# Oral Teratology Study of FM-3422 in Rats T-2253

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| Experiment No.: | 0680TR0010 |
| Conducted At: | Safety Evaluation Laboratory<br>Riker Laboratories, Inc.<br>St. Paul, Minnesota |
| Inclusive Dosing Period: | August 19 to September 4, 1980 |
| Study Director: | E. G. Gortner |

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Court File No. 27-CV-10-28862

Summary

Oral administration of FM-3422 at 75, 37.5 and 25 mg/kg/day to pregnant Sprague-Dawley rats during days 6 through 15 of gestation (period of organogenesis) was teratogenic to rat fetuses. Teratogenic changes included a developmental eye abnormality, cleft palate, blood in the kidney parenchyma and sternebrae malformations. The developmental eye abnormality 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 proportions of fetuses with the lens changes were significantly higher in all FM-3422 groups than in the control group. Cleft palates were produced in the 75 and 37.5 mg/kg/day groups. All three groups receiving compound had fetuses with blood in the kidney parenchyma. The sternebrae changes, although normally considered skeleton aberrations, were viewed as compound-related malformations because of their severity. FM-3422 also produced an increase in other fetal skeleton aberrations.

FM-3422 was not embryotoxic and did not affect the ovaries or reproductive tract contents of the dams.

FM-3422 was maternally toxic to the 75 and 37.5 mg/kg/day dose animals in reducing their group mean body weight gain during the dosing interval. Toxic clinical signs and deaths occurred in only the 75 mg/kg/day dose group.

## Introduction

This teratology study [a] in rats was conducted to evaluate the embryotoxic and teratogenic effects of orally administered FM-3422. 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. Two sets of compound administration groups were dosed between August 19 and September 4, 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 statment). The storage location for specimens, raw data and a copy of the final report is maintained in the Safety Evaluation Laboratory's record archives.

## Methods

Time mated Sprague Dawley derived 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 140 to 240 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 FM-3422 (Lot 784) suspended daily in corn oil at 0, 75, 37.5 or 25 mg/kg/day. FM-3422 was administered daily by oral intubation with a syringe equipped with a ball-tipped intubation needle to the rats on days 6 through 15 gestation (day 0 indicated by sperm-positive vaginal smear). FM-3422 analytical characterization (see Appendix IV) was provided by 3M Commercial Chemical Division, St. Paul, Minnesota.

All surviving animals were sacrificed on day 20 by cervical dislocation and the ovaries and uterus, including its contents, were examined immediately to determine the following: number of corpora lutea, number of viable fetuses, number of resorption sites, pup weights and sex, and any gross fetal abnormalities. Approximately 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. 0680TR0010
[b] Purina Laboratory Chow, Ralston Purina Company, St. Louis, MO

Results and Discussion

FM-3422 was maternally toxic to the high and mid dose groups (75 and 37.5 mg/kg/day) in reducing their group mean body weight gain during the dosing interval. All groups had lower mean weight gain than the controls at all weighings during the dosing interval of days 6 through 15 of gestation (Table 1). In the case of the high dose group at gestation days 9, 12 and 15 and in the case of the mid dose group at gestation days 9 and 15, the group mean weight gains were significantly lower than the mean weight gains of the control group (0 mg/kg/day). The lower mean weight gains of the high and mid dose groups during the dosing interval were responsible for their significantly lower mean body weights between the end of dosing and the termination of the study (Appendix V). The mean body weights and mean weight gains of the low dose group (25 mg/kg/day) were not significantly different from the control.

Abnormal clinical signs were observed and deaths occurred only in the high dose group. Three rats in the high dose group died. One rat died without clinical signs. Two of the rats that died plus one surviving rat had abnormal compound-related clinical signs which included some of the following: thin, lethargic, ataxic, blood in stool, urinary incontinance and bloody nares. The onset of abnormal clinical signs was on day 11 but the signs disappeared in the surviving rat by day 19 of gestation. The remaining 18 high dose rats and the mid and low dose rats did not have abnormal compound-related clinical signs.

The compound 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 and corpora lutea of the three FM-3422 dose groups were not significantly different from the control (Table 2, Appendix VI).

