TWO YEAR ORAL (DIET) TOXICITY / CARCINOGENICITY
STUDY OF FLUOROCHEMICAL FM-3924 IN RATS

(RIKER Experiment No. 0281CR0012)

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TWO YEAR ORAL (DIET) TOXICITY/CARCINOGENICITY STUDY OF FLUOROCHEMICAL FM-3924 IN RATS

(RIKER Experiment No. 0281CR0012)

REPORT SUMMARY

The purpose of this study was to assess the potential chronic toxicity and oncogenicity of FM-3924, an industrial grade of N-ethyl perfluoroocanesulfonamido ethanol, by mixing in the diet and feeding to 50 rats per sex per dose group for two years. An interim termination and evaluation was performed at one year on 15 additional rats per sex from only the control and high-dose groups.

A total of 460 Sprague-Dawley rats (230 of each sex) were equally divided into four groups. The rats were fed diets containing either 0, 10, 30 or 100 ppm of FM-3924 for the duration of the study.

In-life observations performed during the course of the study included: daily observations for abnormal clinical signs; periodic physical examinations; periodic recording of body weight and feed consumption; ophthalmoscopic examinations; and clinical pathology determinations including hematology, clinical chemistry and urinalysis.

Macroscopic postmortem examinations were performed on all animals including those that died or were terminated prior to the end of scheduled dosing. Selected organ weights were obtained from all of the rats at scheduled necropsy at one year as well as from 15 rats/sex/group, randomly selected from the control and all FM-3924-treated groups, at the termination of the study. Selected tissue specimens were harvested from each animal at necropsy, and preserved for future histopathologic evaluation. Microscopic evaluation was performed on tissues preserved from all of the control and high-dose rats, while a similar evaluation was performed on a limited list of tissues obtained from the low- and mid-dose animals.

The major in-life findings associated with FM-3924 administration consisted of a dose related decreased rate of mean body weight gain, an increase in feed consumption per kilogram of mean body weight, a slight
increase in the incidence of ataxia. A slight increase in the incidence of clonic convulsions was seen only in the high-dose male rats when compared to the control group. In the high-dose groups, male mortality values were slightly increased whereas female mortality was slightly decreased when compared to the corresponding control groups.

FM-3924 related hematologic changes were seen in the mid- and high-dose female rats only and consisted of a decrease in red blood cell counts, in hemoglobin concentration, and in hematocrit values: these changes were observed very early in the study, but did not progress into anemia by the end of the two year dosing period. There was an increased incidence of poikilocytosis, microcytosis, and polychromasemia in the high- and mid-dose females at 24 months. The degree of morphological change was generally noted as slight.

Increased liver weights and microscopic findings including hepatomegalocytosis and hepatocellular degeneration with and without necrosis, were the primary dose-related toxicologic findings observed in this study. The hepatomegalocytosis was considered to be the result of chronic hepatocellular metabolic stimulation by FM-3924. The severity and incidence of the liver findings were similar at both the one year and two year necropsies.

The non-neoplastic findings observed in this study were believed to be related to hepatocellular effects and/or were associated with a mild exacerbation of anticipated geriatric endocrine changes in this strain of rat and not considered to be primary test article-related effects. Specific histopathology findings which had an equivocal relationship to treatment with FM-3924 include a dose-related decrease of chronic myocarditis and a statistically significant, dose-related increase in ovarian tubular hyperplasia in the female test article treated groups. Other findings were considered to be spontaneous in origin, and occurred either sporadically or at a generally similar incidence among all groups including controls.

The no observed adverse effect level (NOAEL) feed concentration was judged to be greater than 30 but less than 100 ppm of FM-3924 in the diet. Based on feed consumption data, these NOAEL concentrations corresponded to approximate average daily doses for both sexes of 1.5 and 5.0 milligrams per kilogram of body weight, respectively.
The overall incidence of hepatocellular adenomas and carcinomas was low in both control and FM-3924-treated groups with only the high-dose female rats possibly having a tumor incidence outside historical control limits. The majority of neoplasms were observed in endocrine or endocrine-sensitive organs which are typical neoplastic sites for older rats of this strain. The incidence of these neoplasms was similar among control and test article-treated groups, and did not demonstrate a unique tumor type.

Based on tumor incidence, types of tumors, onset time of tumor appearance, malignancy patterns of tumors and the final mortality values at two years, FM-3924 was not considered to be carcinogenic in the rat under the design and conditions of this study.
TWO YEAR ORAL (DIET) TOXICITY/CARCINOGENICITY STUDY OF FLUOROCHEMICAL FM-3924 IN RATS

INTRODUCTION

This study was designed to evaluate the chronic toxicologic and carcinogenic potential of FM-3924, an industrial grade of N-ethyl perfluorooctanesulfonamido ethanol, in rats following oral administration in the diet for a period of two years. The study was sponsored by the Commercial Chemical Division of 3M Company and was performed by the Pathology and Toxicology Department of Riker Laboratories, Inc., 3M Company, St. Paul, Minnesota, U.S.A. The study and subsequent reporting was coordinated for the sponsor by the 3M Corporate Toxicology Services staff. The in-life or dosing portion of the study began on April 21, 1981, and was completed on May 18, 1983. A copy of the study protocol with amendments is contained in this report as Appendix Item H.

