The NOAEL and LOAEL for F1 generation females are considered to be 10 and 30 mg/kg/day, respectively, based on statistically significant increases in postweaning mortality, delays in sexual maturation (time to vaginal patency), decreases in body weight and body weight gains, and decreases in absolute food consumption, all observed at the highest dose tested.

The NOAEL for the F2 generation offspring was considered to be 30 mg/kg/day. No treatment-related effects were observed at any doses tested in the study. However, it should be noted that the F2 pups were sacrificed at weaning, and thus it was not possible to ascertain the potential post-weaning effects that were noted in the F1 generation.

3.8 Carcinogenicity Studies in Animals

3.8.1 Cancer Bioassays

The carcinogenic potential of APFO has been investigated in a two-year feeding study in rats (3M, 1987). In this study, groups of 50 male and 50 female Sprague-Dawley (Crl:CD BR) rats were fed diets containing 0, 30 or 300 ppm APFO for two years. Groups of 15 additional rats per sex were fed 0, or 300 ppm APFO and evaluated at the one-year interim sacrifice. The mean actual test article consumption was: males, 1.3 and 14.2 mg/kg/day; females, 1.6 and 16.1 mg/kg/day for the low and high-dose groups, respectively.

There was a dose-related decrease in body weight gain in the male rats and to a lesser extent, in the female rats as compared to the controls; the decreases were statistically significant in the high-dose groups of both sexes. The body weight changes are treatment related since feed consumption was actually increased (rather than decreased). There were no differences in mortality between the treated and untreated groups; the survival rates at the end of 104 weeks for the control, low-, and high-dose groups were: male, 70%, 72% and 88%; females, 50%, 48% and 58%. The only clinical sign observed was a dose-related increase in ataxia in the female rats; the incidences in the control, low- and high-dose groups were: 4%, 18% and 30%. Significant decreases in red blood cell counts, hemoglobin concentrations and hematocrit values were observed in the high-dose male and female rats as compared to control values. Clinical chemistry changes indicative of liver toxicity included increases in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AP) in both treated male groups from 3-18 months, but only in the high-dose males at 24 months. Increases in relative liver and kidney weights were noted in both high-dose male and female rats. Significant non-neoplastic lesions were seen primarily in the liver and testis; there were increases in the incidence of liver masses, hyperplastic nodules and foci, and in testicular masses in the high-dose male group. Other liver toxic effects include dose-related increases in the incidence of diffuse hepatomegalocytosis, cystoid degeneration, and portal mononuclear cell infiltration in both male and female treated groups; these increases were statistically significant in the high-dose males. A statistically significant, dose-related increase in the incidence of ovarian tubular hyperplasia was found in female rats; the incidence of this lesion in the control, low-, and high-dose groups was 0%, 14%, and 32%, respectively. Based on these toxic effects, the high dose selected in this study appears to have reached the Maximum Tolerated Dose (MTD). Based on

decreased body weight gain, increased liver and kidney weights and toxicity in the hematological and hepatic systems, the LOAEL for male and female rats is 300 ppm. [Based on increases in the incidence of ataxia and ovarian tubular hyperplasia, the LOAEL for female rats is 30 ppm.]

At the termination of the study, a slight increase in the incidence of various neoplasms (tumors of the liver, testis, thyroid, adrenal and mammary glands, etc.) was seen in the treated animals. Among them, the increased incidences of testicular (Leydig) cell adenomas in the high-dose male rats, and of mammary fibroadenoma in both groups of female rats were statistically significant ($P < 0.05$) as compared to the concurrent controls. The incidence of the Leydig cell tumors (LCT) in the control, low- and high-dose males was 0/50 (0%), 2/50 (4%) and 7/50 (14%), respectively; the respective incidences of mammary fibroadenoma in the female groups were 11/50 (22%), 21/50 (42%) and 24/50 (48%). The increases are also statistically significant as compared to the historical control incidences (LCT, 0.82%; mammary fibroadenoma, 19.0%) observed in 1,340 male and 1,329 female Sprague-Dawley control rats used in 17 carcinogenicity studies (Chandra et al., 1992). The spontaneous incidence of LCT in 2-year old Sprague-Dawley rats in other studies was reported to be approximately 5% (*cited in*: Clegg et al., 1997). Therefore, under the conditions of this study, APFO is carcinogenic in Sprague-Dawley rats, inducing Leydig cell tumors in the male rats and mammary fibroadenomas in the female rats.

