing Procedure #2 of Table 5

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

Gross and Microscopic Lens Observations[a] of Individual Fetuses
Freehand Sectioned Across the Lenses[b]

CD® 25 mg/kg/day	Gross Findings				Microscopic Findings					
					Central Region					
Fetus Number	Dark Streak	Lens Cleft	NVC	Lens Not Sectioned	Lens Vesicle Space	Embryonal Nucleus	Acentric Region	Equatorial Region	Lens Missing	Lens Shattered or Foreign Material
5			RL				L		R	
7			RL		RL	RL				
9			RL		RL	RL				
11			RL			L	R			
26			RL		RL	RL				
28				RL		R		L		
38	L	R			RL	RL				RL
40			RL		L	RL				
77	R	R	L		R	R	L			RL
79		L	R		R	RL				
97			RL			R	L			
98			RL			L		R		
111	RL	RL				L	R			
112			RL				RL			
114			L	R	RL	RL				
116		L		R	RL	RL				
122		RL			L	L	R			
124			L	R			L		R	L
141			R	L		R		L		R
143			RL			R	L			

[a] R = right lens
L = left lens

[b] Tissue processing Procedure #2 of Table 5

Gross and Microscopic Lens Observations[a] of Individual Fetuses
Freehand Sectioned Across the Lenses[b]

CD® 25 mg/kg/day	Gross Findings				Microscopic Findings					
					Central Region					
Fetus Number	Dark Streak	Lens Cleft	MVC	Lens Not Sectioned	Lens Vesicle Space	Embryonal Nucleus	Acentric Region	Equatorial Region	Lens Missing	Lens Shattered or Foreign Material
173	R	R		L			RL			R
176		R	L				RL			
177		R		L	R	R	L			
178			R	L		R		L		
198		R	L		R	R	L			
199	L	L		R	R	RL				RL
207	R	R	L				RL			
208			RL							RL
215			R	L			RL			
216			RL		L	L	R			
221		R	L		L	RL				
223		R		L	R	R		L		
289		RL					RL			
291				RL			L	R		
302	R		L		L	RL				
303	R		L			R	L			R
306			RL			RL				
308			RL		L	RL				
309		L	R		RL	RL				
310		R		L	R	R	L			

[a] R = right lens
L = left lens

[b] Tissue processing Procedure #2 of Table 5

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

Gross and Microscopic Lens Observations[a] of Individual Fetuses
Freehand Sectioned Across the Lenses[b]

CD® 0 mg/kg/day	Gross Findings				Microscopic Findings					
					Central Region					
Fetus Number	Dark Streak	Lens Cleft	NVC	Lens Not Sectioned	Lens Vesicle Space	Embryonal Nucleus	Acentric Region	Equatorial Region	Lens Missing	Lens Shattered or Foreign Material
18		RL					RL			RL
19			R	L	L	L		R		
33			RL							RL
35			R	L			R	L		
49			RL		L	L			R	L
52	L		R		L	L				
85[c]				RL	R	R		L		R
86[c]			R	L			R	L	R	
89[c]	R		L		L	L				L
91[c]		L		R		L		R		
94[d]							L	R		
96	R	L			L	L	R			RL
102			RL			R	L			
103			RL			L		R		R
118			R	L			RL			
119				RL		R		L		
130			RL		L	L	R			
132				RL	R	R	L			
133			RL			L	R			L
135			RL			L	R			

[a] R = right lens
L = left lens

[b] Tissue processing Procedure #2 of Table 5

[c] Data excluded from analysis in Tables 2,3,4 & 5 according to a Protocol Deviation (Appendix I)

[d] Gross findings data lost in transcription

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

Gross and Microscopic Lens Observations[a] of Individual Fetuses
Freehand Sectioned Across the Lenses[b]

CDF®	Gross Findings				Microscopic Findings					
0 mg/kg/day					Central Region					
Fetus Number	Dark Streak	Lens Cleft	NVC	Lens Not Sectioned	Lens Vesicle Space	Embryonal Nucleus	Acentric Region	Equatorial Region	Lens Missing	Lens Shattered or Foreign Material
137		R	L				RL			
139			L	R	L	L	R			L
157	R	L			L	L	R			
160			RL				RL			RL
165	R		L			RL				
167			L	R			RL			
189			L	R		L	R			L
192			RL				RL			
193			RL		L	L	R			
195			R	L		R	L			
209		L	R				RL			
210			RL		R	R	L			
226[c]			RL			RL				
227[c]	RL				L	L	R			
231			RL				L	R		
232			R	L			RL			
264			R	L			RL			
268	R		L				RL			
275			L	R			RL			
276			L	R		L			R	

[a] R = right lens
L = left lens

[b] Tissue processing Procedure #2 of Table 5

[c] Data excluded from analysis in Tables 2,3,4 & 5 according to a Protocol Deviation (Appendix I).