The study was designed to evaluate two separate fluorochemicals, FM-3924 and FC-143, using a common set of control animals. This report will describe the results of the FM-3924 treatment while the results relating to the FC-143 study will be reported separately.

The study was conducted in accordance with the Department's Standard Operating Procedures (ie., SOPs) and in compliance with the Food and Drug Administration's Good Laboratory Practice (GLP) regulations (21 CFR Part 58). Various phases of the study were inspected by the RIKER Quality Assurance Unit; their statement is presented in Appendix Item I of this report. The original signed protocol with amendments, list of study personnel, raw data, study specimens, and other pertinent study samples/documents will be maintained within the Pathology and Toxicology Department archives currently located at 3M Center in St. Paul, Minnesota.
MATERIALS AND METHODS

Test System: Four-hundred and sixty Sprague-Dawley rats [Crl:COBS\textsuperscript{R} CD(SD)BR, Charles River, Portage, MI], 39 to 41 days of age when treatment began, were divided by means of a table of random numbers into four groups. The control and high-dose groups each contained 65 males and 65 females, whereas the mid- and low-dose groups each contained 50 male and 50 female rats.

The rats were housed in hanging stainless steel cages with wire mesh floors and fronts. The males were housed individually, but the females were housed two per cage. The animals were housed in two separate rooms, one containing the control groups and one containing the FM-3924 treatment groups in order to prevent cross contamination by potential vaporization and/or sublimation of the test article. Air samples were taken from each of the animal treatment rooms four months after the initiation of the study in order to assay for the presence of airborne contaminants. The samples were analyzed by the Analytical Section of the 3M Central Research Laboratory and were found to be below detectable limits for the suspected fluorochemicals. In addition to the air monitoring, 30 untreated sentinel rats were placed in each of the two animal rooms. From each animal room, 5 male and 5 female sentinel rats were euthanized during the first week of the study, and at 1 and 3 months after the start of the study. Plasma samples obtained from these rats were analyzed for organic fluorine and were found to contain less than one part per million (see Appendix Item A).

Each animal room was temperature and humidity controlled with the lighting on a 12 hour light/dark cycle. Individual rats were uniquely identified by an animal number on a cage card and on a tag affixed to their ear. Feed (Certified Purina Laboratory Chow, Ralston-Purina Co., St. Louis, MO) and tap water were provided ad libitum.

Test Substance/Diet Preparation: FM-3924 (Lot No. 547) was analyzed by the Commercial Chemicals Divisions (CCD) Analytical Laboratory prior to the start of the study, after approximately one year from the start of the study, and at the termination of the dosing period. No detectable changes were found in the test substance during this time (see CCD Analytical Reports Nos. 318, 347 and 412 in Appendix Item J).
The test substance was a straw-colored waxy solid which was solubilized in ethanol prior to mixing into the diet. The formula and procedures for preparing the test article/diet mixtures are contained in Appendix Item B. Prior to initiating compound administration to any animals, the test substance/diet mixture was assayed. FM-3924 was found to be uniformly blended and stable for one week in the ground feed (see CCD Analytical Report No. 202 in Appendix Item J).

Throughout the study, test article/diet mixtures were prepared fresh weekly and representative samples of each were collected and assayed for test article content and homogeneity during the first month of the study and at 3 month intervals thereafter (see Appendix Item B). The results of these assays indicated that the level of FM-3924 was generally within a few percent of that desired (Table 1).

The rats received either FM-3924-treated or control (i.e., untreated) diets in glass jars 10.2 cm high x 8.9 cm in diameter. A 5.1 cm access hole was cut in the stainless steel lid. On a weekly basis the diet jars were removed and replaced with clean jars containing fresh diet mixtures.

Experimental Design: The study consisted of one control group and three treatment groups. The dosage levels and animal distribution are listed hereinafter.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Dosage Levels (ppm)</th>
<th>Group Size &amp; Animal Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - Control</td>
<td>0</td>
<td>65 (3516-3580)</td>
</tr>
<tr>
<td>2 - High</td>
<td>100</td>
<td>65 (3696-3760)</td>
</tr>
<tr>
<td>3 - Mid</td>
<td>30</td>
<td>50 (3761-3810)</td>
</tr>
<tr>
<td>4 - Low</td>
<td>10</td>
<td>50 (3811-3860)</td>
</tr>
</tbody>
</table>
An interim termination at one year involved 15 male and 15 female rats from both the control and high-dose groups. The remaining 50 animals per sex per group continued on study.

In-Life Observations: All animals were observed daily throughout the two-year dosing period. Weekly physical examinations included palpation for the presence of masses as well as observations for pharmacotoxic signs; mortality was recorded daily. During the study, moribund animals were closely monitored and euthanized when in the judgement of the Study Director death appeared to be imminent, in order to harvest non-autolysed tissue for subsequent histopathologic examination.