FM-3422 was not fetal toxic. However, the combination of reduced maternal body weight gain (Table 1) plus higher numbers of fetuses in the treatment groups than the control group (Table 2 Appendix VI) resulted in mean fetus weights of all FM-3422 groups which were significantly lower than the control mean fetus weight. The reduced mean fetus weights were not associated with an increase in runting or other gross fetus findings (Table 3).

FM-3422 administration resulted in malformations in fetal sternebrae. The changes, although normally considered skeleton aberrations, were interpreted as compound-related malformations because of their severity. The severity and often the incidence of sternebrae malformations were greater in the three treatment groups than the control group. These malformations included the following: sternebrae asymmetrical, sternebrae bipartite, sternebrae scrambled, sternebrae enlarged, sternebrae missing and sternebrae misshapen (Table 4). All three FM-3422 dose groups had significantly higher proportions of fetuses with sternebrae asymmetrical than the control group. In addition, the high dose group had a

significantly higher proportion of fetuses with bipartite sternebrae than the control group.

An increase in other skeleton aberrations also occurred as the result of FM-3422 administration. These skeleton aberrations included nonossification changes of the cranial bones and sternebrae plus other sternebrae and rib changes (Table 4). The high dose group had significantly higher proportions of fetuses with all of these skeleton changes than the control group. The mid and low dose groups had significantly higher proportions of fetuses with some of these changes than the control group; notably nonossification of the cranial bones, sternebrae missing and 13 ribs spurred. The skeleton aberrations found are generally considered minor but they are of appreciable significance in this study with FM-3422 because of the high proportion of fetuses with the abnormalities.

The control group had a higher proportion of fetuses with one or two bodies of the vertebrae bipartite than the three treatment groups (Table 4). This difference was significant in all instances except for the finding of one body of the vertebrae bipartite in the low dose group.

FM-3422 administration produced the teratogenic effect of cleft palate in the high and mid dose groups and blood in the kidney parenchyma in all three dose groups. The proportions of fetuses with cleft palate and blood in the kidney parenchyma were sigificantly higher in the high dose group than in the control group (Table 5). No cleft palates were present in control and low dose fetuses examined.

FM-3422 was teratogenic to the eye of 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 running through the lens to a discoloration of part of the lens and the presence of a cleft beneath the lens epithelium (Table 5). Histologically the discolorations were due to the presence of lens vesicle remnants forming clefts or surrounding the lens nucleus. Also contributing to the discolorations were primary lens fibers which appeared to have not elongated and the possible presence of degenerated epitrichial cells. Secondary lens fiber development progressed normally except immediately surrounding the abnormal embryonal nucleus. Prominant secondary aberrations of secondary lens fibers include V-shaped clefts between the embryonal nucleus and lens epithelium and lens vesicle remnants surrounding the nucleus.

The proportion of fetuses with the lens abnormality in one or both lenses was significantly higher in all groups than in the control group (Table 5).

No lens abnormalities occurred in the control group. A no-effect dose level for the teratogenic abnormality was not established in this study.

Further Discussion on Lens Embryology

Lens structural and functional requirements are met during embryonic development by the differentiation of highly specialized populations of cells from undifferentiated procursers 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. Additional 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[2].

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 (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 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[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 the lens cells[2].

The teratogenic lens effect of FM-3422 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

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Table 1

Oral Teratology Study of FM-3422 in Rats
Mean Body Weight Gains of Pregnant Rats Between Weighings
with Standard Deviations

| Dose Group | | Gestation Day 6 | 9 | 12 | 15 | 20 |
|---|---|---|---|---|---|---|
| 0 mg/kg/day | MEAN | 28 | 17 | 26 | 29 | 71 |
| | STAN. DEV | 5.5 | 7.5 | 5.8 | 4.9 | 12.1 |
| 75 mg/kg/day | MEAN | 30 | 0[a] | 6[a] | 2[a] | 69 |
| | STAN. DEV | 14.2 | 14.6 | 19.8 | 17.0 | 15.1 |
| 37.5 mg/kg/day | MEAN | 28 | 6[a] | 17 | 14[a] | 69 |
| | STAN. DEV | 5.4 | 10.9 | 9.8 | 10.4 | 15.8 |
| 25 mg/kg/day | MEAN | 27 | 11 | 20 | 22 | 73 |
| | STAN. DEV | 11.9 | 15.3 | 8.9 | 5.4 | 11.6 |

