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3M

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

(RIKER Experiment No. 0281CR0012)

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St. Paul, Minnesota 55144-1000 U.S.A.

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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 perfluorooctanesulfonamido 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 polychromasia 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 the high-dose female rats having a tumor incidence that, while not statistically significant, was 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.

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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^{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.

Treatment Groups	Dosage Levels (ppm)	Group Size & Animal Numbers Males (An. Nos.)	Females (An. Nos.)
1 - Control	0	65 (3516-3580)	65 (4576-4640)
2 - High	100	65 (3696-3760)	65 (4756-4820)
3 - Mid	30	50 (3761-3810)	50 (4821-4870)
4 - Low	10	50 (3811-3860)	50 (4871-4920)

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:

Aorta	Liver (2 Sections)
Adrenals (2)	Lung (2 Sections)
Brain (3 Sections including frontal cortex and basal ganglia, parietal cortex and thalamus; cerebellum and pons)	Lymph node (mesenteric)
	Mammary Gland (females)
	Pancreas
	Pituitary
Eyes	Salivary Gland
Gonads	Spinal Cord/Bone Marrow (vertebrae)
Ovaries (2)	Spleen
Testes/Epididymides (2)	Stomach
Heart	Thyroid/Parathyroid/Trachea/Esophagus
Small Intestine (3 Sections)	Urinary Bladder
Large Intestine	Uterus or Prostate
Kidneys (2 Sections)	Any tissue masses (suspected tumors)
	Any gross lesion

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

Table 2

Two Year Oral (Diet) Toxicity-Oncogenicity Study
of Fluorocarbon FM-3924 in Rats

Summary of Mean Body Weights (g) ± % Difference from Control

MALES

Study Week	Control Mean Wt.	100 ppm Mean Wt.	% Diff.	30 ppm Mean Wt.	% Diff.	10 ppm Mean Wt.	% Diff.
0	165.3	168.2	+ 1.8	169.1	+2.3	165.6	0
2	270.9	253.6	- 6.4	262.8	-3.0	258.3	-4.6
4	334.0	308.7	- 7.6	327.7	-1.9	321.3	-3.8
6	390.7	333.1	-14.7	361.6	-7.4	355.3	-9.1
8	429.2	369.8	-13.8	404.8	-5.7	394.5	-8.1
10	456.2	397.2	-12.9	433.8	-4.9	428.3	-6.1
12	475.5	417.1	-12.3	454.8	-4.4	453.2	-4.7
14	493.2	425.5	-13.7	464.7	-5.8	466.2	-5.5
16	502.9	442.4	-12.0	485.0	-3.6	484.2	-3.7
18	515.3	452.7	-12.2	495.0	-3.9	495.0	-3.9
20	526.8	465.0	-11.7	508.9	-3.4	508.0	-3.6
22	542.3	474.6	-12.4	522.5	-3.6	521.1	-3.9
24	550.4	483.7	-12.1	532.1	-3.3	531.9	-3.4
26	561.0	489.0	-12.8	541.4	-5.5	541.1	-3.5
28	567.5	500.1	-11.9	552.4	-2.7	553.0	-2.6
30	576.3	510.5	-11.4	561.3	-2.6	561.9	-2.5
32	580.6	515.9	-11.1	569.2	-2.0	570.1	-1.8
34	582.9	507.1	-13.0	567.1	-2.7	566.3	-2.8
36	591.0	518.8	-12.2	576.8	-2.4	575.8	-2.6
38	599.4	524.9	-12.4	578.2	-3.5	578.0	-3.6
40	608.4	530.4	-12.8	586.8	-3.6	588.7	-3.2
42	612.9	536.5	-12.4	592.0	-3.4	590.7	-3.6
44	619.8	541.7	-12.6	598.8	-3.4	595.9	-3.9
46	623.5	548.8	-12.0	604.8	-3.0	601.3	-3.6
48	626.1	552.3	-11.8	611.0	-2.4	605.2	-3.3
50	639.2	559.9	-12.4	619.1	-3.1	614.1	-3.9
52	638.6	566.7	-11.2	623.8	-2.3	618.1	-3.2

Table 2 (concluded)

Two Year Oral (Diet) Toxicity-Oncogenicity Study
of Fluorocarbon FM-3924 in Rats

Summary of Mean Body Weights (g) ± % Difference from Control

MALES

Study Week	Control Mean Wt.	100 ppm Mean Wt.	% Diff.	30 ppm Mean Wt.	% Diff.	10 ppm Mean Wt.	% Diff.
54	654.3	573.4	-12.4	627.3	-4.1	620.7	-5.1
56	661.6	579.0	-12.5	633.5	-4.2	625.7	-5.4
58	668.0	583.6	-12.6	634.6	-5.0	635.2	-4.9
60	673.2	584.5	-13.2	637.3	-5.3	639.4	-5.0
62	674.4	589.2	-12.6	640.3	-5.1	641.2	-4.9
64	664.5	589.5	-11.3	641.7	-3.4	642.8	-3.3
66	650.8	591.0	- 9.2	644.7	-0.9	646.0	-0.7
68	656.6	592.9	- 9.7	646.2	-1.6	650.6	-9.1
70	661.9	601.2	- 9.2	650.5	-1.7	650.5	-1.7
72	658.3	592.1	-10.1	640.5	-2.7	642.9	-2.3
74	660.8	588.1	-11.0	638.7	-3.3	641.6	-2.9
76	667.7	600.2	-10.1	651.9	-2.4	648.3	-2.9
78	668.8	598.9	-10.5	654.8	-2.1	650.6	-2.7
80	670.6	607.2	- 9.5	656.5	-2.1	652.5	-2.7
82	663.1	602.6	- 9.1	657.5	-0.8	648.8	-2.2
84	668.1	612.3	- 8.4	657.3	-1.6	648.9	-2.9
86	675.6	610.2	- 9.7	653.4	-3.3	653.4	-3.3
88	678.9	613.4	- 9.6	648.2	-4.5	655.9	-3.4
90	690.0	617.7	-10.5	646.9	-6.2	656.8	-4.8
92	686.0	610.9	-10.9	644.7	-6.0	655.4	-4.5
94	678.3	595.3	-12.2	634.3	-6.5	657.3	-3.1
96	677.5	592.6	-12.5	630.9	-6.9	651.1	-3.9
98	675.7	594.2	-12.1	627.6	-7.1	649.2	-3.9
100	671.1	594.7	-11.4	630.0	-6.1	642.1	-4.3
102	665.9	588.8	-11.6	629.0	-5.5	651.1	-2.2
104	642.0	568.6	-11.4	628.9	-2.0	645.6	+0.6

Table 3

Two Year Oral (Diet) Toxicity-Oncogenicity Study
of Fluorocarbon FM-3924 in Rats

Summary of Mean Body Weights (g) ± % Difference from Control

FEMALES

Study Week	Control Mean Wt.	100 ppm Mean Wt.	% Diff.	30 ppm Mean Wt.	% Diff.	10 ppm Mean Wt.	% Diff.
0	138.2	138.7	+ 0.4	135.8	-1.7	135.6	-1.9
2	159.2	168.5	+ 5.8	163.3	+2.6	158.3	-0.6
4	209.5	211.4	+ 0.9	213.6	+2.0	211.0	+0.7
6	238.7	224.6	- 6.0	234.3	-1.8	237.9	-0.3
8	256.9	237.7	- 7.4	252.0	-2.0	256.8	0.0
10	269.2	243.2	- 9.7	261.6	-2.8	260.8	-3.1
12	279.5	241.6	-13.6	264.4	-5.4	270.4	-3.3
14	289.7	251.1	-13.3	275.0	-5.1	283.2	-2.2
16	291.8	256.9	-12.0	283.4	-2.9	291.7	0.0
18	306.8	263.2	-14.2	292.3	-4.7	301.8	-1.6
20	311.6	269.4	-13.5	299.3	-3.9	308.0	-1.2
22	319.1	273.2	-14.4	303.5	-4.9	317.0	-0.7
24	324.5	278.1	-14.3	310.3	-4.4	323.1	-0.4
26	327.3	279.9	-14.5	312.9	-4.4	327.7	+0.1
28	333.7	285.0	-14.6	321.7	-3.6	332.5	-0.4
30	338.3	282.2	-16.6	318.6	-5.8	332.7	-1.7
32	345.5	289.8	-16.1	325.7	-5.7	337.6	-2.3
34	350.7	292.1	-16.7	331.6	-5.4	342.1	-2.4
36	355.5	300.5	-15.4	337.8	-5.0	351.6	-1.1
38	359.3	302.1	-15.9	341.5	-5.0	358.3	-0.3
40	363.2	306.3	-15.7	345.4	-4.9	359.6	-1.0
42	371.2	311.4	-16.1	353.7	-4.7	369.3	-0.5
44	380.1	313.9	-17.4	358.3	-6.0	376.3	-1.0
46	385.7	317.8	-17.6	363.8	-5.7	379.6	-1.6
48	392.3	321.1	-18.1	368.6	-6.0	386.9	-1.4
50	398.6	324.6	-18.6	373.9	-6.2	391.3	-1.8
52	406.1	328.8	-19.0	379.3	-6.6	400.5	-1.4

Table 3 (concluded)