Body weights and feed consumption were recorded once per week for the first six months, and then once every two weeks for the remainder of the study.

Eye examinations using indirect ophthalmoscopy and/or slit lamp biomicroscopy were performed on the control and high-dose rats by the Staff Veterinarian prior to compound administration and at approximately one year. The eyes of the surviving control and high-dose animals were examined 2-3 weeks prior to the termination of the study by a consulting Veterinary Ophthalmologist (see Appendix Item G).

Clinical pathology determinations included hematology, clinical chemistry (plasma) and urinalysis. Tests were conducted on samples obtained from 15 rats per sex from each group at 3, 6, 12, 18 and 24 months; animals were randomly selected at each time interval. Hematologic tests included total red and white blood cell counts, hemoglobin, hematocrit, and a differential white blood cell count. Clinical chemistry parameters included total bilirubin, total protein, albumin, blood urea nitrogen (BUN), glucose, alkaline phosphatase (AP), creatine phosphokinase (CPK), aspartate aminotransferase (AST-formerly SGOT), alanine aminotransferase (ALT-formerly SGPT), and calcium. Urine tests included pH, specific gravity, albumin, glucose, bilirubin, occult blood and ketones.

Blood samples were collected from the retrobulbar venous plexus of anesthetized rats which had been fasted overnight. Blood was generally collected from the right eye. Urine samples were obtained by placing each rat in an individual metabolism cage for 20-22 hours. The specific methods
used for hematology, clinical chemistry and urinalysis are outlined in Appendix C. The mean hematology and clinical chemistry values from the treated groups were compared to both the concurrent control group as well as normal ranges for these parameters obtained from historical control animal data generated in this laboratory (Appendix C).

Metabolic Examination: Overnight urine and fecal samples were collected at 2, 5, 11 and 23 months from five rats per sex per group for total organic fluoride analysis, and for the presence of FM-3924 and/or FC-95 (a FM-3924 metabolite). At the scheduled one and two year necropsies, samples of liver, blood, kidney, spleen, lung and bone marrow (ie. from the femur) were saved from five rats/sex/group. After collection, all specimens were frozen by the RIKER Drug Metabolism Department. The results of these analyses will be reported separately.

Postmortem Examinations: Gross postmortem examinations were performed on all rats which died or were terminated in extremis, on all rats terminated at the one year interim necropsy, and on all rats surviving to the end of the scheduled dosing period. At necropsy, an examination was made of the external body surface and body orifices. The carcass was then opened and the contents of the abdomen, thorax and cranium were examined in situ and following removal from the body.

Organ weights (ie. wet tissue) were obtained at the interim termination from both the control and high-dose groups, and from the control and all FM-3924-treated groups at the two year necropsy. The weights of the adrenal glands, brain, testes, heart, kidneys, liver, spleen and uterus were recorded for 15 randomly selected rats/sex/group. Body weights were obtained just prior to necropsy from the same rats in order to calculate organ weights relative to whole body weights.

Representative samples of the following tissues and organs from each rat were fixed in 10% neutral, buffered formalin for subsequent histologic processing:
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
<td>Liver (2 Sections)</td>
</tr>
<tr>
<td>Adrenals (2)</td>
<td>Lung (2 Sections)</td>
</tr>
<tr>
<td>Brain (3 Sections including frontal cortex and basal ganglia, parietal cortex and thalamus; cerebellum and pons)</td>
<td>Lymph node (mesenteric)</td>
</tr>
<tr>
<td>Brain (3 Sections including frontal cortex and basal ganglia, parietal cortex and thalamus; cerebellum and pons)</td>
<td>Membrany Gland - (females)</td>
</tr>
<tr>
<td>Eyes</td>
<td>Pancreas</td>
</tr>
<tr>
<td>Gonads</td>
<td>Pituitary</td>
</tr>
<tr>
<td>Ovaries (2)</td>
<td>Salivary Gland</td>
</tr>
<tr>
<td>Testes/Epididymides (2)</td>
<td>Spinal Cord/Bone Marrow (vertebrae)</td>
</tr>
<tr>
<td>Heart</td>
<td>Spleen</td>
</tr>
<tr>
<td>Small Intestine (3 Sections)</td>
<td>Stomach</td>
</tr>
<tr>
<td>Large Intestine</td>
<td>Thyroid/Parathyroid/Trachea/Esophagus</td>
</tr>
<tr>
<td>Kidneys (2 Sections)</td>
<td>Urinary Bladder</td>
</tr>
<tr>
<td></td>
<td>Uterus or Prostate</td>
</tr>
<tr>
<td></td>
<td>Any tissue masses (suspected tumors)</td>
</tr>
<tr>
<td></td>
<td>Any gross lesion</td>
</tr>
</tbody>
</table>

Light microscopic examination was performed on hematoxylin and eosin stained, paraffin-embedded tissue sections from all tissues listed above, when available, and from all rats in the control (Group 1) and high-dose (Group 2) populations regardless of the cause of death. Microscopic examination of tissues from the middle- (Group 3) and low- (Group 4) dose rats included the tissues listed above except: aorta, brain, eyes, small and large intestines, lymph node(s), and spinal cord/bone marrow. The histopathologic examination and evaluation of these tissues was performed by Dr. Robert G. Geil, consulting Veterinary Pathologist (see Appendix Item D).

