DUPONT

January 9, 1997

DuPont Haskell Laboratory

HAZARD CHARACTERIZATION FOR HUMAN HEALTH C8 EXPOSURE

CAS REGISTRY NO. 3825-26-1

Prepared by: L. B. Biegel, Ph.D.
Senior Research Toxicologist

MAB000010

EID080808

Table of Contents

MAB000011

INTRODUCTION

This document is a Hazard Characterization of C8 for human health. C8 is also known as ammonium perfluorooctanoate (AFPO; CAS # 3825-26-1) and is the primary ingredient in FC-143 FLUORAD Brand Fluorochemical Surfactant. Within this document the chemical will be referred to as C8. However, it is acknowledged that many of the studies discussed actually tested the product FC143, which is a mixture of several straight-chain perfluorocarboxylic acids containing approximately 93.0-97.0% C8.

I. MAMMALIAN TOXICOLOGY

I.1. Acute Toxicity Studies

I.1.a. Acute Oral Toxicity

Numerous acute oral toxicity studies, in several species (rats, mice, guinea pigs, dogs), have been conducted with C8 (see Table I-1). The results of the various studies have been consistent in their results. Administration of a single dose of 12 mg/kg to 3 rats produced no clinical signs of toxicity. Studies demonstrate that newborn and older adult rats appear to be more sensitive than weanlings and young adults. Additionally, while mice and rats appear to be equally sensitive to the acute toxicity of C8, guinea pigs are more sensitive than mice or rats. In the rat, acute oral exposure generally results in enlarged livers, elevations of liver enzyme levels, gastrointestinal irritation, and weight loss. C8 is considered to have moderate acute oral toxicity.

In addition to the numerous studies listed below, several other studies were conducted which investigated the effects of C8 alone or on animals pre-exposed to other chemicals or drugs. Pre-treatment of rats with phenobarbital sodium or proadifen hydrochloride does not result in an alteration of the LD_{50} of C8 (478 mg/kg). Pre or post-dosing with Dowex® 1-X2-C1 Ion Exchange Resin at 1000 mg/kg reduced the mortality compared to rats dosed with C8 alone. A study was conducted to determine if pre-treatment with ethanol (a single dose of 60% or a 15% aqueous solution (v/v) in drinking water for 14 days) modifies the effects of C8 on liver weight. This study determined that pre-treatment with ethanol did not alter C8's effect on liver to body weight ratios.

MAB000012

Table I-1

SUMMARY OF ACUTE ORAL TOXICITY STUDIES WITH C8

Study Type	Species	#/sex/dose	Dose (mg/kg)	Vehicle	Results mg/kg	Reference
ALD	Rat	10 Males	200, 480, 670	Corn oil	480	HL-565-81
ALD	Rat	1 Male	1.5, 12, 40, 120, 200, 300, 450, 670, 1000, 1500, 2250	Water	670	HL-55-61
ALD	Rat	1 Male	1.0, 1.5, 2.3, 3.4, 5.1, 26, 40, 60, 77, 90, 120, 130, 170, 200, 300, 450, 670, 2250	Water	670	HL-128-68
LD_{50}	Rat	5	100, 215, 464, 1000, 2150	Acetone(49%) Corn oil (60%)	Males: 680 Females: 430	(Griffith and Long, 1980)
LD_{50}	Rat	10	200, 400, 450, 500, 670, 1000	Corn oil	Males: 470 Females: 482	HL-295-81
LD_{50} as a function of age	Rat					HL-788-82
		10 Weanling males	350, 400, 450, 525, 670, 710	Corn oil	573	
		10 Weanling females	350, 400, 450, 670		580	
		10 Young adult females	350, 425, 500, 670		453	
		10 Mature adult males	200, 240, 300, 350, 400, 500, 720		336	
		10 Mature adult females	225, 350, 400, 450, 670		343	
		10 Newborn males	130, 200, 240, 280, 330, 370		243	
		10 Newborn females	130, 180, 200, 220, 240, 280, 320		258	
LD_{50} castrated vs. intact adults	Rat					HL-600-81
		10 intact males	200, 480, 670	Corn oil	439	
		10 intact females	200, 480, 670		491	
		10 orchidectomized males	200, 480, 670		459	
		10 ovariectomized females	200, 480, 670		400	
LD_{50}	Rat	10 males	400, 500, 650	Corn oil	478	HL-567-81
LD_{50}	Rat				590	Hazleton Laboratory America, Inc. 2-6-87
LD_{50}	Mice	10	250, 500, 750, 1000, 2000, 4000	Corn oil	457	HL-329-81
Liver function	Dog	3 Males	450, 200	Not stated	Lethal at 450 mg/kg by 48 hours Elevated GPT and GOT which normalized within 1 week at 200 mg/kg	HL-123-65
LD_{50}	Guinea Pig	10	150, 200, 250, 300, 400, 670	Corn oil	Males: 178 Females: 217	HL-291-81