The induction of Leydig cell tumors was confirmed in a follow-up 2-year mechanism study of PFOA in male Sprague-Dawley (CD) rats at a dietary level of 300 ppm (Cook et al., 1994; Biegel et al. 2001). A significantly increased LCT incidence was observed in the treated rats (8/76, 11%) as compared to the controls (0/80, 0%). In addition, PFOA also caused significantly increased incidences of liver tumors and pancreatic acinar cell tumors. The incidences of liver adenomas in the control and treated groups were 2/80 (3%) and 10/76 (13%), respectively, whereas those for the pancreatic acinar cell adenomas were 0/80 (0%) and 7/76 (9%). There was one pancreatic acinar cell caricinoma in 76 of the treated rats and none in 80 controls. The incidence of combined pancreatic acinar cell adenoma/carcinoma in the treated rats (8/76, 11%) was also significantly increased as compared to the controls (0/80, 0%).

PFOA has also been shown to promote liver carcinogenesis in rodents (Abdellatif et al.,1991; Nilsson et al., 1991).

3.8.2 Mode of Action Studies

The mechanism(s) of toxicological/carcinogenic action of PFOA is not clearly understood. PFOA was not mutagenic in the Ames test using five strains of *Salmonella typhimurium*, or in an assay with *Saccharomyces cerevisiae* (Griffith and Long, 1980). Short-term genotoxicity assays appear to suggest that PFOA is not a DNA-reactive compound. However, when tested with metabolic activation, PFOA induced significant increases in chromosomal aberrations and in polyploidy in CHO cells (Murli, 1996). The significance of these genotoxic effects is unclear. Available data appear to indicate that the induction of tumors by PFOA is due to a non-genotoxic mechanism, involving activation of receptors and perturbations of the endocrine system. The

REPEAT DOSE DATA

Title: Two Year Oral (Diet) Toxicity/Carcinogenicity Study of Fluorochemical FC-143 in Rats

TEST SUBSTANCE

Identity: Fluorad® Fluorochemical FC-143, also referred to as PFOA ammonium salt, ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, FC-143, or as a major component of FX-1003 (octanoic acid, pentadecafluoro-, ammonium salt, CASRN 3825-26-1)

Remarks: The test substance, a white powder, was analyzed 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. The composition and purity of the test substance were not indicated in the main body of the study report.

METHOD

Method/guideline followed: Guideline number not stated

Study duration: Two years

GLP (Y/N): Yes

Year study performed: 1981 - 1983

Species/strain: Sprague-Dawley rat [Crl:COBS^{R} CD(SD)BR]

Sex: Male/female

Number of animals per dose group: The control and high-dose groups contained 65 rats/sex and the low-dose group contained 50 rats/sex.

Route of administration: Diet

Doses tested and frequency: Low-dose: 1.3 mg/kg/day (males), 1.6 mg/kg/day (females)
High-dose: 14.2 mg/kg/day (males), 16.1 mg/kg/day (females)

Post-treatment observation period: None

Statistical methods used: Bartlett's test for homogeneity of variance was used to analyze the test data. If this test was not significant at alpha = 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 alpha = 0.05 significance level.

Remarks: Test animals were 39 to 41 days of age when treatment began. An interim termination at one year included 15 rats/sex from both the control and high-dose groups. All animals were observed daily throughout the dosing period. Weekly physical examinations included palpation for any masses present and pharmacotoxic observations. Body weights and feed consumption were recorded weekly or bi-weekly. Eye examinations using indirect ophthalmoscopy and/or slit lamp biomicroscopy were performed at the one-year period. Clinical pathology determinations included hematology, clinical (serum) chemistry and urinalysis. Tests were conducted on samples obtained at 3, 6, 12, 18, and 24

months from randomly selected animals of each dose group. 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, and calcium. Urine tests included pH, specific gravity, albumin, glucose, bilirubin, occult blood and ketones. Metabolic examinations involved collection of urine and fecal samples. Post mortem examinations were performed on all animals and the weights of the adrenal glands, brain, testes, heart, kidneys, liver, spleen, and uterus were recorded from 15 randomly selected rats/sex/group. Samples of many different tissues were collected and observed microscopically from these animals.

RESULTS

Survival rates:
-Generally, survival rates for the FC-143-treated rats were good during the full two years of the study. Fewer deaths were seen in high-dose males and females than in the controls.