**Biostatistical Methods:** The means and standard deviations for body weights, feed consumption, absolute organ weight, relative organ weight-to-whole body weight, organ weight-to-brain weight ratios and other laboratory data were determined separately for each sex and dose group.

These data were analyzed using Bartlett’s test for homogeneity of variance. If this test was not significant at p = 0.001, the data were further analyzed by comparing each treated group to the control group using a two-tailed Dunnett’s test at the p = 0.05 significance level. The
results of Dunnett's test have been indicated by asterisks on the mean tables. If Bartlett's test was significant at $p = 0.001$, the data were ranked and a two-tailed Dunnett's test was performed on the ranks. These results have been indicated by the pound sign (#) on the mean tables.

In addition, for each organ/lesion classification the sexes were analyzed separately using a two-tailed Fisher's Exact Test comparing each treated group to the controls. An $p = 0.05$ significance level with Bonferroni's adjustment for multiple comparisons was used within each organ/lesion/sex category. If the expected value of each cell was greater than 20, then Yates' corrected Chi-Square test was used. An asterisk on the summary tables indicates a significant difference between the controls and the treated group.

Internal RIKER memoranda pertaining to these biostatistical procedures are presented within Appendix Item E.
RESULTS

In-Life Findings: Body weight gains were depressed in the FM-3924-treated male rats compared to the control male animals for only the first month and a half of the study. There was an approximate 14% decrease in the high-dose body weights by study week six and this difference was maintained until the end of the study. Further, a 2-6% decrease occurred in the low- and mid-dose male groups throughout the study (see Table 2, Figure 1 and Appendix Item F).

Body weight gains were also depressed in the FM-3924 dosed female rats compared to the control female animals. However, there were several differences between the sexes regarding this parameter. The onset for this change was slower, the effects were clearly dose dependent, the degree of difference from control values was greater, and the changes in body weight gain continued throughout the study for the female groups. The body weight differences compared to control values at the end of the study were -20.8%, -15.3% and -3.5% for the high-, mid- and low-dose groups, respectively (see Table 3, Figure 2 and Appendix Item F).

Feed consumption, measured as daily mean consumption per kilogram of mean body weight, was generally increased in all of the FM-3924-treated male and female groups over control animals. While more pronounced in the treated males, feed consumption increased gradually during the two year test period when compared to control group feed intake. In the males, this change was most pronounced in the high-dose group where there was roughly a 15% increase noted with sporadic values going as high as 33% during the two year test period. In the females, the pattern was less consistent during the first 94 weeks. There was an increase in feed consumption during the last 10 weeks of the study, particularly in the mid-dose group, but also in the high-dose females. During this specific period, feed consumption increased as much as 35% in the mid-dose animals (see Tables 4 & 5 and Figures 3 & 4).

Actual feed consumption (without regard for body weight change) was decreased in the high-dose males, but increased in the mid- and low-dose male groups during the first year of the study. During the second year, all male dose groups tended to consume more feed than the comparable
control animals. In the female treated groups, only the low-dose group showed a very slight increase in feed consumption. During the second year, all of the female dose groups tended to consume less feed than the comparable controls with the exception of the mid-dose group which consumed considerably more feed than the controls during the last ten weeks of the study (see Tables 6 & 7 and Appendix Item F).

Test substance concentration measured as parts per million in the diet was determined at three month intervals with duplicate analyses performed on two separate occasions when aberrant values were detected. The mean deviations from the target concentration of the high-, mid- and low-dose groups were +3.4%, -1.8% and +1.4%, respectively (Table 1).

Actual test substance consumption was determined for every two week period for each sex and each experimental group, and expressed as milligrams per kilogram per day (mg/kg/day). The mean test substance consumption was approximately 4.5, 1.3, and 0.4 mg/kg/day for males, and 5.5, 1.6 and 0.5 mg/kg/day for females regarding the high-, mid- and low-dose groups, respectively. Mean test substance consumption values calculated at two week intervals for the entire study are presented in Table 8.

Overall survival rates, particularly for the male rats, were not affected during the two year test period. At the end of the first year, 15 rats/sex from the control and high-dose groups were terminated to fulfill protocol requirements. Therefore, the final survival rates based on 50 rats/sex/group were 70%, 64%, 70% and 78% for males, and 50%, 68%, 51% and 44% for females regarding the control, high-, mid- and low-dose groups, respectively. A possible test substance effect may have slightly lowered the survival rate in the high-dose male group; however, the survival rate in the high-dose females was higher when compared to control rats (see Table 9).