a). Weanling = 21-days old b). Young adult = 8-10 weeks old c). Mature adult = >10 weeks old d). Newborn = < 2 days old

EID0080811

MAB000013

I.1.b. Acute Dermal Toxicity

Acute dermal toxicity and irritation studies in rats and rabbits have been conducted with C8. C8 is considered to be mild - moderately irritating to the skin and moderately toxic by the dermal route of exposure. Rat skin showed less irritation than rabbit and in general the effects were more pronounced in males than in females. In addition to dermal irritation several clinical signs of toxicity were observed in both rats and rabbits in response to C8 exposure. These observations included body weight loss, wet and/or stained perineal area, cyanosis (rabbits only), diarrhea (rabbits only), lethargy (rabbits only), labored breathing (rabbits only), and chromodacryorrhea (rats at 7500 mg/kg)

Table I-2

SUMMARY OF ACUTE DERMAL TOXICITY/IRRITATION STUDIES WITH C8

Study Type	Species	#/sex/dose	Dose (mg/kg)	Results	Reference
LD_{50}	Rat	5	3000, 5000, 7500	Male LD_{50} = 6959 mg/kg Female LD_{50} = >7500 mg/kg	HL-659-79 (Kennedy, 1985)
Skin Absorption LD_{50}	Rat	5 Females	5000 and 7500	LD_{50}>7500 mg/kg Mild skin irritation	HL-682-80
Skin Absorption LD_{50}	Rat	5 Females	5000 and 7500	LD_{50}>7500 mg/kg Mild skin irritation	HL-682-80
LD_{50}	Rabbit	5 Males (2 at 7500)	1500, 3000, 5000, 7500	LD_{50} = 4278 mg/kg	HL-659-79 (Kennedy, 1985)
	Rabbit	4	100, 1000, 2000	Lethal 4/4 at 2000 3/4 at 1000 0/4 at 100	Riker Laboratories Report No. 09790AB0485
Skin Irritation	Rabbit	6	500 mg on intact and abraded sites	Non-irritating	(Griffith and Long, 1980)
Skin Irritation	Rabbit	6 Males	500 mg	Mild-moderate irritation at 24 hours Slight-moderate irritation at 48 hours	HL-636-79

I.1.c. Acute Ocular Toxicity

Eye irritation studies in rabbits have been conducted with C8. C8 is considered to be moderately irritating to the eye. Instillation of solid C8 into rabbit eyes produced moderate corneal opacity, iritis, and conjunctivitis. These ocular effects gradually receded over time. Prompt washing of the eye reduced the effects and provided a more rapid recovery. In addition to the eye irritation studies that have been conducted, rats

MAB000014

EID080812

exposed to C8 during a 4-hour inhalation period exhibited corneal opacity and ulceration, which were microscopically evident 42 days post-exposure.