Two Year Oral (Diet) Toxicity-Oncogenicity Study
of Fluorocarbon FM-3924 in Rats

Summary of Mean Body Weights (g) ± % Difference from Control

FEMALES

Study Week	Control Mean Wt.	100 ppm Mean Wt.	% Diff.	30 ppm Mean Wt.	% Diff.	10 ppm Mean Wt.	% Diff.
54	414.1	334.2	-19.3	388.0	-6.3	407.6	-1.6
56	419.1	340.9	-18.7	396.0	-5.5	412.6	-1.6
58	420.9	346.9	-17.6	404.1	-4.0	421.8	+0.2
60	423.6	350.0	-17.4	408.0	-3.7	423.9	0.0
62	426.6	351.0	-17.7	411.5	-3.5	425.6	-0.2
64	426.1	351.8	-17.4	416.5	-2.3	429.0	+0.7
66	424.9	350.4	-17.5	413.7	-2.6	425.5	+0.1
68	427.5	357.7	-19.5	422.4	-1.2	434.7	+1.7
70	431.4	360.8	-16.4	423.5	-1.8	440.6	+2.1
72	435.2	366.4	-15.8	429.9	-1.2	449.9	+3.4
74	446.5	371.4	-16.8	435.1	-2.6	455.9	+2.1
76	455.2	373.4	-18.0	442.1	-2.9	458.2	+0.7
78	464.8	369.3	-20.5	438.3	-5.7	458.7	-1.3
80	474.9	378.7	-20.3	444.9	-6.3	466.9	-1.7
82	484.0	378.2	-21.9	436.4	-9.8	467.1	-3.5
84	484.8	383.9	-20.8	443.8	-8.5	475.7	-1.9
86	492.7	378.3	-23.2	443.9	-9.9	490.2	-0.5
88	499.1	389.7	-21.9	445.5	-10.7	486.9	-2.4
90	500.6	389.7	-22.2	442.1	-11.7	494.6	-1.2
92	512.5	392.6	-23.4	448.9	-12.4	487.1	-5.0
94	505.7	399.1	-21.1	440.1	-13.0	486.1	-3.9
96	506.9	402.6	-20.6	445.6	-12.1	499.3	-1.5
98	500.0	402.6	-19.5	444.9	-11.0	494.6	-1.1
100	501.2	396.9	-20.8	435.5	-13.1	492.1	-1.8
102	503.6	402.3	-20.1	438.6	-12.9	490.4	-2.6
104	502.0	397.8	-20.8	425.2	-15.3	484.5	-3.5

Figure 1

Two Year Oral (Diet) Toxicity-Oncogenicity Study of Fluorocarbon FM-3924 in Rats

Mean Body Weights (g) Males

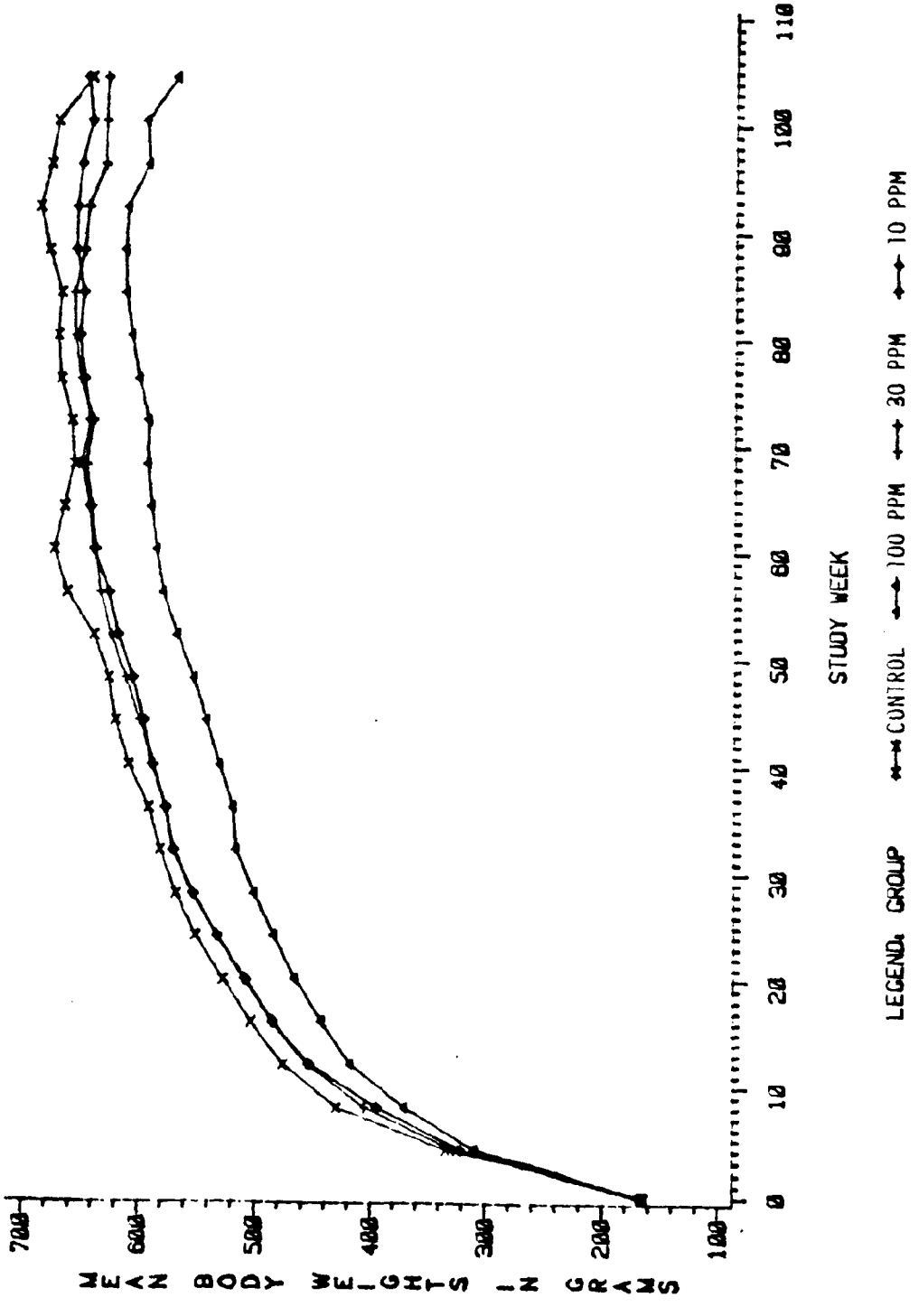
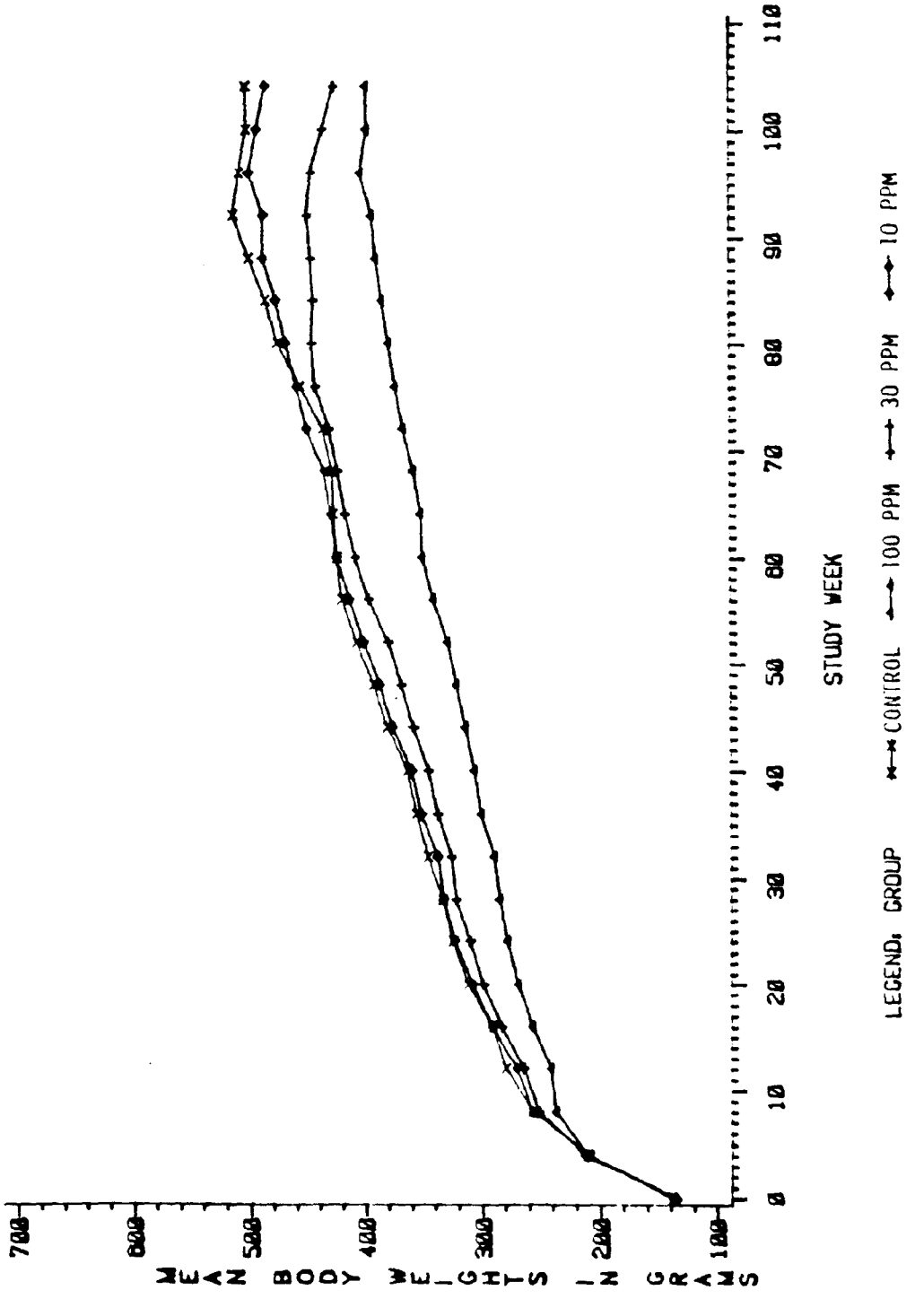