Neoplastic effects:

Percent Neoplastic Lesions in Males

	Control	Low	High
Adrenal			
Pheochromocytoma, benign	4	8	8
Pheochromocytoma, malig.	0	2	0
Liver			
Hepatocellular carcinoma	6	2	10
Pituitary			
Adenoma	35	36	28
Testes/Epididymis			
Leydig cell adenoma	0	4	14*
Thyroid			
C-cell adenoma	0	4	9
C-cell carcinoma	5	0	0

Source: Table 19
*Significantly different ($p < 0.05$) from controls

Percent Neoplastic Lesions in Females

	Control	Low	High
Adrenal			
Pheochromocytoma, benign	4	0	0
Pheochromocytoma, malig.	0	0	2
Liver			
Hepatocellular carcinoma	0	0	2
Mammary gland			
Adenocarcinoma	15	31	11
Adenoma	7	0	0
Carcinoma	2	0	0
Fibroadenoma	22	42	48*
Lymphangiosarcoma	0	0	2
Pituitary			
Adenoma	72	83	72
Thyroid			
C-cell adenoma	2	0	0
C-cell carcinoma	0	0	0

Source: Table 19
*Significantly different ($p < 0.05$) from controls

Statistical analysis of neoplastic effects (i.e., percent that was statistically significantly different from controls; $p < 0.05$):

Females (16.1 mg/kg):
Mammary gland fibroadenomas
Males (14.2 mg/kg):
Leydig cell adenomas in testis

Nonneoplastic effects:

NOAEL (dose and effect): none

LOAEL (dose and effect):

1.3 mg/kg/day (males) – based upon salivary gland sialadenitis (note that the study authors implied an association of this lesion with a suspected outbreak of sialodacryoadenitis viral infection; however, the presence of a virus was not confirmed)

1.6 mg/kg/day (females) – based upon ovarian tubular hyperplasia (and ataxia, a clinical sign).

Percent Non-neoplastic Lesions in Males

	Control	Low	High
Adrenal			
Nodular hyperplasia	4	2	18
Sinusoidal ectasis	22	26	32
Heart			
Myocarditis, chronic	28	36	34
Liver			
Cystoid degeneration	8	14	56*
Hepatocellular alt. basophil.	4	2	12
Hyperplastic nodule	0	0	6
Megalocytosis	0	12	80*
Portal mononuclear cell infil.	74	64	96*
Necrosis	6	10	10
Lung			
Alveolar macrophages	20	32	62*
Hemorrhage	20	28	44*
Perivas. mono. infil.	42	6*	14*
Vascular mineralization	86	86	94
Pneumonia, interstitial	32	10*	14
Testis/epididymis			
Tubular atrophy	14	20	22
Vascular min.	0	6	18*
Thyroid			
C-cell hyperlasia	2	13	2
Pancreas			
Acinar atrophy	13	20	22
Salivary gland			
Sialadenitis, chronic	2	27*	30*
Spleen			
Hemosiderosis	32	8*	44

Source: Table 20

*Significantly different ($p < 0.05$) from controls

Percent Non-neoplastic Lesions in Females

	Control	Low	High
Adrenal			
Nodular hyperplasia	0	6	2
Sinusoidal ectasis	84	86	82
Heart			
Myocarditis, chronic	32	10*	20
Liver			
Cystoid degeneration	0	2	2
Hepatocellular alt. basoph.	16	16	4
Hyperplastic nodule	2	0	4
Megalocytosis	0	2	16*
Portal mono. cell infil.	38	22	38
Necrosis	10	12	4
Lung			
Alveolar macrophages	28	20	38
Hemorrhage	28	26	38
Perivas. mono. infil.	26	4*	28
Vascular mineralization	44	76*	52
Pneumonia, interstitial	14	6	18
Testis/epididymis			
Tubular atrophy			
Vascular min.			
Ovary			
Cyst	13	18	11
Tubular hyperplasia	0	14*	32*
Thyroid			
C-cell hyperlasia	0	2	7
Uterus			
Cystic glands	14	24	10
Pancreas			
Acinar atrophy	12	12	9
Salivary Gland			
Sialadenitis, chronic	2	2	5
Spleen			
Hemosiderosis	50	6*	24*

Source: Table 20

*Significantly different ($p < 0.05$) from controls

List of statistically different non-neoplastic effects (increased compared with controls, unless indicated; $p < 0.05$):