A summary of the most commonly observed clinical signs is contained in Table 10. The only clinical sign that occurred more frequently in FM-3924-treated groups than control groups was clonic convulsions, which occurred at a slightly higher incidence in high-dose males. In this case, ten animals were affected whereas clonic convulsions were seen only in three, three and four male rats in the control, mid- and low-dose groups, respectively. There was a very slight increase in the incidence of ataxia
in both sexes of the treated groups; however, there also was a background incidence of this clinical finding in the controls.

The FM-3924 treated population of rats experienced a suspected outbreak of sialodacryoadenitis (SDA) viral infection between the first and second months of the study. Clinical signs included swollen submandibular salivary glands combined with occasional ocular manifestations observed in eight male and three female high-dose animals, four male and three female mid-dose animals, and five male and 13 female low-dose rats. The submandibular swelling resolved within 10 days, and the incidence of residual ocular changes was extremely low. The control population had comparable signs during the sixteenth month of the study. Thirteen males and 13 females demonstrated signs of this condition which persisted for about 16 days from the time of onset. One male and three females developed ocular opacities during this period.

The incidence of palpable tissue masses during the in-life phase of the study was not increased in any of the FM-3924-treated groups when compared to the control rats (Table 10).

The results of the final ophthalmoscopic examinations were negative relative to any FM-3924 treatment-related effects. Changes which were observed included a random distribution of cataracts considered to be typical geriatric changes along with some cases of chronic uveitis and superficial keratitis which were also considered to be within normal limits for aging populations of rats (see Table 11 and Appendix Item G). Many of the rats which exhibited ocular lesions were those used to obtain blood samples via the retrobulbar venous plexus.

The high-dose female red blood cell counts were decreased below control values throughout the two year study with statistically significant decreases observed at 6, 12 and 18 months (Table 13). In contrast, the female mid-dose erythrocyte counts were statistically increased at 3 and 6 months, but were not significantly different from control values during the last year of the study. There was an increased incidence of poikilocytosis, microcytosis and polychromasia in the high- and mid-dose females at 24 months. The degree of morphological change was generally noted as slight. Hemoglobin values were statistically decreased in the high-dose females from month 3 to month 24. Hematocrit values were statistically decreased in the high-dose females at the 3 month interval.
Changes seen in leukocytic parameters did not suggest a meaningful test substance effect at any FM-3924 dose levels (Tables 12 & 13). Increased white blood cell counts were seen in the male FM-3924 treated groups during the first three months of the study, but the increase was only statistically significant at the high-dose and still within an acceptable range for all groups. The increase in leukocytes was generally due to small increases in neutrophils and/or leukocytes. At six months, leukocyte counts were again slightly elevated in all male FM-3924 treated groups with elevated neutrophil counts in the mid-dose, decreased neutrophils in the low-dose, and significantly elevated lymphocyte counts in both the mid- and low-dose groups. The only change seen in the FM-3924 treated females was a nonspecific decrease in leukocytes as a result of a decrease in lymphocytes during the first three months.

Clinical chemistry findings at three months included slight increases in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP) and blood urea nitrogen (BUN) in all of the FM-3924-treated male groups. The treated female groups at this same time showed only a minor rise in BUN when compared to concurrent control values. From six months until the end of the study, the high-dose (and occasionally mid-dose) male ALT and AST values were increased above both the concurrent controls and the historical control values for the laboratory. AP values were also increased during this same time period, but rarely did these values exceed historical control limits. After six months, no meaningful changes were noted in the female groups (Tables 14 & 15).

Urinalysis findings demonstrated a general, time related increase in incidence and severity of albumin and occult blood in all of the male and female control and treated groups. These findings were more pronounced in the males than in the females at the end of the study. Other than an occasional incident of slight ketonuria in both control and treated animals, there were no other remarkable urinalysis findings (Table 16).

Postmortem Findings: The consulting Veterinary Pathologist's complete report is presented in Appendix Item D. The important gross pathology findings seen at the one year interim termination were limited to the high-dose male rats in which there was an increase in the incidence of pale and/or tan livers. This change was considered to be most likely a

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compound related effect. There were no specific necropsy findings in the high-dose female animals suggestive of any alterations related to the administration of FM-3924.

FM-3924 related gross findings seen in male and female high-dose rats which were either found dead, terminated in extremis, or terminated at the end of the study were also limited primarily to the liver. These findings included an increased incidence of hepatic masses, nodules and raised lesions, mottled livers, and yellow or pale focal lesions. There were no gross findings suggestive of a test substance related effect seen in the low- and mid-dose animals necropsied at comparable periods of time. Those gross pathologic findings which were observed were not related to test article administration, and were typical of findings seen in aging rats of this strain. These findings included mammary masses, pituitary masses and foci, ulcers on the hind footpads, pale livers, and pale, pitted and enlarged kidneys.