Figure 2

Two Year Oral (Diet) Toxicity-Oncogenicity Study of Fluorocarbon FM-3924 in Rats
Mean Body Weights (g) Females



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 in 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

Table 1

Two Year Oral (Diet) Toxicity - Oncogenicity Study
of Fluorocarbon FM-3924 in Rats

Analytical Analysis of FM-3924[a]
(% Above/Below Desired Concentration)

Approximate Study Month	CCD Analytical Report Numbers	Dosage Levels (ppm) 200 Diet Concentrate	100	30	10
0 (Initial)	213	+ 7.0%	+ 7.0%	- 4.3%	± 0.0%
1	229	+ 4.0%	+ 4.0%	± 0.0%	+ 9.0%
4	251	+ 7.0%	+10.0%	+ 2.2%	+ 7.0%
7	290	+ 8.5%	+ 5.0%	- 1.0%	- 3.0%
10	320	- 1.0%	+ 5.3%	- 6.7%	[b] -21.7%
11	327	-	-	-	+ 0.7%
13	341	- 0.5%	- 1.3%	- 3.3%	+11.0%
16	367	- 3.5%	[c] -19.3%	- 4.4%	+ 6.7%
16	368	-	+ 3.3%	-	-
19	383	+11.5%	+ 7.3%	+11.0%	+13.0%
22	394	-16.5%	- 8.0%	-10.0%	-17.0%
24	403	+ 4.0%	+ 1.0%	- 2.0%	-13.0%
Mean Deviation for the study		+ 2.0%	[d] ± 3.4%	- 1.8%	[d] ± 1.4%

[a] Commercial Chemicals Division (CCD)
[b] Incorrect values given at this level due to instrument malfunction; this dosage level was reanalyzed after new detector was installed.
[c] Incorrect values given possibly due to a variation in analytical procedure; percentage near lower acceptable limit, this dosage level reanalyzed (two weeks later).
[d] Excluding from mean deviation the percentages near ± 20% acceptable limit.

Table 9

TWO YEAR ORAL(DIET) TOXICITY-ONCOGENICITY STUDY OF FLUOROCARBON FM-3924 IN RATS
MORTALITY DATA

DOSE GROUP	INITIAL NO.	WEEK OF STUDY 1-4	5-8	9-12	13-16	17-20	21-24	25-28	29-32	33-36	37-40	41-44	45-48	49-52	53-56
MALES															
CONTROL 0 PPM	50	0	0	0	0	0	0	0	0	1	0	0	0	0	1
HIGH DOSE 100 PPM	50	0	0	0	1	0	0	0	0	0	0	0	1	1	0
MID DOSE 30 PPM	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LOW DOSE 10 PPM	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FEMALES															
CONTROL 0 PPM	50	0	0	0	0	0	0	1	0	0	1	0	0	1	1
HIGH DOSE 100 PPM	50	0	0	0	0	0	0	0	0	0	0	0	0	1	0
MID DOSE 30 PPM	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LOW DOSE 10 PPM	50	0	0	0	0	0	0	0	0	0	0	0	0	0	1

Table 9 (Concluded)

TWO YEAR ORAL (DIET) TOXICITY-ONCOGENICITY STUDY OF FLUOROCARBON FM-3924 IN RATS
MORTALITY DATA

DOSE GROUP	INITIAL NOS.	WEEK OF STUDY 57-60	61-64	65-68	69-72	73-76	77-80	81-84	85-88	89-92	93-96	97-100	101-104	105-108	1-108 WEEK NOS.	1-108 WEEK TOTALS %
MALES																
CONTROL 0 PPM	50	0	1	0	0	1	0	1	4	1	2	1	2	1	16	32
HIGH DOSE 100 PPM	50	0	1	1	1	1	2	3	0	0	4	2	0	1	19	38
MID DOSE 30 PPM	50	0	0	0	1	2	1	1	0	1	2	3	4	0	15	30
LOW DOSE 10 PPM	50	1	0	0	1	1	0	1	3	0	1	1	2	1	12	24
FEMALES																
CONTROL 0 PPM	50	0	3	0	0	1	3	2	2	1	3	5	1	0	25	50
HIGH DOSE 100 PPM	50	0	0	1	1	1	3	1	1	3	2	0	2	0	16	32
MID DOSE 30 PPM	50	1	0	1	1	0	0	3	6	2	5	3	5	0	27	54
LOW DOSE 10 PPM	50	1	0	1	0	2	5	4	2	2	3	3	3	0	27	54

CHI-SQUARE TESTS FOR DOSE GROUP DIFFERENCES FROM CONTROL

DOSE GROUP	MALES CHI-SQUARE	MALES PROB.	FEMALES CHI-SQUARE	FEMALES PROB.
100 PPM	0.18	0.678	2.85	0.099
30 PPM	0.00	1.000	0.04	0.823
10 PPM	0.45	0.512	0.04	0.823

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

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

TABLE 19

Two Year Oral (Diet) Toxicity - Oncogenicity Study of Fluorochemical FM-3924 in Rats

Summary of Major Microscopic Findings - Percent Incidence at Two Years

NEOPLASTIC LESIONS[a]

	Controls		High		Mid		Low	
Organ/Findings	Male 50[b]	Female 50	Male 50	Female 50	Male 50	Female 50	Male 50	Female 50
ADRENAL								
Pheochromocytoma, Benign	4	4	0	2	8	0	8	2
Pheochromocytoma, Malignant	0	0	0	0	0	2	2	0
LIVER								
Hepatocellular Carcinoma	6	0	4	6	2	0	2	0
Hepatocellular Adenoma	0	0	0	8	0	0	0	0
MAMMARY GLAND								
Adenocarcinoma	-	15	-	21	-	24	-	13
Adenoma	-	7	-	0	-	0	-	0
Carcinoma	-	2	-	0	-	0	-	0
Fibroadenoma	-	22	-	13	-	33	-	36
PITUITARY								
Adenoma	35	72	43	71	60	82	47	76
THYROID								
C-cell Adenoma	0	2	2	2	4	3	7	2
C-cell Carcinoma	5	0	2	4	2	3	0	2
UTERUS								
Polyp	-	2	-	2	-	9	-	8

[a] - No statistically significant differences ($\alpha = 0.05$) were found between rats fed diets with FM-3924 and control group animals.

[b] - 50 rats/sex/group were at risk; % values derived from actual tissues examined.

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

TABLE 20

Two Year Oral (Diet) Toxicity - Oncogenicity Study of Fluorochemical FM-3924 in Rats

Summary of Major Microscopic Findings - Percent Incidence at Two Years

NON-NEOPLASTIC LESIONS

	Controls		High		Mid		Low	
Organ/Findings	Male 50[a]	Female 50	Male 50	Female 50	Male 50	Female 50	Male 50	Female 50
ADRENAL								
Nodular Hyperplasia	4	0	22*	2	0	4	10	0
Sinusoidal Ectasia	22	84	30	68	40	78	10	72
Cortical Vacuolation	48	10	36	22	30	14	20*	10
HEART								
Myocardial Fibrosis	10	4	10	2	12	10	20	6
Myocarditis, Chronic	28	32	30	4*	50	14	40	18
KIDNEY								
Nephropathy, Chronic	88	60	86	36	80	52	88	44
Pelvic Mineralization	8	60	0	66	16	48	16	46
LIVER								
Cystoid Degeneration	8	0	30*	8	16	0	12	0
Hepatocellular Alt.Vac.	20	26	18	12	32	12	24	12
Hepatocellular Vac.	44	60	78*	82	38	50	24	54
Hyperplastic Nodule	0	2	10	18*	2	0	0	0
Megalocytosis	0	0	84*	88*	56*	2	54*	0
Necrosis	6	10	20	8	0	12	4	10
LUNG								
Alveolar Macrophages	20	28	46*	54*	30	30	36	28
Perivas, Mono.Infil.	42	26	14*	38	40	20	30	18
Vascular Mineralization	86	44	96	78*	88	84*	90	80*
Pneumonia, Interstitial	32	14	12	18	14	12	8*	20
TESTIS/EPIDIDYMIS								
Polyarteritis	12	-	4	-	4	-	8	-
Vascular Mineralization	0	-	12	-	4	-	14*	-
OVARY								
Tubular Hyperplasia	-	0	-	31*	-	23*	-	13*
THYROID								
C-cell Hyperplasia	2	0	9	9	6	11	4	0
URINARY BLADDER								
Epithelial Hyperplasia	7	14	4	4	13	5	2	0*
UTERUS								
Cystic Glands	-	14	-	29	-	13	-	17

[a] = 50 rats/sex/group were at risk; % values derived from actual tissues examined.

* = Statistically significant difference ($\alpha = 0.05$) from controls.

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, although not statistically significant, 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

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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.

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CONCLUSIONS

The pertinent findings after administering FM-3924 to rats in the diet at concentrations of 0, 10, 30 and 100 ppm for up to two years under the conditions of this study may be summarized as follows:

1. FM-3924 related hematologic changes were seen in the high-dose female rats only and consisted of a decrease in hemoglobin concentration, and in hematocrit values with some evidence of altered red blood cell morphology in blood smears obtained at the end of the study. While the decrease in hemogram values was observed very early in the study, it did not progress into anemia by the end of the two year dosing period.