Males (1.3 mg/kg):
Chronic sialadenitis (salivary gland)
Perivascular mono. infil. (lung)[a]
Interstitial pneumonia (lung)[a]
Hemosiderosis (spleen)[a]

Males (14.2 mg/kg):
Cystoid degeneration (liver)
Megalocytosis (liver)
Portal mononuclear cell infiltration (liver)
Alveolar macrophages (lung)
Hemorrhage (lung)
Vascular mineralization (testis/epididymis)
Chronic sialadenitis (salivary gland)
Perivascular mono. infil. (lung)[a]

Females (1.6 mg/kg):
Vascular mineralization (lung)
Tubular hyperplasia (ovary)
Chronic myocarditis[a]
Perivascular mono. infil. (lung)[a]
Hemosiderosis (spleen)[a]

Females (16.1 mg/kg):
Megalocytosis (liver)
Tubular hyperplasia (ovary)
Hemosiderosis (spleen)[a]

[a]Decreased incidence relative to controls

Genetic toxicity studies (study type and results):

None

Remarks:

-Dose-related decreased in mean body weights in excess of 10% was observed in high-dose males and females.
-Mean feed consumption (as grams diet/kg bw) was increased in all of the FC-143 treated males throughout the study when compared to male control feed consumption. Overall, the variations were related to the variation in body weight among groups. Actual mean feed consumption was decreased in high-dose males relative to controls for the first year of the study.
-Dose-related occurrence of ataxia in females was the only clinical sign observed.
-A statistically significant ($p<0.05$) decrease in red blood cell parameters was noted in the high-dose males as compared to the controls.
-A statistically significant ($p<0.05$) increase in relative liver and kidney weights was found in high-dose males and an increase in relative kidney weights was found in high-dose females.

-Histopathological effects were noted in the liver of high-dose males and females.
-Urinary findings included increased incidence and severity of albumin and occult blood in all male and female control and FC-143-treated groups at 12, 18, and 24 months. These findings were more pronounced in males than in females at the termination of the study.
-Rats given the test article experienced a suspected outbreak of sialodacryoadenitis (SDA) viral infection between the first and second months of the study; however, the presence of a virus was not confirmed.

CONCLUSIONS

The study results are summarized as follows:

Treatment-related changes were found more commonly in males than in females of each of the two treatment groups, which were supported by earlier pharmacokinetic studies demonstrating a higher retention of FC-143 by males than females.

The test material was considered to be carcinogenic in the rat, inducing testicular/Leydig cell tumors in the males and mammary gland tumors in females.

Based on decreases in body weight gain, increase in liver and kidney weights and toxicity in the hematological and hepatic systems, the LOAEL for male and female rats is 300 ppm (male:14.2 mg/kg/day ; female:16.1 mg/kg/day). [The LOAEL for male rats is 1.3 mg/kg/day if salivary gland sialadenitis is based upon; the LOAEL for female rats is 1.6 mg/kg/day if increases in the incidences of ataxia (a clinical sign) and of ovarian tubular hyperplasia (may be reversible) are based upon].

The dose-dependent increases in neoplastic and non-neoplastic lesions were as follows:

- testicular Leydig cell adenoma ($p < 0.05$ at high dose) and vascular mineralization of the testes ($p < 0.05$ at high dose)
- thyroid C-cell adenomas in low-dose males
- thyroid C-cell hyperplasia in high-dose females
- mammary gland fibroadenomas in females ($p < 0.05$ at high dose)
- lung lesions in males ($p < 0.05$ at high dose)
- salivary gland sialadenitis in males ($p < 0.05$ at low and high doses)
- ovarian tubular hyperplasia in females ($p < 0.05$ at low and high doses)
- megalocytosis in the liver of males and females ($p < 0.05$ at high dose) with increases in relative liver weight and elevations of serum enzyme activities indicative of liver toxicity
- cystoid degeneration and portal mononuclear cell infiltration in the liver of males ($p < 0.05$ at high dose)

Remarks: Influence of potential viral infection in male Sprague-Dawley rats at both doses on the response to the test substance is not clear. Sialodacryoadenitis virus (SDAV) is a common viral infection of F344 rats; evaluation of 29 diet control rat groups at 5 different laboratories with and without viral infection found no consistent influence of viral infection on body weight, survival, or tumor prevalence (Rao, et.al., 1988).

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