Table I-3

SUMMARY OF EYE IRRITATION STUDIES WITH C8

Species	#/sex/dose	Dose (mg)	Results	Reference
Rabbit	2 (1 unwashed, 1 washed)	38.3	Unwashed eye Moderate-severe corneal opacity Moderate iritis Moderate conjunctivitis At 21-28 days Corneal opacity Mild vascularization Washed eye Slight-moderate corneal opacity Slight-moderate conjunctivitis At 7 days Mild conjunctival redness At 14 days Normal	HL-635-79
Rabbit	6 unwashed 6 washed	100	Unwashed eye Moderate irritation Conjunctivitis Iritis Washed eye Conjunctivitis At 7 days 4/6 eyes were free of irritation	Biosearch, Inc. Report No. T1395 (Griffith and Long, 1980)

I.1.d. Acute Inhalation Toxicity

Acute inhalation toxicity studies in rats have been conducted with C8. Acute exposure to C8 by inhalation is considered to be highly toxic, with a 4-hour approximate lethal concentration (ALC) in rats of 0.8 mg/L. At concentrations of 2.2 mg/L C8 and higher, all rats died within 48 hours of exposure. At concentrations between 0.38 and 0.83 mg/L C8, rats experienced an initial weight loss following exposure and an increased liver-to-body weight ratio which returned to the high end of the normal range 42 days post-exposure. Additionally, all rats exposed to 0.81 mg/L C8 and higher showed corneal opacity and corrosion.

MAB000015

EID080813

Table I-4

SUMMARY OF ACUTE INHALATION STUDIES WITH C8

Species	#/sex/dose	Concentration (mg/L)	Results	Reference
Rat	6 Males	4-hour exposure to: 0.38, 0.81, 0.83, 2.2, 4.8, 5.7	4-hour ALC = 0.8 mg/L LC_{50} = 0.98 mg/L	HL-160-69 (Kennedy, et al., 1986)
Rat	5	1-hour exposure to:18.6 mg/L	No deaths Eye and respiratory irritation	(Griffith and Long, 1980)

I.1.e. Acute Injection Toxicity

Acute toxicity of C8 when administered by intraperitoneal injection was assessed in mice (3M, 1979). The LD_{50} by intraperitoneal injection in mice is 192 mg/kg.

I.2. Subchronic Toxicity Studies

I.2.a. Subchronic Oral Toxicity

Numerous subchronic oral toxicity studies in several species (rats, mice, and monkeys), have been conducted with C8 (see Table I-5). The results of the various studies have been quite consistent in their results. Administration of C8 in the diet or by daily gastric intubation produced death at concentrations of 1000 ppm and higher for rats and mice and at 30 mg/kg/day for monkeys. The primary target organ for toxic responses in all species studied is the liver. C8 produces increased liver weights, increased liver enzyme activity, hepatocellular hypertrophy, and hepatic peroxisome proliferation.

In addition to the numerous studies listed below, other studies were conducted which investigated the mechanism of action of C8. These studies are summarized in Section III. Mechanisms of Action.

MAB000016

EID080814

Table I-5

SUMMARY OF SUBCHRONIC ORAL TOXICITY STUDIES WITH C8

Study Type	Species	#/sex/dose	Concentration (ppm unless specified)	Results	Reference
14-day feeding	Mice	5	10, 30, 100, 300, 1000, 3000, 10000	100% mortality at ≥3000; deaths at 1000; increased liver weight/body weight ratio at ≥10	HL 560-81
14-day feeding	Mice	5	30, 300, 3000	100% mortality at 3000; deaths at 300; weight loss at ≥ 300 increased liver weight/body weight ratios at ≥ 30	HL12-82 (Kennedy, 1987)
21-day feeding	Mice	5	0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30	Significantly increased liver weight at 30	HL 323-82 (Kennedy, 1987)
14-day feeding	Mice	5	30	Increased liver weight; when C8 was combined with an equal amount of nonadecafluorodecanoic acid, a similar effect was produced	HL 537-82
9-dose gavage	Mice	5	0.1, 1.0, 10 mg/kg	Weight loss, death in 10 mg/kg females, increased liver weight at 1 and 10 mg/kg	HL 138-83
	Rat	5	0.1, 1.0, 10 mg/kg	Weight loss, increased liver weight in 10 mg/kg males	
14-day feeding with a 2-week recovery period	Rat	6 Males	25% Teflon® with C8 as the dispersing agent	Slightly increased liver weights following the recovery period	HL 56-61
14-day feeding With a 56 day recovery period	Rat	5 Males	30, 300	Decreased body weights at 300; increased liver weights at the end of the feeding period at 30 and 300 and on recovery days 7 and 28 (300 ppm only; elevated blood fluoride levels out to recovery day 7 (final day tested)	HL 326-95