2. Increased liver weights and microscopic findings including hepatomegalocytosis, which was considered to be the result of chronic hepatocellular metabolic stimulation by FM-3924, and hepatocellular degeneration with and without necrosis, were the primary dose-related toxicologic findings observed in this study. These liver changes were seen at the one year necropsy, but showed very little evidence of progression during the study.

3. The non-neoplastic findings observed in this study were not considered to be primary test article related effects, but were believed to be related to hepatocellular effects and/or were associated with a mild exacerbation of anticipated geriatric endocrine lesions in this strain of rat. Specific histopathology findings which had an equivocal relationship to treatment with FM-3924 included a statistically significant, dose-related increase in ovarian tubular hyperplasia observed with the female rat 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.

4. 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.

5. The overall incidence of hepatocellular adenomas and carcinomas was low in both control and FM-3924 exposed groups with only the high-dose female rats possibly having a tumor incidence outside historical control limits. The majority of neoplasms observed originated from 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 treated groups, and did not demonstrate a unique tumor type.

6. Based on the incidence and types of neoplasms, time of onset of tumor appearance, malignancy patterns of tumors found at two years and the final mortality values, FM-3924 was not considered to be carcinogenic in the rat.

PRINCIPAL PERSONNEL INVOLVED WITH THE CONDUCT AND REPORTING OF RIKER EXPERIMENT NO. 0281CR0012 - TWO YEAR ORAL (DIET) TOXICITY/CARCINOGENICITY STUDY OF FLUOROCHEMICAL FC-143 IN RATS

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C.D. King, D.V.M., Ph.D.	External Consultant/Draft Report Preparation
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L.A. Marschke	Advanced Secretary/ Report Typing
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W.C. McCormick, III, B.S.	Toxicology Specialist/Sponsor Representative and Project Coordinator
J.R. Nelson, H.T.	Histotechnologist/Microscope Slide Preparation
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L.O. Wiseth	Senior Laboratory Technician/ Assisted With Study Data

SIGNATURE PAGE

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

RIKER Experiment No. 0281CR0012

From the Pathology and Toxicology Department
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METHODS

Gross Pathology

All rats which were sacrificed at the 1 year interim or the 2 year terminal sacrifice or which died or were sacrificed *in extremis* during the course of study were necropsied. 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 after removal from the carcass. Representative tissues and organs from each rat were fixed in 10% buffered neutal formalin for subsequent histologic processing. At the 1 and 2 year sacrifices, wet weights were obtained on adrenals, brain, testes, heart, kidneys, liver, spleen and uterus from 15 randomly selected rats/sex/group. Body weights were obtained prior to necropsy from the same rats for calculation of relative organ weights.

Histopathology

Microscopic examination was made of hematoxylin and eosin stained paraffin tissue sections of the following tissues and organs, where available, from all rats from the control group (0 ppm) and the 100 ppm group which were sacrificed at 1 and 2 years or which died or were sacrificed *in extremis* during the course of study.

- aorta
- adrenals (2)
- brain (3 sections including frontal cortex and basal ganglia, parietal cortex and thalamus; cerebellum and pons)
- eyes
- gonads
  - ovaries (2)
  - testes/epididymides (2)
- heart
- small intestine (3 sections)
- large intestine
- kidneys (2 sections)
- liver (2 sections)
- lung (2 sections)
- lymph node (mesenteric)
- mammary gland (females)
- pancreas
- pituitary
- salivary gland
- spinal cord/bone marrow (vertebrae)
- spleen
- stomach
- thyroid/parathyroid/trachea/esophagus
- urinary bladder
- uterus or prostate
- any tissue masses (suspected tumors)
- any other gross lesions

Microscopic examination of tissues from the 30 and 10 ppm groups included all the above listed tissues except aorta, brain, eyes, small and large intestine, lymph node and spinal cord/bone marrow.

RESULTS

Gross Pathology

At the 1 year interim sacrifice, an increased incidence of pale/tan color livers described in male rats from the 100 ppm group was considered possibly compound related. There were no necropsy findings suggestive of a compound effect in female rats from the 100 ppm group which were sacrificed at 1 year. In rats which were sacrificed at study termination or which died or were sacrificed in extremis during the course of study (TS/DOS), an increased incidence was noted for liver masses, nodules and raised lesions, mottled livers and yellow or pale liver foci and lesions at the 100 ppm level in males and females. No gross findings suggestive of a compound effect were seen in any TS/DOS rats from the 30 or 10 ppm groups. Other gross pathologic findings were typical of findings in ageing rats of this strain. These included mammary masses, pituitary masses and foci, ulcers on the hind footpads, pale livers and pale, pitted and enlarged kidneys.

Organ Weights

There were several statistically significant ($p < 0.05$) variations in absolute and relative organ weights (organ:body, organ:brain weight ratios) in male and females rats from the 100 ppm group which were sacrificed at 1 year. Increases in absolute liver weight and in liver:brain weight ratios in males and females from the 100 ppm group correlated with compound related morphologic changes and were considered toxicologically significant. A significant decrease in group mean body weight in male and female rats from the 100 ppm group at 1 year also was considered toxicologically significant. None of the other organ weight variations in 100 ppm rats at the 1 year interim was considered of toxicologic significance.

At the terminal sacrifice, statistically significant increases occurred in absolute liver weight and in liver:body and liver:brain weight ratios in males and females at the 100 ppm level. A significant increase in liver:body weight ratio also occurred in 30 ppm males and females. These increases in liver weight correlated with morphologic liver changes and were considered toxicologically significant. A statistically significant decrease in body weight in males and females at 100 ppm also was considered toxicologically significant. No morphologic correlates were observed for other statistically significant organ weight variations and they were not considered toxicologically significant.

Histopathology

Compound related microscopic changes were observed in the livers of male and female rats from the 100 ppm group at the 1 year interim sacrifice, in male and female TS/DOS rats from the 100 and 30 ppm groups and in male TS/DOS rats from the 10 ppm group. The principal compound related liver change was megalocytosis, which was characterized by an increase in size of liver parenchymal cells due to an increase in cytoplasmic volume. The increased cytoplasm was of a finely granular "ground glass" appearance. The coarser cytoplasmic organelles were relatively decreased and were displaced to the cell membrane. The nucleus:cytoplasm ratio was decreased by the increase in cytoplasmic volume in affected cells. Most or all lobules in affected livers were involved and the centrilobular cells were more severely affected. At the 1 year interim, megalocytosis occurred to a mild to marked degree in 15/15 males and to a minimal to moderate degree in 14/15 females from the 100 ppm group. The lesion was accompanied by mild to moderate hepatocyte vacuolation, probably fatty, in 14/15 males and by minimal to mild necrosis in 6/15. In females, minimal to mild hepatocyte vacuolation occurred in 5/15 controls and 15/15 100 ppm rats but necrosis was not seen. Association of hepatocyte vacuolation and necrosis with megalocytosis suggests that the progression of lesions is megalocytosis to fatty degeneration to necrosis.

In TS/DOS rats, megalocytosis occurred in males from the 100, 30 and 10 ppm groups with a dose response evident in incidence and severity. In female TS/DOS rats, megalocytosis occurred only at the 100 and 30 ppm levels; the 10 ppm level was free of compound related microscopic lesions in females. The incidence of megalocytosis in TS/DOS males was 0/50, 42/50, 28/50 and 27/50 at the 0, 100, 30 and 10 ppm levels, respectively. In females, the incidence was 44/50 and 1/50 at the 100 and 30 ppm levels. The incidence of megalocytosis was statistically significant in male rats at the 100, 30 and 10 ppm levels and in females at the 100 ppm level. Incidence and severity of hepatocyte vacuolation and necrosis also was increased in 100 ppm TS/DOS males; the increased incidence of these lesions was statistically significant at this level. Five hyperplastic nodules occurred in males at the 100 ppm level and 1 was seen at the 30 ppm level. Presence of hyperplastic nodules indicated regeneration was occurring in these livers. There were 9 hyperplastic nodules in 100 ppm females and 1 in a control female; this increase was statistically significant. Cystoid degeneration, characterized by areas of multilocular microcysts in the liver parenchyma,

also was increased in male rats from the 100 and 30 ppm groups and in females from the 100 ppm group. Incidence of this lesion in males was 4/50, 15/50, 8/50 and 6/50 at 0, 100, 30 and 10 ppm, respectively. The increased incidence at the 100 ppm level was statistically significant. In females, the incidence of cystoid degeneration was 4/50 in the 100 ppm group and 0/50 in all other groups.

Occurrence of these liver histomorphologic changes correlated with elevations in alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase levels observed in 100 ppm male rats. The markedly reduced incidence and severity of histomorphologic liver changes in female rats parallels the absence of change in liver specific serum biochemical values in females.

There was no direct or indirect compound effect in tissues other than liver at the 1 year interim sacrifice. In TS/DOS rats, changes which were considered secondarily compound related, probably through FM-3924 altered liver metabolism of endogenous steroids, were observed in the adrenals and ovaries. In the adrenals, a statistically significant increased incidence of nodular hyperplasia of the cortex occurred in males at the 100 ppm level. Incidence of this lesion was 2/49, 11/50, 0/49 and 5/50 at the 0, 100, 30 and 10 ppm levels, respectively. In females, there was a dose related occurrence of tubular hyperplasia of the ovarian stroma. Tubular hyperplasia is a non-neoplastic diffuse increase in tubular elements of the ovarian stroma which was usually bilateral and associated with absent or markedly reduced follicular development. The incidence of this condition, which was statistically significant at all dose levels, was 0/48, 15/48, 11/48 and 6/47 at the 0, 100, 30 and 10 ppm levels, respectively. An increased incidence of accumulations of foamy alveolar macrophages in the lung, which was probably compound related, was statistically significant in males and females at the 100 ppm level. The incidence of this condition at the 0, 100, 30 and 10 ppm levels, respectively, was 10/50, 23/50, 15/50 and 18/50 in males and 14/50, 27/50, 15/50 and 14/50 in females.