Appendix V (Continued)
Special Lens Oral Teratology Study of
T-2999CoC in Two Strains of Rats

Gross and Microscopic Lens Observations[a] of Individual Fetuses
Freehand Sectioned Across the Lenses[b]

CDF® 25 mg/kg/day	Gross Findings				Microscopic Findings					
					Central Region					
Fetus Number	Dark Streak	Lens Cleft	NVC	Lens Not Sectioned	Lens Vesicle Space	Embryonal Nucleus	Acentric Region	Equatorial Region	Lens Missing	Lens Shattered or Foreign Material
14	R		L			RL				
15			RL			R	L			
23			RL				RL			
24		L	R			L	R			
46			RL			L		R		
48			RL			L	R			
53	L		R			RL				R
55			R	L			RL			RL
61	R	R	L			L	R			RL
62			L		R					
65			L	R		L		R		
68		L		R	L	L	R			RL
70	L	L	R			L				R
71			L	R			L		R	
73			RL				L	R		
75	R		L				RL			RL
83	RL				L	L	R			
84	L		R		L	RL				
107		L	R		R	RL				
108	RL	RL			L	L		R		

[a] R = right lens
L = left lens

[b] Tissue processing Procedure #2 of Table 5

Appendix V (Concluded)
Special Lens Oral Teratology Study of
T-2999CoC in Two Strains of Rats

Gross and Microscopic Lens Observations[a] of Individual Fetuses
Freehand Sectioned Across the Lenses[b]

CDF® 25 mg/kg/day	Gross Findings				Microscopic Findings					
					Central Region					
Fetus Number	Dark Streak	Lens Cleft	NVC	Lens Not Sectioned	Lens Vesicle Space	Embryonal Nucleus	Acentric Region	Equatorial Region	Lens Missing	Lens Shattered or Foreign Material
127			RL				RL			
128	L			R		L		R		
145			RL			RL				RL
147				RL	R	R	L			
201	R		L			R	L			
203			RL				RL			
237	L			R		L		R		
240			RL				RL			
243			L	R			L	R		
244		RL								RL
245			L	R			RL			
246	RL	L				L			R	L
249			L	R					R	L
252			RL				L		R	
253			RL				L		R	
254			L	R			L	R		
269			L	R	L	L	R			
272	L		R			RL				RL
313			RL					L	R	
314	R			L			RL			

[a] R = right lens
L = left lens

[b] Tissue processing Procedure #2 of Table 5

Appendix VI

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

Microscopic Lens Observations[a] of Individual Fetuses
Not Freehand Sectioned Across the Lenses[b]

mg/kg/day	Central Region					
tus mber	Lens Vesicle Space	Embryonal Nucleus	Acentric Region	Equatorial Region	Lens Missing	Lens Shattered or Foreign Material
1		RL				
2		RL				
31	R	R	L			
32		R	L			
42			RL			
43			RL			
59		R	L			
60			RL			
49	RL	RL				
50	RL	RL				
53	R	R	L			
56			RL			
62	R	R	L			
64			RL			
70	L	L	R			
72			RL			
82	L	L	R			R
83	RL	RL				
85			RL			
87	R	R	L			

= left lens
= right lens
issue Processing Procedure #1 of Table 5

Appendix VI (Continued)

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

Microscopic Lens Observations[a] of Individual Fetuses
Not Freehand Sectioned Across the Lenses[b]

mg/kg/day	Central Region					
tus mber	Lens Vesicle Space	Embryonal Nucleus	Acentric Region	Equatorial Region	Lens Missing	Lens Shattered or Foreign Material
217	L	L		R		
220	RL	RL				
233		R	L			
234		R	L			R
257		L	R			
258		RL				
261		L	R			
263			RL			
279		L	R			
280	L	L	R			
282	L	L	R			
284		L	R			
286			RL			
287			L	R		
293		L	R			
294			RL			
298			RL			
299			RL			
317	R	R	L			
318			RL			

L = left lens
R = right lens
Tissue Processing Procedure # 1 of Table 5

Appendix VI (Continued)

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

Microscopic Lens Observations[a] of Individual Fetuses
Not Freehand Sectioned Across the Lenses[b]

5 mg/kg/day	Central Region					
tus mber	Lens Vesicle Space	Embryonal Nucleus	Acentric Region	Equatorial Region	Lens Missing	Lens Shattered or Foreign Material
6	L	L		R		
8	RL	RL				
10		L		R		
12			RL			
25	L	L	R			
27			RL			
37	L	L		R		
39	R	RL				
78		R	L			
80			RL			
99			RL			
100			RL			
109	RL	RL				
110	RL	RL				
113			RL			R
115			RL			
121	R	RL				
123			RL			
142		RL				RL
144		RL				