EID0080815

MAB000017

Table I-5 (Con't)

SUMMARY OF SUBCHRONIC ORAL TOXICITY STUDIES WITH C8

Study Type	Species	Sex (#/sex/dose)	Concentration (ppm unless specified)	Results	Reference
28-day feeding	Rat	5	30, 100, 300, 1000, 3000, 10000, 30000	100% mortality at ≥ 3000; decreased body weights at ≥ 1000 and 3000 for females; increased liver weight/body weight ratios at ≥ 30 for males and ≥ 300 for females	(Griffith and Long, 1980)
	Mice	5	30, 100, 300, 1000, 3000, 10000, 30000	100% mortality at ≥ 1000; deaths at ≥ 30; decreased body weights at ≥ 30; cyanosis and muscle weakness at ≥ 3000; increased liver weight/body weight ratios at ≥ 30; panlobular diffuse hypertrophy of hepatocytes	
90-day feeding	Rat	5	10, 30, 100, 300, 1000	Decreased body weights at ≥ 300; increased liver weights at ≥ 300; panlobular diffuse hypertrophy of hepatocytes at ≥ 1000 with males more affected than females; serum fluoride concentration increased 75 to 226 fold with higher concentrations observed in males	
90-day gavage	Monkey	2	3, 10, 30, 100 mg/kg/day	100% mortality at ≥ 100; deaths at ≥ 30 (females only); decreased body weights at ≥ 30; no signs of toxicity at 3 mg/kg/day; dose dependent increases in serum and liver fluorine levels (no apparent sex difference)	
90-day feeding with an 8-week recovery period	Rat	55 Males	1, 10, 30, 100	Reduced body weigh at 100; increased palmitoyl CoA oxidase activity at ≥ 30 and transient increases at 10; palmitoyl CoA oxidase activity returned to normal after the 8 weeks of recovery; increased liver weights and hepatocellular hypertrophy at ≥ 10 which was reversible following the recovery period. Serum estradiol, testosterone and lutienizing hormone levels were not affected by dietary exposure to C8, while estradiol levels were slightly elevated at 100 ppm at week 5. The NOAEL = 100 ppm; the NOEL = 1 ppm	(Perkins, 1992)

EID080816

MAB000018

I.2.b. Subchronic Inhalation Toxicity

Similar to the oral toxicity studies, inhalation exposure to C8 produces reduced body weight, increased liver weights, increases in plasma enzymes indicative of liver injury, and pathological lesions in the liver. Measurement of the blood fluoride levels (indicative of the presence of C8) determined that the blood half-life of C8 in the rat is 5-7 days following inhalation exposure.

Table I-6

SUMMARY OF SUBCHRONIC INHALATION TOXICITY STUDIES WITH C8 IN THE RAT

Study Type	Sex (#/sex/dose)	Concentration	Results (mg/kg)	Reference
10 exposure with a 42-day recovery	20	11, 83 mg/m^3 for 6 hours/day	Dose related decrease in body weight, suppression of body weight maintained during the 42-day recovery period at 83; increased plasma enzymes indicative of liver injury present up to 28 days following the last exposure; granular degeneration of hepatocytes; increased liver weights, no ocular effects were observed. The liver effects were not observed after 14, 32, or 42 days of recovery.	HL 253-79
10 exposure with an 84-day recovery	24	1, 8, 84 mg/m^3 for 6 hours/day	Deaths at 84; increased lung, liver and testes weights, no ocular effects observed; increased plasma enzymes indicative of liver injury; increased liver weights at $\geq$ 8 mg/m^3; panlobular and centrilobular hepatocellular hypertrophy and necrosis. The liver effects were reversible following a 28-day recovery period. Dose related presence of C8 in the blood, which decreased with time during the recovery period but was still detectable after 84 days of recovery. NOAEL = 1 mg/m^3, although 13 ppm organofluoride was detected immediately following exposure to 1 mg/m^3	HL 205-81 (Kennedy, Hall et al., 1986)