There was no increased incidence of neoplasia suggestive of a compound effect in male rats. Incidence of primary liver tumors (hepatocellular carcinomas) in males was 3/50, 2/50, 1/50 and 1/50 at 0, 100, 30 and 10 ppm, respectively. In females, the only occurrence of primary liver tumors was at the 100 ppm level where there were 3 hepatocellular carcinomas and 4 hepatocellular adenomas. This increased incidence of these primary liver tumors in high dose female rats was not statistically significant. There was no other incidence of neoplasia

suggestive of a compound effect in female rats.

Other microscopic lesions in tissues from FM-3924 treated rats were typical of naturally occurring inflammatory, degenerative and neoplastic lesions in an ageing population of Charles River CD rats. Commonly occurring inflammatory lesions included chronic nonsuppurative myocarditis with associated myocardial fibrosis, mononuclear inflammatory cell infiltrate into the portal triads of the liver which was frequently associated with portal bile duct proliferation and perivascular mononuclear inflammatory cell infiltrate and multifocal chronic interstitial pneumonia in the lung. There were a number of non-neoplastic lesions which, although they had a statistically significant variation in incidence compared to control group levels, were not considered to be of toxicological significance. These included decreased incidence of chronic myocarditis in 100 ppm females, decreased incidence of perivascular mononuclear cell infiltrate in the lung of 100 ppm males, increased incidence of vascular mineralization in the lung of 100, 30 and 10 ppm females, decreased incidence of interstitial pneumonia in 10 ppm males, increased incidence of vascular mineralization in the testes of 10 ppm males and decreased incidence of epithelial hyperplasia of the urinary bladder epithelium of 10 ppm females.

Some of the more commonly occurring degenerative lesions in this study included adrenal cortical vacuolation and sinusoidal ectasia, focal mineralization in large pulmonary artery branches in the lung, focal acinar atrophy in the pancreas and chronic progressive nephropathy. Pituitary adenomas were the most common neoplasm in male and female rats. Mammary adenocarcinomas and fibroadenomas also were frequently observed in female rats. Overall, the health of the rats in this study was excellent, with organs free of lesions of common infectious diseases which would have had an impact upon survival or the validity of the study.

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9/27/81
Date

good discussion + correlation of path findings - [illegible] in report

Neoplastic Incidence for Males
Riker Laboratories, Inc. 3M
FM-3924: Two Year Oral Toxicity-Oncogenicity Study in Rats

Table: 11

Project Number: 0281CR0012 Species: Rat

Tissue/ Diagnosis/ Modifier	Group 1		Group 2		Group 3		Group 4	
	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
Adrenal	(15)	(34)	(19)	(31)	(14)	(35)	(12)	(38)
Carcinoma, cortical	0	1	0	1	0	0	0	0
Pheochromocytoma, benign	1	1	0	0	1	3	1	3
Pheochromocytoma, malignant	0	0	0	0	0	0	0	1
Hemolymphoreticular neoplasm present	0	0	1	0	0	0	0	0
Bone	(0)	(2)	(1)	(1)	(0)	(0)	(1)	(0)
Osteosarcoma	0	0	1	0	0	0	0	0
Brain	(16)	(34)	(18)	(31)	(0)	(0)	(0)	(0)
Astrocytoma, benign	0	1	0	1	0	0	0	0
Oligodendroglioma, benign	1	0	0	0	0	0	0	0
Hemolymphoreticular neoplasm present	0	0	1	0	0	0	0	0
Eye	(12)	(33)	(17)	(31)	(1)	(1)	(1)	(4)
Hemolymphoreticular neoplasm present	0	0	1	0	0	0	0	0
Heart	(16)	(34)	(19)	(31)	(15)	(35)	(12)	(38)
Hemolymphoreticular neoplasm present	1	0	2	0	1	0	0	0
Kidney	(16)	(34)	(19)	(31)	(15)	(35)	(12)	(38)
Adenoma	0	0	0	0	0	0	0	1
Hemolymphoreticular neoplasm present	1	0	1	0	1	0	1	0
Liver	(16)	(34)	(19)	(31)	(15)	(35)	(12)	(38)
Hepatocellular carcinoma	2	1	1	1	0	1	1	0
Hemolymphoreticular neoplasm present	2	1	2	0	1	1	1	0
Lung	(16)	(34)	(19)	(31)	(15)	(35)	(12)	(38)
Adenoma	0	0	0	1	0	0	0	0
Hemolymphoreticular neoplasm present	3	1	2	0	1	1	1	0
Lymph Node (abdominal)	(0)	(0)	(0)	(0)	(0)	(1)	(0)	(0)
Hemolymphoreticular neoplasm present	0	0	0	0	0	1	0	0
Lymph Node (mesenteric)	(12)	(33)	(13)	(29)	(1)	(1)	(0)	(1)
Hemangiosarcoma	0	0	0	0	0	0	0	1
Hemolymphoreticular neoplasm present	1	0	1	0	1	0	0	0
Pancreas	(13)	(33)	(15)	(29)	(14)	(35)	(11)	(37)
Adenoma	0	0	1	0	0	0	0	0
Islet cell adenoma	0	1	1	1	1	2	0	1
Islet cell carcinoma	0	0	0	1	0	0	0	0
Hemolymphoreticular neoplasm present	1	0	1	0	1	0	0	0
Parathyroid	(6)	(16)	(13)	(16)	(9)	(18)	(9)	(26)
Adenoma	0	0	0	1	0	1	0	0
Pituitary	(15)	(33)	(17)	(30)	(15)	(33)	(11)	(36)
Adenoma	6	11	4	16	11	18	6	16
Salivary Gland	(12)	(32)	(15)	(30)	(13)	(33)	(10)	(32)
Hemolymphoreticular neoplasm present	0	0	1	0	0	0	0	0

Titles:
Group 1 CONTROL 0 ppm
Group 2 FM-3924 100 ppm
Group 3 FM-3924 30 ppm
Group 4 FM-3924 10 ppm

() = Total Examined
DOS= Unscheduled Death(s)
SAC= Protocol Scheduled Sacrifice(s)

Neoplastic Incidence for Males (continued)
Riker Laboratories, Inc. 3M
FM-3924: Two Year Oral Toxicity-Oncogenicity Study in Rats

Table: 11

Project Number: 0231CR0012 Species: Rat

Tissue/ Diagnosis/ Modifier	Group 1		Group 2		Group 3		Group 4	
	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
Skin	(8)	(7)	(3)	(3)	(6)	(10)	(1)	(5)
Basal cell tumor, benign	0	0	0	0	0	1	0	0
Fibroma	1	0	0	1	0	1	0	1
Fibrosarcoma	1	0	1	0	0	0	0	0
Keratoacanthoma	1	2	0	0	0	0	0	0
Liposarcoma	1	0	0	0	0	0	0	0
Papilloma	0	1	0	0	0	0	0	0
Squamous cell carcinoma	0	0	0	0	1	0	0	0
Hemolymphoreticular neoplasm present	1	0	0	0	0	0	1	0
Small Intestine	(14)	(34)	(17)	(31)	(0)	(1)	(0)	(0)
Hemolymphoreticular neoplasm present	1	0	0	0	0	0	0	0
Soft Tissues (head)	(0)	(0)	(2)	(0)	(0)	(0)	(0)	(0)
Hemangiosarcoma	0	0	1	0	0	0	0	0
Zymbal gland carcinoma	0	0	1	0	0	0	0	0
Soft Tissues (neck)	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(1)
Fibroma	0	0	0	0	0	0	0	1
Fibrosarcoma	0	0	0	0	0	0	1	0
Soft Tissues (thorax)	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)
Liposarcoma	0	0	0	0	0	0	1	0
Spleen	(16)	(34)	(19)	(31)	(15)	(35)	(12)	(38)
Hemangiosarcoma	0	1	0	0	0	0	0	0
Hemolymphoreticular neoplasm present	1	1	1	0	1	1	0	0
Stomach	(16)	(33)	(19)	(31)	(15)	(35)	(11)	(37)
Hemolymphoreticular neoplasm present	1	0	0	0	0	0	0	0
Testis/Epididymis	(16)	(33)	(19)	(31)	(14)	(35)	(12)	(38)
Mesothelioma, benign	0	0	0	0	0	0	0	1
Thymus	(1)	(0)	(1)	(0)	(0)	(0)	(0)	(0)
Hemolymphoreticular neoplasm present	1	0	1	0	0	0	0	0
Thyroid	(12)	(31)	(18)	(27)	(15)	(33)	(10)	(36)
C cell adenoma	0	0	1	0	0	2	0	3
C cell carcinoma	0	2	1	0	0	1	0	0
Follicular adenoma	0	0	0	0	0	1	0	0
Urinary Bladder	(13)	(33)	(18)	(30)	(13)	(34)	(11)	(38)
Papilloma	0	0	0	0	0	0	0	1
Hemolymphoreticular neoplasm present	1	0	0	0	0	0	0	0
Hemolymphoreticular System [# affected]	[3]	[1]	[3]	[0]	[1]	[1]	[1]	[0]
Malignant lymphoma, lymphocytic	0	0	2	0	0	0	0	0
Malignant lymphoma, histiocytic	3	1	1	0	0	1	1	0
Malignant lymphoma, mixed	0	0	0	0	1	0	0	0

Titles:
Group 1 CONTROL 0 ppm
Group 2 FM-3924 100 ppm
Group 3 FM-3924 30 ppm
Group 4 FM-3924 10 ppm