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

Table 2

Oral Teratology Study of FM-3422 in Rats
Mean Litter Data with Fetus Weights and Standard Deviations

| 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 | 18 | 3.6 | 5.4 | 8.9 | 0.0 | 0.7 | 9.6 | 9.9 | 4.4 |
| | | 1.6 | 1.8 | 2.6 | 0.0 | 1.0 | 2.5 | 2.1 | 0.5 |
| 75 mg/kg/day | 17 | 5.1 | 4.7 | 9.8 | 0.1 | 0.5 | 10.4 | 10.5 | 3.7[a] |
| | | 2.1 | 2.3 | 2.1 | 0.2 | 0.6 | 1.9 | 2.2 | 0.5 |
| 37.5 mg/kg/day | 20 | 4.4 | 5.4 | 9.7 | 0.0 | 0.7 | 10.4 | 10.5 | 4.0[a] |
| | | 2.1 | 2.1 | 1.9 | 0.0 | 0.9 | 1.6 | 1.7 | 0.3 |
| 25 mg/kg/day | 21 | 4.3 | 5.8 | 10.1 | 0.0 | 0.5 | 10.7 | 11.3 | 4.0[a] |
| | | 1.6 | 1.9 | 1.9 | 0.0 | 0.5 | 2.0 | 1.9 | 0.3 |

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



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Table 3

Oral Teratology Study of FM-3422 in Rats
Number of Fetuses with Gross Findings[a]

| Finding | 0 mg/kg/day | 75 mg/kg/day | 37.5 mg/kg/day | 25 mg/kg/day |
|---|---|---|---|---|
| Total Fetuses Examined | 161 | 167 | 195 | 213 |
| Runted | --- | 2 | --- | 2 |
| Umbilical hernia | 1 | --- | --- | 2 |
| Total Normal Fetuses | 160 | 165 | 195 | 209 |
| Total Abnormal Fetuses | 1 | 2 | 0 | 4 |

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

Table 4

Oral Teratology Study of FM-3422 in Rats
Number and Percent of Fetuses with Skeleton Findings

| Skeleton Finding | 0 mg/kg/day | | 75 mg/kg/day | | 37.5 mg/kg/day | | 25 mg/kg/day | |
|---|---|---|---|---|---|---|---|---|
| Fontanelle not closed | 27 | (24) | 26 | (22) | 25 | (18) | 28 | (19) |
| Holes in parietal | 1 | (1) | 1 | (1) | | | | |
| Parietal scalloped | 1 | (1) | | | | | | |
| Frontal nonossified | 21 | (19) | 62 | (53)[a] | 70 | (51)[a] | 75 | (50)[a] |
| Parietal nonossified | 21 | (19) | 62 | (53)[a] | 70 | (51)[a] | 74 | (50)[a] |
| Interparietal nonossified | 14 | (12) | 54 | (47)[a] | 46 | (33)[a] | 59 | (40)[a] |
| Occipital nonossified | | | 1 | (1) | | | | |
| Sternebrae nonossified | 80 | (71) | 100 | (86)[a] | 102 | (74) | 111 | (75) |
| Sternebrae asymmetrical | 10 | (9) | 42 | (36)[a] | 34 | (25)[a] | 36 | (24)[a] |
| Sternebrae bipartite | 2 | (2) | 37 | (32)[a] | 6 | (4) | 5 | (3) |
| Sternebrae scrambled | | | 1 | (1) | 1 | (1) | | |
| Sternebrae enlarged | | | 1 | (1) | | | | |
| Sternebrae misshapen | | | | | 1 | (1) | | |
| One sternebrae missing | 23 | (20) | 32 | (28) | 31 | (22) | 33 | (22) |
| Two sternebrae missing | 2 | (2) | 16 | (14)[a] | 9 | (7) | 16 | (11)[a] |
| Three sternebrae missing | | | 1 | (1) | | | | |
| One body vertebrae missing | | | 1 | (1) | | | | |
| 13 ribs | 1 | (1) | 3 | (3) | 3 | (2) | 5 | (3) |
| 13 ribs spurred | 3 | (3) | 32 | (28)[a] | 28 | (20)[a] | 9 | (6) |
| Wavy ribs | 5 | (4) | 8 | (7) | 4 | (3) | 2 | (1) |
| Protrusion on ribs | 8 | (7) | 12 | (10) | 5 | (4) | 7 | (5) |
| One body of the vertebrae bipartite | 29 | (26) | 15 | (13)[b] | 21 | (15)[b] | 30 | (20) |
| Two bodies of the vertebrae bipartite | 17 | (15) | 4 | (3)[b] | 5 | (4)[b] | 3 | (2)[b] |
| Three bodies of the vertebrae bipartite | | | | | 1 | (1) | 2 | (1) |
| Four bodies of the vertebrae bipartite | | | | | | | 1 | (1) |
| Five bodies of the vertebrae bipartite | | | | | | | 1 | (1) |
| Total Normal Fetuses | 9 | (8) | 2 | (2) | 6 | (4) | 7 | (5) |
| Total Abnormal Fetuses | 104 | (92) | 114 | (98) | 132 | (96) | 142 | (95) |
| Total Fetuses Examined | 113 | | 116 | | 138 | | 149 | |