Organ weights presented as either absolute or relative values (ie. ratio of organ/body weight or organ/brain weight) are contained in Tables 17 and 18. At the one year interim sacrifice where the only groups examined were the high-dose and controls (n = 15/sex/group), the most obvious changes in absolute weights were a statistically significant decrease in body weight and an increase in liver weight for both male and female high-dose animals compared with their respective control values. Statistically significant decreases (p = <0.05) in heart and spleen weights were also noted for both the males and females of the high-dose group, and statistically significant decreases were seen in adrenal and pituitary weights in the high-dose males only. Relative weight comparisons confirmed the increased liver weight in both sexes of the high dose group and in addition showed a significant increase in kidney and brain weights. Other statistically significant altered relative values in the high-dose group included increased uterine and adrenal weights in the females, and increased testicular and decreased pituitary weights in the males.

Organ weights were obtained from all four groups at the end of the study (n = 15/sex/group). Statistically significant decreased body weights and increased liver weights when compared to control values were seen only in the high-dose male and female animals. The only other significant change was a decrease in spleen weights in the high- and mid-dose male
rats. Statistically significant relative organ weight changes found in both males and females included increased liver weights in the high- and mid-dose groups, and increased kidney weights in the high-dose group; statistically significant increased spleen weights were noted only in high-dose males. Liver to brain weight ratios were increased in all of the FM-3924 treated groups, but only in the high-dose groups was the change statistically significant (p = <0.05). Uterine and testicle to brain weight ratios were slightly increased in the FM-3924 treated groups.

Details of the histopathologic findings are contained in Appendix Item D and a summary of the major neoplastic and non-neoplastic microscopic changes found after two years of continuous oral administration of FM-3924 are enumerated in Tables 19 and 20.

Histopathologic evaluation of the tissues obtained from the animals necropsied at one year indicated the major FM-3924 effects were confined to the liver. Diffuse megalocytosis (ie. hypertrophy) and vacuolation of hepatocytes were the most common findings in essentially all of the male and female rats in the high-dose group. In addition, hepatocytic necrosis was found in 6/15 high-dose males, but was not seen in any of the high-dose females.

The majority of neoplasms observed after two years of dosing with FM-3924 involved either the liver or one of several endocrine or endocrine-sensitive organs (see Table 19). Hepatocellular carcinomas were found in 6%, 4%, 2% and 2% of the male rats from the control, high-, mid- and low-dose groups, respectively. For the females, hepatocellular carcinomas and adenomas were found only in the high-dose group with incidence values of 6% and 8%, respectively. The organ with the largest incidence of tumors was the pituitary gland in which adenomas in the control animals were 35% for males and 72% for females. Sporadic increases in incidence for this neoplasm were noted primarily for the FM-3924 treated males; however, there did not appear to be any meaningful relationship of these to the dose administered.

Mammary gland adenocarcinomas were present in both control and treated female rats at a level of 15%, 21%, 24% and 13% for the control, high-, mid- and low-dose groups, respectively. Similarly, fibroadenomas were seen in 22%, 13%, 33% and 36% of the female rats at the end of the study. Mammary gland adenomas (7%) and carcinomas (2%) were observed only in the
female controls. The incidence of uterine polyps, benign growths of the endometrium, was slightly increased in the mid- and low-dose females. Likewise, there were minor variations in the tumor incidence in the adrenal glands (ie. pheochromocytomas) and thyroids, both of which commonly exhibit spontaneous tumors in geriatric rats of this strain. Only the incidence of C-cell carcinomas of the thyroid in female rats appeared to show a minimal dose dependent increase with an incidence of 0%, 4%, 3% and 2% for the control, high-, mid- and low-dose groups, respectively. However, in contrast, the male control animals showed a greater incidence of C-cell carcinomas than any of the FM-3924 treated male groups (ie. 5%, 2%, 2% and 0%, respectively).

Non-neoplastic histopathologic changes at the end of the study were found in the adrenal, heart, kidney, liver, lung, testes, ovary, thyroid, urinary bladder and uterus (see Table 20). As noted in the one year interim histopathologic evaluation, the liver was the primary target organ for FM-3924 related effects, and there was a remarkable consistency in the type of findings observed after the second full year of test article administration at the high-dose level. Megalocytosis and hepatocyte vacuolation (both findings considered to be reversible biological events) were the major changes seen in both males and females treated with the highest doses of FM-3924. Megalocytosis was also found at an incidence exceeding 50% in the mid- and low-dose males, but was essentially absent from the corresponding female groups. Hepatocyte vacuolation was observed at an incidence less than control values in the mid- and low-dose male and female groups.

Hepatic cystoid degeneration, a condition characterized by areas of multilocular microcysts in the liver parenchyma, was seen at an incidence of 8%, 30%, 16% and 12% in males from the control, high-, mid- and low-dose groups, respectively, while only the high-dose females were affected at an incidence of 8%. Hepatocellular necrosis was found at an incidence of between 6-10% in the control animals of both sexes, but the high-dose males demonstrated an incidence rate for this lesion of 20%, with 0% and 4% in the mid- and low-dose groups, respectively. The incidence of liver necrosis for females was 8%, 12% and 10% for the high to low treatment groups. Hyperplastic nodules, a localized proliferation of hepatic parenchymal cells, was observed essentially only in the high-dose group.
with an incidence of 10% in the males and 18% in the females as compared to 0% and 2% in the control male and female rats, respectively. The only other hyperplastic nodule seen was found in a mid-dose male animal.