() = Total Examined
DOS= Unscheduled Death(s)
SAC= Protocol Scheduled Sacrifice(s)

Neoplastic Incidence for Females
Riker Laboratories, Inc. 3M
FM-3924: Two Year Oral Toxicity-Oncogenicity Study in Rats

Table: 12

Project Number: 0281CR0012 Species: Rat

Tissue/ Diagnosis/ Modifier	Group 1		Group 2		Group 3		Group 4	
	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
Adrenal	(25)	(25)	(15)	(34)	(26)	(23)	(27)	(22)
Adenoma	0	0	0	0	0	0	0	1
Adenoma, cortical	0	0	0	0	1	0	0	0
Carcinoma, cortical	0	0	0	0	1	0	0	0
Pheochromocytoma, benign	0	2	0	1	0	0	1	0
Pheochromocytoma, malignant	0	0	0	0	0	1	0	0
Hemolymphoreticular neoplasm present	2	0	1	0	1	0	2	0
Aorta	(21)	(23)	(14)	(33)	(0)	(0)	(0)	(0)
Hemolymphoreticular neoplasm present	2	0	0	0	0	0	0	0
Bone Marrow	(25)	(25)	(16)	(34)	(0)	(0)	(0)	(0)
Hemolymphoreticular neoplasm present	2	0	0	0	0	0	0	0
Brain	(25)	(25)	(16)	(34)	(3)	(0)	(1)	(1)
Oligodendroglioma, benign	0	1	0	0	0	0	0	0
Esophagus	(15)	(21)	(13)	(30)	(25)	(20)	(24)	(21)
Hemolymphoreticular neoplasm present	1	0	0	0	0	0	0	0
Eye	(20)	(23)	(12)	(34)	(2)	(2)	(2)	(2)
Hemolymphoreticular neoplasm present	1	0	0	0	0	0	0	0
Heart	(25)	(25)	(16)	(34)	(27)	(23)	(28)	(22)
Hemolymphoreticular neoplasm present	1	0	1	0	0	0	1	0
Kidney	(25)	(25)	(16)	(34)	(27)	(23)	(28)	(22)
Hemolymphoreticular neoplasm present	3	0	1	0	0	0	2	0
Large Intestine	(24)	(23)	(14)	(34)	(1)	(0)	(3)	(0)
Leiomyosarcoma	0	0	1	0	0	0	0	0
Hemolymphoreticular neoplasm present	1	0	0	0	0	0	1	0
Liver	(25)	(25)	(16)	(34)	(27)	(23)	(28)	(22)
Hepatocellular adenoma	0	0	0	4	0	0	0	0
Hepatocellular carcinoma	0	0	1	2	0	0	0	0
Hemolymphoreticular neoplasm present	5	0	1	0	0	0	4	0
Lung	(25)	(25)	(16)	(34)	(27)	(23)	(28)	(22)
Hemolymphoreticular neoplasm present	5	0	1	0	0	0	3	0
Lymph Node (mesenteric)	(19)	(23)	(9)	(29)	(8)	(3)	(1)	(0)
Hemolymphoreticular neoplasm present	3	0	0	0	1	0	1	0
Lymph Node (thoracic)	(0)	(0)	(0)	(0)	(1)	(0)	(0)	(0)
Hemolymphoreticular neoplasm present	0	0	0	0	1	0	0	0
Mammary Gland	(23)	(23)	(14)	(33)	(23)	(23)	(25)	(22)
Adenocarcinoma	4	3	4	6	5	6	3	3
Adenoma	2	1	0	0	0	0	0	0
Carcinoma	0	1	0	0	0	0	0	0
Fibroadenoma	6	4	1	5	7	8	6	11
Hemolymphoreticular neoplasm present	2	0	0	0	0	0	1	0

Titles:
Group 1 CONTROL 0 ppm
Group 2 FM-3924 100 ppm
Group 3 FM-3924 30 ppm
Group 4 FM-3924 10 ppm

() = Total Examined
DOS= Unscheduled Death(s)
SAC= Protocol Scheduled Sacrifice(s)

Neoplastic Incidence for Females (continued)
Riker Laboratories, Inc. 3M
FM-3924: Two Year Oral Toxicity-Oncogenicity Study in Rats

Table: 12

Project Number: 0281CR0012 Species: Rat

Tissue/ Diagnosis/ Modifier	Group 1		Group 2		Group 3		Group 4	
	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
Mesentary	(0)	(0)	(0)	(0)	(0)	(1)	(0)	(0)
Leiomyosarcoma	0	0	0	0	0	1	0	0
Ovary	(25)	(23)	(14)	(34)	(25)	(23)	(26)	(21)
Leiomyoma	1	0	0	0	0	0	0	0
Tubular adenoma	0	4	0	3	0	2	0	0
Hemolymphoreticular neoplasm present	2	0	0	0	0	0	1	0
Pancreas	(24)	(25)	(14)	(34)	(17)	(17)	(21)	(20)
Acinar adenoma	0	0	0	1	0	0	0	0
Islet cell adenoma	0	1	0	0	0	2	0	0
Islet cell carcinoma	0	1	0	0	0	0	0	0
Hemolymphoreticular neoplasm present	2	0	0	0	1	0	2	0
Parathyroid	(5)	(0)	(6)	(23)	(16)	(15)	(19)	(18)
Adenoma	0	0	0	1	0	0	0	0
Pituitary	(22)	(24)	(15)	(34)	(23)	(21)	(25)	(21)
Adenocarcinoma	0	0	0	0	0	1	0	0
Adenoma	16	17	12	23	20	16	20	15
Skin	(3)	(3)	(0)	(2)	(5)	(4)	(5)	(1)
Fibroma	1	2	0	0	3	0	0	0
Fibrosarcoma	0	0	0	0	1	0	0	0
Lipoma	0	0	0	0	1	1	0	0
Hemolymphoreticular neoplasm present	0	0	0	0	0	0	2	0
Small Intestine	(24)	(25)	(14)	(34)	(1)	(0)	(1)	(0)
Hemolymphoreticular neoplasm present	0	0	0	0	0	0	1	0
Soft Tissues (leg)	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(0)
Hemolymphoreticular neoplasm present	0	0	1	0	0	0	0	0
Spinal Cord	(25)	(23)	(15)	(34)	(0)	(0)	(0)	(0)
Hemolymphoreticular neoplasm present	1	0	0	0	0	0	0	0
Spleen	(25)	(25)	(16)	(34)	(27)	(23)	(23)	(22)
Hemolymphoreticular neoplasm present	3	0	0	0	0	0	2	0
Stomach	(25)	(25)	(16)	(34)	(26)	(23)	(28)	(22)
Hemolymphoreticular neoplasm present	1	0	0	0	1	0	1	0
Thymus	(0)	(0)	(0)	(0)	(1)	(0)	(0)	(0)
Hemolymphoreticular neoplasm present	0	0	0	0	1	0	0	0
Thyroid	(25)	(25)	(13)	(33)	(20)	(18)	(23)	(22)
Adenocarcinoma	0	0	0	0	0	1	0	0
C cell adenoma	0	1	0	1	0	1	1	0
C cell carcinoma	0	0	1	1	0	1	0	1
Hemolymphoreticular neoplasm present	2	0	0	0	0	0	0	0
Urinary Bladder	(25)	(25)	(13)	(34)	(23)	(21)	(25)	(22)
Transitional cell carcinoma	0	0	0	0	0	0	1	0
Hemolymphoreticular neoplasm present	1	0	0	0	1	0	2	0

Titles:
Group 1 CONTROL 0 ppm
Group 2 FM-3924 100 ppm
Group 3 FM-3924 30 ppm
Group 4 FM-3924 10 ppm

() = Total Examined
DOS= Unscheduled Death(s)
SAC= Protocol Scheduled Sacrifice(s)

Microscopic Incidence Page: 12

Table: 12

Project Number: 0281CR0012 Species: Rat

Tissue/ Diagnosis/ Modifier	Group 1		Group 2		Group 3		Group 4	
	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
Uterus	(25)	(25)	(14)	(34)	(24)	(23)	(26)	(22)
Adenoma	0	1	0	0	0	0	0	0
Hemangioma	0	0	0	0	1	0	0	0
Leiomyoma	0	0	0	0	0	0	0	1
Polyp	0	1	0	1	2	2	1	3
Hemolymphoreticular neoplasm present	2	0	0	0	1	0	0	0
Hemolymphoreticular System [# affected]	[5]	[0]	[1]	[0]	[3]	[0]	[4]	[0]
Malignant lymphoma, histiocytic	2	0	1	0	1	0	4	0
Malignant lymphoma, lymphocytic	3	0	0	0	0	0	0	0
Lymphosarcoma	0	0	0	0	2	0	0	0

Titles:
Group 1 CONTROL 0 ppm
Group 2 FM-3924 100 ppm
Group 3 FM-3924 30 ppm
Group 4 FM-3924 10 ppm

() = Total Examined
DOS= Unscheduled Death(s)
SAC= Protocol Scheduled Sacrifice(s)

REPORT AMENDMENT NO. 1

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

Experiment No.0281CR0012

The attached pages include revisions in the first paragraph on page 3 of the Summary and in the third paragraph of page 22 of the Discussion. The revisions were made to clarify that the incidence of benign hepatic adenomas found in the high-dose females was not statistically significant, although it was outside the historical limit.