[a] Significantly higher than the control (Chi-square $p < 0.05$)
[b] Significantly lower than the control (Chi-square $p < 0.05$)
( ) = percent of total examined

Table 5

Oral Teratology Study of FM-3422 in Rats
Number and Percent of Fetuses with Internal Findings

| Internal Finding | 0 mg/kg/day | | 75 mg/kg/day | | 37.5 mg/kg/day | | 25 mg/kg/day | |
|---|---|---|---|---|---|---|---|---|
| Fetuses with eye abnormalities | 0 | | 35 | (69)[a] | 29 | (51)[a] | 27 | (42)[a] |
| Discoloration running through the lens of one eye | | | 7 | (13) | 2 | (4) | 1 | (2) |
| Discoloration running through the lens of both eyes | | | | | | | 1 | (2) |
| Discoloration running 1/2 to 3/4 through the lens of one eye | | | 16 | (31)[a] | 13 | (23)[a] | 10 | (16)[a] |
| Discoloration running 1/2 to 3/4 through the lens of both eyes | | | 5 | (10) | 1 | (2) | 5 | (8) |
| Discoloration in back of lens | | | | | | | 2 | (3) |
| Bubble on outside of lens and discoloration running through the lens of one eye | | | 1 | (2) | | | | |
| Cleft in the lens and discoloration running through the lens of one eye | | | 5 | (10) | 7 | (12)[a] | 4 | (6) |
| Cleft in the lens and discoloration running through the lens of both eyes | | | | | 1 | (2) | | |
| Bubble on outside of lens cleft in the lens of one eye | | | | | 1 | (2) | 1 | (2) |
| Cleft in the lens of one eye | | | 1 | (2) | 5 | (9) | 3 | (5) |
| Open space in the rear of the lens of one eye | | | | | | | 1 | (2) |
| Small eyes | | | 1 | (2) | | | | |
| Cleft palate | | | 7 | (14)[a] | 3 | (5) | | |
| Enlarged atriums | | | | | | | 2 | (3) |
| Enlarged renal pelvis area in the kidney | 5 | (10) | 1 | (2) | | | | |
| Blood in the kidney parenchyma | | | 11 | (22)[a] | 3 | (5) | 3 | (5) |
| Abdominal cavity full of blood | 1 | (2) | 3 | (6) | | | 1 | (2) |
| Total Normal Fetuses | 42 | (87.5) | 8 | (16) | 25 | (44) | 32 | (50) |
| Total Abnormal Fetuses | 6 | (12.5) | 43 | (84) | 32 | (56) | 32 | (50) |
| Total Fetuses Examined | 48 | | 51 | | 57 | | 64 | |

[a] Significantly different from the control (Chi-square $p < 0.05$)