There was a statistically significant and dose dependent increase in tubular hyperplasia of the ovarian stroma of female rats. Tubular hyperplasia is a diffuse, non-neoplastic increase in stromal tubular elements which is usually bilateral and associated with decreased or absent follicular development. The incidence of this change was 0%, 31%, 23% and 13% in the control, high-, mid- and low-dose groups, respectively. Cystic glands of the uterine endometrium were found at a higher incidence in the high-dose females (29%) when compared to the control animals (14%).

The incidence of nodular hyperplasia of the adrenal cortex was significantly increased in the high-dose males.

A statistically significant increased incidence of foamy macrophage accumulation in the lung of the high-dose male and female rats was considered a possible test article effect.

The remaining major non-neoplastic lesions (Table 20) and other histopathologic findings (Appendix Item D) had varying incidences either similar to, increased, or decreased from control values; however, essentially all of these findings are commonly associated with either endemic diseases and/or geriatric changes found in this strain of rat. The changes presented hereinafter were not considered test-article related findings, although their incidence varied from that observed in the control rats.

In the lung, the incidences of alveolar macrophages and vascular mineralization were increased above control levels while the incidence of interstitial pneumonia was essentially equal to or less than the control incidence at the end of the study. Likewise, the characteristic lesions of an endemic renal disease seen in old rats appeared to be somewhat diminished in the high-dose males and females. The incidences of adrenal gland changes, usually found in old rats, were inconsistent, being either higher or lower than the control values. The incidence of nodular hyperplasia of the adrenal cortex was significantly increased in the high-dose males; the incidence of sinusoidal ectasia was increased in the mid- and high-dose males but decreased in the females of the same group;
and the incidence of adrenal cortical vacuolation was decreased in the treated males but generally increased in the FM-3924 treated females.

The incidence of chronic myocarditis was reduced in a dose dependent fashion in the female rats while being increased above the control incidence in the mid- and low-dose males. Polyarteritis, a spontaneous lesion in the testes of old male rats, was found in this study in more control animals than in any of the FM-3924-treated groups. However, the incidence of testicular vascular mineralization was increased in the high- and low-dose animals.

The incidence of epithelial hyperplasia of the urinary bladder was decreased in all of the FM-3924-treated female groups, while there was an equivocal increase in the incidence for the mid-dose male group only.

Finally, the incidence of thyroid C-cell hyperplasia was moderately increased in both the male and female high- and mid-dose groups.
DISCUSSION

The purpose of this study was to define any long term toxicity and to profile the potential oncogenicity of FM-3924, a perfluoroalkylsulfonamido alcohol used in a variety of industrial manufacturing processes. The results of the study included a series of biological events which when taken as separate findings may appear to confound test substance dose, sex and/or time relationships. However, considering an overview of all of these results, there appears to be a common pattern of test substance related effects which may be generalized as follows.

The general well being of a rat exposed to the experimental conditions of a two year feeding study may be examined at the beginning of the test by evaluating body weight gains and feed consumption compared to the study control animal population. Body weights of the males decreased as early as the second week of the study. The females also showed a decrease, but the time of onset was delayed and the early body weight differences were not as pronounced as those seen in the males. This effect did not appear to be associated with decreased palatability of the diet due to the addition of test article since feed consumption based on weight of diet consumed versus body weight was actually increased. The possible effects that the apparent SDA viral infection might have had on body weight gains were considered, but discounted since the FM-3924 treated animals continued to show weekly increases in body weight despite the fact that the total gains were less than control gains. Since there was a dose and sex dependent change in this parameter, the decrease in body weight gain could be associated with a direct test article effect, possibly through varying degrees of altered hepatocellular metabolic activity as suggested by the microscopic hepatocellular changes observed in tissues obtained at both the one and two year necropsies. Regardless of this assumption, mortality rates were not obviously affected by the decreased body weight gains.

The concentration of the test substance in the diet was maintained during the full two years of the study at 10, 30 and 100 ppm for the low-, mid- and high-dose groups, respectively. The average daily dosage of FM-3924 for the same time period and for both sexes combined was estimated to be about 0.5, 1.5 and 5.0 mg/kg/day, respectively. Both in-life and postmortem results confirmed the systemic absorption of the test substance.
and the 100 ppm dosage level appeared to adequately comply with the concept of a maximum tolerated dose for a long term study in this particular strain of rat.

The only clinical signs seen during the study which were suggestive of a possible test article effect were ataxia and sporadic convulsions. The incidence of convulsions was increased very slightly only in the high-dose males, while ataxia was seen more frequently in both males and females of the FM-3924 treated groups as compared to control animals. These clinical signs were also seen in the control population but at a lower incidence. There were no cellular lesions found in the central or peripheral nervous system at the end of the study which could support a treatment related effect.