Study Director: Leonard J. Sibinski 10/24/88
Leonard J. Sibinski, B.A. Date
Senior Toxicologist

Amendment Reviewed By: J L Allen 10-24-88
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Diplomate, A.B.T.
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Amendment Approved By: Steve V. Elrod 10-25-88
Steve V. Elrod, Ph.D. Date
Diplomate, A.B.T.
Manager, Pathology/Toxicology and
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The overall incidence of hepatocellular adenomas and carcinomas was low in both control and FM-3924-treated groups with the high-dose female rats having a tumor incidence that, while not statistically significant, was 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.

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, although not statistically significant, 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.

SIGNATURE PAGE

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

RIKER Experiment No. 0281CR0012

From the Pathology and Toxicology Department
RIKER Laboratories, Inc./3M Company
St. Paul, Minnesota U.S.A.

Study Director: Leonard J. Sobinski 4/6/88
Leonard J. Sobinski, B.A. Date
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Report Reviewed By: Steve V. Elrod 4-5-88
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PRINCIPAL PERSONNEL INVOLVED WITH THE CONDUCT AND REPORTING OF RIKER EXPERIMENT NO. 0281CR0012 - TWO YEAR ORAL (DIET) TOXICITY/CARCINOGENICITY STUDY OF FLUOROCHEMICAL FC-143 IN RATS

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J.R. Nelson, H.T.	Histotechnologist/Microscope Slide Preparation
G.C. Pecore	Supervisor, Laboratory Animal Care
S.L. Westmark, A.A.S.	Laboratory Technician
L.O. Wiseth	Senior Laboratory Technician/ Assisted With Study Data

Animal #	Gross observations	Histopath. findings
Control Males		
1R 3521	pale	hepatocyte vacuolation, centrilobular, moderate
1R 3525	raised pale circular lesion, 2 cm. dia., in medial lobe	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild hepatocellular carcinoma large, poorly differentiated, anaplastic, infiltrative. adenoid areas. multipletumor emboli in lung.
1R 3529	raised pale mass, 1.2 cm dia.,in left lateral lobe.	portal mononuclear cell infiltrate, mild hepatocellular carcimoma trabecular pattern, fairly well differentiated.
1R 3534	pale with yellowish circular lesion 0.3 cm diameter in right anterior lobe	hepatocyte vacuolation, diffuse, marked
1R 3537	very pale	hepatocyte vacuolation, diffuse, marked malignant lymphoma, histiocytic
1R 3538	multiple pale foci, all lobes	malignant lymphoma, histiocytic
1R 3545	slightly pale	within normal limits
1R 3547	pale	portal mononuclear cell infiltrate, mild hepatocyte vacuolation, periportal, moderate
1R 3549	slightly pale	hepatocyte vacuolation, multifocal, mild
1R 3560	pale, appears enlarged	malignant lymphoma, histiocytic
1R 3565	pale	hepatocyte vacuolation, multifocal, mild necrosis, multifocal, moderate vascular mineralization, mild
1R 3571	pale	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild hepatocyte vacuolation, multifocal, mild
1R 3574	slightly pale	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild
1R 3579	raised pale 2 cm dia. mass in right lateral lobe	portal bile duct proliferation, mild hepatocellular carcinoma portal mononuclear cell infiltrate, mild

Animal #	Gross observations	Histopath. findings
High dose	Males 100 ppm	
1R 3697	mottled	portal mononuclear cell infiltrate, minimal portal bile duct proliferation, minimal hepatocyte vacuolation, diffuse, moderate megalocytosis, marked
1R 3698	possibly slightly pale and mottled	portal mononuclear cell infiltrate, mild hepatocyte vacuolation, multifocal, mild cystoid degeneration, multifocal, mild
1R 3701	pale raised circular lesion, 1.1 cm dia., median lobe	hepatocyte vacuolation, diffuse, moderate hepatocyte alteration, vacuolated, mild megalocytosis, moderate hyperplastic nodule ✓
1R 3702	mottled	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild hepatocyte vacuolation, diffuse, moderate megalocytosis, marked
1R 3706	slightly pale	portal mononuclear cell infiltrate, mild hepatocyte vacuolation, centrilobular, marked cystoid degeneration, multifocal, mild megalocytosis, marked
1R 3707	scattered pale foci and pink raised mass, 1 cm dia.,in left lateral lobe	portal mononuclear cell infiltrate, mild portal bile duct proliferarion, mild hepatocyte vacuolation, multifocal, moderate cystoid degeneration, focal, minimal hepatocyte alteration, vacuolated, moderate megalocytosis, marked hyperplastic nodule ✓
1R 3709	scattered yellowish foci	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild hepatocyte vacuolation, diffuse, moderate megalocytosis, mild hepatocyte alteration, basophilic, mild

Animal #	Gross observations	Histopath. findings
High dose male con't.		
1R 3712	appears enlarged and mottled	hepatocyte vacuolation, diffuse, moderate cystoid degeneration, focal, minimal megalocytosis, marked
1R 3716	pale	portal bile duct proliferation, minimal hepatocyte vacuolation, diffuse, marked megalocytosis, moderate
1R 3718	mottled	portal mononuclear cell infiltrate, mild hepatocyte vacuolation, diffuse, moderate megalocytosis, marked hepatocyte alteration, basophilic, moderate
1R 3719	slightly pale	hepatocyte vacuolation, multifocal, mild necrosis, focal, mild hematopoiesis, extramedullary, mild
1R 3723	enlarged with scattered foci	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild hepatocyte vacuolation, multifocal, moderate hepatocyte alteration, vacuolated, mild megalocytosis, marked
1R 3724	small whitish lesion,0.2 cm dia., median lobe;small pale yellowish lesion,0.4 cm. dia.,left lateral lobe	hepatocyte vacuolation, centrilobular,moderate megalocytosis, mild malignant lymphoma, lymphocytic two grossly described nodules composed oflarge lymphoblastic cells with vesicular nuclei and high mitotic activity.Different from osteosarcoma cells.
1R 3726	small scattered pale foci	portal mononuclear cell infiltrate, mild hepatocyte vacuolation, multifocal, mild hepatocyte alteration, vacuolated, moderate megalocytosis, moderate

Animal #	Gross observations	Histopath. findings
High dose males con't.		
1R 3728	pale raised mass,1.3 x 0.8 cm,anterior right lateral lobe	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild hepatocyte vacuolation, centrilobular, moderate cystoid degeneration, focal, mild megalocytosis, moderate hyperplastic nodule large lesion with good preservation oflobular structure. Most cells of nodule vacuolated.
1R 3738	scattered pale foci	hepatocyte vacuolation, multifocal, mild cystoid degeneration, multifocal, mild hepatocyte alteration, vacuolated, moderate " " , basophilic, moderate megalocytosis, mild
1R 3739	scattered pale foci	portal bile duct proliferation, mild hepatocyte vacuolation, multifocal, mild megalocytosis, mild metastatic neoplasm
1R 3742	appears somewhat pale and mottled; possible area of infarction in right lateral lobe	hepatocyte vacuolation, diffuse, marked necrosis, diffuse, marked necrosis, centilobular, mild megalocytosis, moderate
1R 3743	pale and mottled	portal bile duct proliferation, mild. hepatocyte vacuolation, periportal, moderate
1R 3744	circular pale lesion, 0.4 cm dia., median lobe	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild hyperplastic nodule
1R 3749	mottled	hepatocyte vacuolation, multifocal, mild necrosis, centrilobular, moderate megalocytosis, moderate

Animal #	Gross observations	Histopath. findings
High dose males con't.		
1R 3750	raised, 1.5 cm dia.nodule in left lateral lobe	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild necrosis, multifocal, mild megalocytosis, marked hepatocellular carcinoma ✓ large well differentiated neoplasm. solid moderate mitotic activity.
1R 3752	pale, raised lesion,0.7 cm dia.,in right lateral lobe	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild hepatocellular carcinoma ✓ megalocytosis, minimal
1R 3753	enlarged and mottled with a circular raised lesion,1.7 cm dia., on one medianlobe and another circular lesion 0.8 cm dia. on the other median lobe	hepatocyte vacuolation, centrilobular, marked malignant lymphoma, lymphocytic megalocytosis, moderate necrosis, centrilobular, moderate
Mid dose male 30 ppm		
1R 3765	slightly pale	portal bile duct proliferation, minimal portal mononuclear cell infiltrate, minimal
1R 3783	pale	hepatocyte vacuolation, diffuse, moderate
1R 3786	dark lesion,0.3 cm dia., in median lobe	portal mononuclear cell infiltrate, mild hepatocyte alteration, vacuolated,local areas, moderate megalocytosis, minimal
1R 3790	raised pale mass, 1.5 x 1 cm dia., caudatelobe	megalocytosis, mild hepatocellular carcinoma ✓
1R 3792	possibly slightly pale	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild

Animal #	Gross observations	Histopath. findings
Mid dose males con't.		
1R 3802	mottled with irregular surface	portal bile duct proliferation, mild hepatocyte vacuolation, centrilobular,moderate malignant lymphoma, histiocytic
1R 3804	pale	hepatocyte vacuolation, local areas, mild
1R 3808	large mass, 5 x 3 x 2 cm,in median lobe	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild cystoid degeneration, focal, mild hyperplastic nodule ✓
Low dose males 10 ppm		
1R 3815	median and left lateral lobes appear shrunken with very rough surface; left lateral and caudate lobes pale with a multilobular mass, 3.5 x 2.5 x 2.5 cm.	portal mononuclear cell infiltrate, moderate hepatocellular carcinoma ✓ atrophy, marked fibrosis, portal, marked
1R 3831	small pale foci in several lobes	malignant lymphoma, histiocytic
1R 3834	slightly pale	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild hepatocyte vacuolation, multifocal, mild cystoid degeneration, focal, mild megalocytosis, minimal
1R 3840	pale	within normal limits
1R 3842	pale mottled	hepatocyte vacuolation, periportal, moderate
1R 3847	possibly slightly mottled	portal mononuclear cell infiltrate, mild
1R 3856	pale and mottled	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild hepatocyte vacuolation, periportal, marked