L = left lens
R = right lens
Tissue Processing Procedure #1 of Table 5

Appendix VI (Continued)

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

Microscopic Lens Observations[a] of Individual Fetuses
Not Freehand Sectioned Across the Lenses[b]

CD ●

25 mg/kg/day	Central Region					
Fetus Number	Lens Vesicle Space	Embryonal Nucleus	Acentric Region	Equatorial Region	Lens Missing	Lens Shattered or Foreign Material
174			RL			L
175	L	L	R			
179	R	R	L			
180	L	L	R			
197	L	L	R			
200					RL	
205		RL				RL
206						
213	L	L	R			
214	RL	RL				
222	L	L		R		
224			RL			
290	L	L	R			
292			RL			
301	RL	RL				
304		L	R			
305		L	R			
307		L	R			
311		RL				
312	L	RL				

L = left lens
R = right lens
Tissue Processing Procedure #1 of Table 5

Appendix VI (Continued)

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

Microscopic Lens Observations[a] of Individual Fetuses
Not Freehand Sectioned Across the Lenses[b]

CDF●

0 mg/kg/day	Central Region					
Fetus Number	Lens Vesicle Space	Embryonal Nucleus	Acentric Region	Equatorial Region	Lens Missing	Lens Shattered or Foreign Material
17			RL			
20		L	R			
34			RL			
36			RL			
50			RL			
51		L		R		
87[c]		R	L			
88[c]			R	L		
90[c]		L	R			
92[c]			RL			
93			L	R		
95			RL			
101			RL			
104			RL			RL
117			RL			
120	RL	RL				
129	L	L	R			
131			RL			
134			RL			
136		R	L			

L = left lens
R = right lens
Tissue Processing Procedure #1 of Table 5
Data excluded from analysis in Table 5 according to a Protocol Deviation (Appendix I)

Appendix VI (Continued)

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

Microscopic Lens Observations[a] of Individual Fetuses
Not Freehand Sectioned Across the Lenses[b]

CDF®

0 mg/kg/day	Central Region					
Fetus Number	Lens Vesicle Space	Embryonal Nucleus	Acentric Region	Equatorial Region	Lens Missing	Lens Shattered or Foreign Material
138	L	L	R			
140			RL			
158		L	R			
159	L	L	R			
166		RL				RL
168			RL			RL
190			L			R
191		L	R			L
194	L	RL				
196			RL			
211	L	RL				
212	L	RL				
225[c]		R	L			
228[c]		R	L			
229			RL			
230	L	L				RL
265			RL			
267			RL			
273			RL			
274		L	R			

L = left lens
R = right lens
Tissue Processing Procedure #1 of Table 5
Data excluded from analysis in Table 5 according to a Protocol Deviation (Appendix I)

Appendix VI (Continued)

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

Microscopic Lens Observations[a] of Individual Fetuses
Not Freehand Sectioned Across the Lenses[b]

OF●

5 mg/kg/day

etus umber	Central Region: Lens Vesicle Space	Central Region: Embryonal Nucleus	Central Region: Acentric Region	Central Region: Equatorial Region	Lens Missing	Lens Shattered or Foreign Material
13			L	R		
16	L	L	R			
21			RL			
22	L	L	R			
45			RL			
47			RL			
54		L	R			
56			RL			
63			L	R		
64		L	R			
66			RL			
67			L	R		
69			RL			
72		L	R			
74			L	R		
76			RL			
81			RL			
82		R		L		
105		RL				RL
106		RL				RL

L = left lens
R = right lens
Tissue Processing Procedure #1 of Table 5

Appendix VI (Concluded)

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

Microscopic Lens Observations[a] of Individual Fetuses
Not Freehand Sectioned Across the Lenses[b]

CDF®

25 mg/kg/day	Central Region					
Fetus Number	Lens Vesicle Space	Embryonal Nucleus	Acentric Region	Equatorial Region	Lens Missing	Lens Shattered or Foreign Material
125			RL			
126			RL			
146		RL				
148		L	R			
202		RL				
204		L	R			
238					RL	
239			RL			
241		RL				RL
242		L	R			L
247		R	L			R
248		R				RL
250	R	R	L			
251		RL				
255			RL			
256	R	R	L			
270			RL			
271			RL			
315		L	R			
316		R	L			R

[a] L = left lens
R = right lens

[b] Tissue Processing Procedure #1 of Table 5

Appendix VII

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

T-2999CoC Composition, Purity and Stability

References: Commercial Chemicals Analytical Requests #16474, 18319, Analytical Report #318

A light, straw-colored waxy solid labeled FM-3924, Lot 547 was received for analysis for Riker teratology study #0681TR0362. The material has been analyzed with the following results:

Gas Chromatography of Sample As Received

All in Area %

	QC(14310	12/3/80	3/31/81	3/5/82	5/27/82
Inerts		0.4	0.7	0.4	0.7
Amide	0.5	0.6	0.6	0.6	0.2
C_3		1.2	1.3	1.2	1.3
C_4	10.6 for	1.8	1.7	1.8	2.2
C_5	all low	1.5	1.6	1.5	1.9
C_6	boilers	4.8	4.3	4.8	4.9
C_7,C_8	88.9	88.9	88.9	88.9	88.9
High Boilers	-	0.6	0.8	0.6	0.2

All values are within experimental and integration parameters. Values meet QC specifications.