In the high-dose females there was an apparent decrease in hemoglobin; however, these values were within the normal ranges for this laboratory. There were no other meaningful compound-related effects on the hematologic parameters.

Elevation of serum alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase activities, primarily in the FM-3924 treated male rats, suggested the presence of hepatocellular alterations. Most of the increases in enzyme activity were more pronounced in the earlier phases of the study, subsequently resolved in the mid- and low-dose males, and persisted in the high-dose group until the end of the study. The mean values in the mid- and low-dose groups from which these observations were derived were influenced commonly by only one or two animals with aberrant serum enzyme activities. However, there was no meaningful exacerbation of the liver changes even into the geriatric period of the rat’s life span. These findings were substantiated by the organ weight and histopathologic comparisons obtained at the one and two year necropsies.

Mean blood urea nitrogen values were elevated in the male FM-3924 treated groups at three months, but not thereafter. If there were alterations in kidney function at that time, they did not persist until the end of the study since there was a decrease in the incidence of chronic nephropathy in the FM-3924 treated males at two years when compared with control males.

Changes in the quality or character of the urine specimens from both control and FM-3924 treated rats demonstrated a change in several urinary
parameters that were progressive over time. These findings were considered to be associated with the progressive degenerative changes of naturally occurring chronic renal disease commonly found in rats of this strain.

The primary test article effect occurred in the liver as increased organ weight (both absolute and relative), as gross findings at necropsy, and as histopathologic alterations. These changes were observed at the one year necropsy, but showed remarkably little progression one year later at the two year necropsy. The high-dose males and females had essentially equal incidences of hepatic findings, while the mid- and low-dose males were more markedly affected than were the females from the corresponding dose groups.

Hepatomegalocytosis and hepatocellular vacuolation are characteristic of increased metabolic activity in the rat. It is recognized that these lesions may progress to cystoid degeneration and, ultimately, hepatocellular necrosis. The incidence of hepatic necrosis in this study was slightly increased only in the high-dose males. Since the liver in the rat rarely repairs parenchymal cell loss with fibrosis or scar tissue, a more typical cellular reaction is hepatocellular hyperplasia. In this study, the incidence of hyperplastic nodules were increased only in the high-dose male and female animals (ie., 10% and 18%, respectively) compared to the control groups (ie. 0% and 2%, respectively). It is important to note that no proliferative hepatic lesions (ie. hyperplasia nor neoplasia) were seen in any of the high-dose rats after receiving FM-3924 for one year.

Primary liver neoplasms observed in this study after two years consisted of hepatocellular adenomas and carcinomas. The overall incidence of these neoplasms was low, with carcinomas occurring in both the control and FM-3924 treated groups as follows: males 6%, 4%, 2% and 2%; and females 0%, 6% 0% and 0% for the control, high-, mid- and low-dose groups, respectively. Benign hepatic neoplasms (ie. adenomas) were not found in any group except the high-dose females where an incidence of 8% was recorded. Considering the chronic liver stimulation noted in both the high-dose male and female groups during their life span, the incidence of hepatic neoplasia appears to be within reasonable limits with the possible exception of the high-dose female group. Combined malignant and benign incidence values are within reasonable historical control limits for the
high-dose males while the high-dose females appear to be slightly outside these limits only because there were no liver tumors seen in the control group. Based on these findings, FM-3924 was not considered to be a hepatic carcinogen in the rat.

Most of the neoplasms observed in this study originated from endocrine or endocrine-sensitive organs including the adrenal glands, mammary gland, pituitary, thyroid and uterus. These are common sites for spontaneous or naturally occurring oncogenesis in this strain of rat as evidenced by the specific tumor incidence in the control group (see Table 19). Deviations from the control incidence for these neoplasms were neither numerically meaningful nor did they involve a unique tumor type not commonly seen in this strain of rat.

The non-neoplastic findings reported from the histopathologic evaluation of all of the animals scheduled for the two year necropsy were mostly geriatric lesions typical for this strain of rat. The organs in which these lesions were found included adrenal glands, heart, kidneys, lung, testes, ovaries, thyroid, urinary bladder and uterus. Specific deviations from the control incidence seen in FM-3924 treated groups were addressed in the results section of this report. However, the following changes were considered equivocal test article related effects. The incidence of nodular hyperplasia of the adrenal cortex was significantly increased (22%) in the high-dose males compared to the same finding in the controls (4%), while the high-dose female rats showed a much lower incidence (2%).

Lung changes were also sporadic in occurrence with the incidences of the accumulation of alveolar macrophages increased in the high-dose males and females. The incidence of ovarian (stromal) tubular hyperplasia was increased in a statistically significant and dose dependent manner. The interpretation of this finding is uncertain, but a possible explanation is that it was secondary to the hepatic changes which may evoke endocrine related effects in older rats. Likewise, the increase in uterine cystic glands in the high-dose females could be a manifestation of this biological phenomenon.