Animal #	Gross observations	Histopath. findings
Control Females		
1R 4576	enlarged; mottled yellow color	malignant lymphoma, lymphocytic
1R 4579	slightly pale	hepatocyte vacuolation, multifocal, mild
1R 4581	possibly slightly pale and swollen	portal mononuclear cell infiltrate, mild hepatocyte vacuolation, multifocal, mild hepatocyte alteration, basophilic, mild hematopoiesis, extramedullary, mild
1R 4596	very pale	hepatocyte vacuolation, diffuse, marked
1R 4599	appears mottled	malignant lymphoma, histiocytic necrosis, local areas, marked
1R 4600	appears mottled	portal mononuclear cell infiltrate, moderate portal bile duct proliferation, mild hepatocyte vacuolation, multifocal, mild hepatocyte alteration, eosinophilic, moderate telangiectasis, mild
1R 4602	circular raised lesion, 0.6 cm dia., left lateral lobe	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild hepatocyte alteration, vacuolated, mild
1R 4607	possibly slightly pale	malignant lymphoma, lymphocytic
1R 4613	appears pale	hepatocyte vacuolation, periportal, mild necrosis, multifocal, mild hematopoiesis, extramedullary, mild
1R 4617	pale; left lateral lobe dark red	portal mononuclear cell infiltrate, mild necrosis, diffuse, marked massive necrosis of one lobe with compensatory increase in mitoses in another lobe.
1R 4619	pale with an irregular surface on all lobes	portal bile duct proliferation, marked necrosis, local areas, moderate malignant lymphoma, histiocytic
1R 4621	appears mottled	portal mononuclear cell infiltrate, minimal hepatocyte vacuolation, periportal, mild

Animal #	Gross observations	Histopath. findings
Control Females con't.		
1R 4633	very pale	hepatocyte vacuolation, centrilobular, marked
1R 4636	slightly raised dark mass,0.6 cm dia., left lateral lobe	hepatocyte vacuolation, multifocal, mild hepatocyte alteration, mild hepatocyte alteration, basophilic, mild
1R 4637	possibly pale	hepatocyte vacuolation, periportal, moderate
1R 4639	slightly pale	portal mononuclear cell infiltrate, mild
High dose females 100 ppm		
1R 4759	appears mottled	hepatocyte vacuolation, periportal, moderate megalocytosis, mild
1R 4762	dark in color; raised circular lesion 1.5 cm dia.,median lobe;circular " 1 cm dia.,right anterior lobe;circular lesion, 0.9 cm dia.,caudate lobe.	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild hepatocyte vacuolation, diffuse, moderate hepatocyte alteration, vacuolated, mild hyperplastic nodule megalocytosis, moderate
1R 4765	appears mottled	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild hepatocyte vacuolation, local areas, mild megalocytosis, mild
1R 4768	slightly pale and mottled	hepatocyte vacuolation, periportal, moderate megalocytosis, mild
1R 4770	appears mottled	hepatocyte vacuolation, periportal, moderate megalocytosis, moderate pigment, hepatocyte, minimal
1R 4771	appears dark brown in color with multiple pale foci on all lobes.	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild hepatocyte vacuolation, periportal, moderate

Animal #	Gross observations	Histopath. findings
High dose females con't.		
1R 4772	circular mass, 2 x 1.2 cm, in right posterior	megalocytosis, moderate hyperplastic nodule ✓ hepatocellular adenoma ✓ large tumor with areas of necrosis. Tumor cells many vacuolated.
1R 4773	pale, slightlyraised mass, 3 x 2 cm, in median lobe	hyperplastic nodule ✓ hepatocyte alteration, vacuolated, multifocal, mild megalocytosis, moderate hepatocyte vacuolation, multifocal, mild
1R 4774	somewhat pale and mottled	portal mononuclear cell infiltrate, minimal portal bile duct proliferation, minimal hepatocyte vacuolation, diffuse, marked megalocytosis, moderate necrosis, focal, mild
1R 4781	small pale foci, approx. 0.2 cm dia., in left lateral lobe.	megalocytosis, moderate necrosis, multifocal, mild
1R 4784	multiple pale foci in left lateral lobe	hepatocyte vacuolation, multifocal, mild megalocytosis, mild
1R 4786	raised mass, 3 x 1.5 cm in right lateral lobe	hepatocyte vacuolation, periportal, moderate megalocytosis, mild hepatocellular carcinoma ✓ well differentiated, trabecular pattern. Areas of necrosis. Moderate mitotic activity.
1R 4789	scattered small pale foci in most lobes	hepatocyte vacuolation, periportal, mild megalocytosis, moderate necrosis, multifocal, mild
1R 4795	raised pale mass, 2 cm dia., in left lateral lobe.	portal bile duct proliferation, mild megalocytosis, mild hepatocellular adenoma ✓
1R 4797	appears pale and mottled	hepatocyte vacuolation, diffuse, moderate megalocytosis, marked

Animal #	Gross observations	Histopath. findings
High dose females con't.		
1R 4802	raised pale circular lesion, 0.7 cm dia.,in median lobe; multiple raised pale lesions, 0.5 - 1 cm, in left lateral lobe.	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild hepatocyte vacuolation, multifocal, mild megalocytosis, mild hepatocellular adenoma ✓
1R 4804	multiple pale foci in all lobes;large pale mass, 4 x 3 x 2 cm in left lateral lobe.	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild hepatocyte vacuolation, multifocal, mild megalocytosis, mild hepatocellualr carcinoma ✓ hematopoiesis, extramedullary, mild
1R 4805	pale	hepatocyte vacuolation, periportal, moderate megalocytosis, mild
1R 4807	small pale lesion, 0.4 cm dia., right anterior lobe.	megalocytosis, mild hyperplastic nodule
1R 4809	several pale lesions in all lobes.	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild megalocytosis, moderate hepatocellular adenoma ✓ hyperplastic nodule, multiple vacuolation of hepatocytes in some nodules.
1R 4811	pale and mottled	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild hepatocyte vacuolation, periportal, moderate megalocytosis, moderate pigment, brown, hepatocytes, mild
1R 4813	pale and mottled	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild hepatocyte vacuolation, multifocal. mild hepatocyte alteration, vacuolated, multifocal,mild megalocytosis, mild

Animal #	Gross observations	Histopath. findings
High dose females con't.		
1R 4817	pale raised circular mass, 2 cm dia., median lobe	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild hepatocyte vacuolation, diffuse, marked cystoid degeneration, focal, mild hepatocellular carcinoma ✓
1R 4819	pale circular lesion, 1.4 cm dia., median lobe	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild megalocytosis, moderate hyperplastic nodule ✓
1R 4820	mottled, enlarged	malignant lymphoma, histiocytic extensive nodular and diffuse proliferation with vascular involvement.
Mid dose Females 30 ppm		
1R 4822	scattered pale foci	necrosis, local areas, moderate hematopoiesis, extramedullary, mild
1R 4833	appears enlarged	within normal limits
1R 4838	pale	hematopoiesis, extramedullary, minimal
1R 4840	pale and mottled	portal mononuclear cell infiltrate, minimal portal bile duct proliferation, minimal hepatocyte vacuolation, local areas, mild hematopoiesis, extramedullary, minimal
1R 4841	pale	portal bile duct proliferation, minimal
1R 4845	slightly pale	portal mononuclear cell infiltrate, mild portal bile duct proliferation, moderate hepatocyte vacuolation, diffuse, moderate

Animal #	Gross observations	Histopath. findings
Mid dose females con't.		
1R 4848	pale	portal bile duct proliferation, minimal
1R 4851	pale	necrosis, centrilobular, moderate hematopoiesis, extramedullary, moderate
1R 4857	pale	hematopoiesis, extramedullary, minimal
1R 4862	pale and mottled	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild hepatocyte vacuolation, diffuse, marked necrosis, focal, minimal
Low dose Females 10 ppm		
1R 4875	pale and mottled	portal bile duct proliferation, mild necrosis, centrilobular, mild
1R 4879	slightly enlarged with multiple pale lesions,0.1-0.5 cm dia.,on all lobes	malignant lymphoma, histiocytic necrosis, local areas, moderate
1R 4886	enlarged with multiple pale foci	necrosis, centrilobular, moderate
1R 4887	appears pale and mottled	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild hepatocyte vacuolation, periportal, moderate hepatocyte alteration, basophilic,local areas,moderate
1R 4890	appears pale	hepatocyte vacuolation, diffuse, marked
1R 4891	small yellowish lesion, 0.3 x 0.2 cm on the outer area near fissure of median lobe.	hepatocyte vacuolation, centrilobular,local areas,mild
1R 4900	slightly pale with rough surface	malignant lymphoma, histiocytic
1R 4904	pale	hepatocyte vacuolation,centrilobular,moderate necrosis,centrilobular,mild
1R 4909	pale,mottled with scattered yellowish foci and an irregular surface.	malignant lymphoma, histiocytic

Animal #	Gross observations	Histopath. findings
Low dose females con't.		
1R 4915	pale	hepatocyte vacuolation, diffuse, marked
1R 4918	pale	portal mononuclear cell infiltrate, mild portal bile duct proliferation, mild hepatocyte vacuolation, diffuse, mild
1R 4920	pale, scattered white nodules on all lobes, 0.1 - 1 cm dia.	malignant lymphoma, histiocytic