Elemental Analysis

	% Theor.	% Found 3/5/82	% Found 5/27/82
C	25.2	24.6	25.0
H	1.75	1.5	1.8
N	2.45	2.6	2.7
S	5.6	5.3	6.1
F	56.66	57.0	55.3
O by difference	8.4	9.0	9.1

Appendix VII (Concluded)

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

T-2999CoC Composition, Purity and Stability

MW = 571

Melting Point: Initial 38-48°C
Recheck 38-48°C
5/28/82 38-48°C

Spectroscopic:

Infrared spectra 16474-2,3,4 and 18318-1 are identical to each other and match reference spectra.

16474-2 was obtained	11/14/80
16474-3 was obtained	3/31/81
16474-4 was obtained	3/5/82
18318-1 was obtained	5/28/82.

NMR spectra H8385, H8387, F8390, F8391 were obtained on 12/1/80 and 12/2/80. These were to confirm structure and not repeated. Structure was confirmed. The isomer distribution was found to be 69.6% straight chain, 10.9% $(CF_3)_2CF$ branched, and 19.5% backbone branching.

Conclusions:

FM-3924, Lot 547 has an analysis within specifications, is stable and is representative of commercial material.

RMP/hc

cc: M. T. Cabe
E. G. Gortner (original + 1)
~~F. D. Griffith~~/W. C. McCormick
F. Keller
E. G. Lamprecht
W. H. Pearlson
R. A. Prokop
V. Pothapragada/L. Winter
L. O. Wiseth

TITLE: Protocol for Special Lens Oral Teratology Study of T-2999CoC[a] in Two Strains of Rats
(Riker Experiment Number 0681TR0362).

OBJECTIVE: Changes were detected in day 20 fetal lenses of Sprague-Dawley rats in teratology studies run on T-2999CoC. The Sprague-Dawley strain of rat may be predisposed to develop the lens change. In addition, the threshold for development of the lens change may be lowered by handling procedures used in conducting teratology studies. One objective of this study is to compare the incidence of the lens change in fetuses of both control and T-2999CoC treated Sprague-Dawley and Fisher strain dams. The second objective is to compare the incidence of lens changes of fetuses not exposed in utero to dosing and handling procedures used in teratology studies (Sprague-Dawley non-treated group) with the incidence of lens changes in the Sprague-Dawley control group. 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: Fourth quarter, 1981

TEST SYSTEM: Ninety sexually mature, time mated Sprague-Dawley derived female rats and sixty Fisher 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. These strains of rats will be used because time mated females are readily available and they will provide a comparison between two strains. 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. The Sprague-Dawley non-treated group will be identified only by a number 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] FM-3924

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

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

Strain	Dose Level	Group Size
Sprague-Dawley	25 mg/kg/day	30
Fisher	25 mg/kg/day	30
Sprague-Dawley	0 mg/kg/day	30
Fisher	0 mg/kg/day	30
Sprague-Dawley	No treatment	30

The oral route of administration will be used because it is the route of exposure in previous studies in which lens changes were observed. No dietary contaminants are known to interfere with the test article.

The T-2999CoC treated and control groups 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 Sprague-Dawley non-treated group will only be observed for a live/dead check from day 3 through 20 of gestation. 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 could be sent to histopath for microscopic examination.

DATA ANALYSIS AND FINAL REPORT: The proposed statistical method to be used for analysis of the data is the Fisher exact probability and Chi-square for percent of abnormalities. The proposed date for the final report is 2-3 months after the pup eye exams have been completed (approximately fourth quarter, 1981).

E. G. Gortner 9-2-81
E. G. Gortner Date
Senior Research Technologist
Animal Teratology-Reproduction
Study Director

Elden G Lamprecht 9-3-81
E. G. Lamprecht, DVM, PhD Date
Research Veterinary Pathologist

Marvin T Case 9/8/81
M. T. Case, DVM, PhD Date
Manager, Pathology-Toxicology
Safety Evaluation Laboratory

William C McCormick 9/14/81
W. C. McCormick, MS Date
Toxicologist
Sponsor Representative