I.2.c. Subchronic Dermal Toxicity

The subchronic dermal toxicity of C8 has been studied in the rat and rabbit (see Table I-7). Similar to the oral toxicity studies, dermal exposure to C8 produces reduced body weight, increased liver weights, increases in plasma enzymes indicative of liver injury and lesions in the liver. Measurement of blood fluoride levels (indicative of the presence of C8) determined that the blood half-life of C8 in the rat is 5-7 days following dermal exposure. A comparison of the dermal exposure studies to the feeding studies leads to the conclusion that the rates of absorption of C8 by these two routes are not significantly different.

MAB000019

Table I-6

SUMMARY OF SUBCHRONIC INHALATION TOXICITY STUDIES WITH C8 IN THE RAT

Study Type	Species (#/sex/dose)	Concentration (mg/kg)	Results (mg/kg)	Reference
10 dose with an 84 day recovery	15 Male Rats	20, 200, 2000 for 6 hours/day, 5 days/week	Skin irritation at ≥ 200; reversible reduction in body weight at ≥ 200; increased plasma enzymes indicative of liver injury; increased liver weights at ≥ 20; hepatocellular hypertrophy and necrosis at 20 ; no ocular effects observed The liver effects were generally reversible following a 42-day recovery period at ≤ 200. Dose related presence of C8 in the blood, which decreased with time during the recovery period but was still detectable after 42 days of recovery.	HL 589-80 (Kennedy, 1985)
Range-finder	4 Rabbits (sex not specified)	100, 1000, 2000	Lethal to 4 of 4 at 2000, 3 of 4 at 1000, 0 of 4 at 100	Riker Laboratories, Report 09790AB0485, March 15, 1981
10 exposure with a 14 day recovery	10 Rabbits	100 for 6 hours/day, 5 days/week	Reversible reduction in body weight; Blood fluorine levels were 5.4, 6.8, 4.6 ppm for males and 10.1, 12.1, and 3.5 for females at 7, 14, and 28 days of the study, respectively.	

I.3. Developmental Toxicity

Developmental toxicity studies have been conducted in rats and rabbits (See Table I-7). The original developmental toxicity study in rats indicated that C8 might be a teratogen in rats. However, because the results were questionable, additional studies were conducted to clarify the result. The additional studies did not confirm the original result. Overall, C8 is not considered to be uniquely hazardous to the conceptus.

The two areas of question were apparent lens abnormalities and skeletal alterations. In the original study, the lens alterations consisted of the following: large lens cleft, dark streak running ½ to ¾ of the way through the lens; or disorganized lens fibers. In the subsequent studies, the lens alterations were determined to be an artifact created in the lens during freehand sectioning. Processing Bouin's-fixed fetal heads that were trimmed on either side of the orbit, instead of through the center of the eye, essentially eliminated this artifact. Examination of the eyes of offspring using focal illumination, indirect ophthalmoscopy, and slitlamp microscopy were also used and did not detect any C8-related eye alterations. The skeletal alterations included ossification sites on the first lumbar vertebrae in rats and 13 ribs in rabbits. Both of these alterations are considered to represent stress-related changes indirectly related to C8-administration.

MAB000020

EID080818

Table I-7

SUMMARY OF DEVELOPMENTAL/REPRODUCTIVE TOXICITY STUDIES WITH C8[a]

Species (#/dose)	Concentration	Results mg/kg	Reference
Rats (# not specified)	25, 50, 75, 100, 150 mg/kg by gavage	Reduced maternal body weight gain and clinical signs of toxicity at 150; eye abnormalities at 25 and 150	3M Report M-601 (1981)
25 Rats[b]	100 mg/kg by gavage	Maternal deaths, decreased maternal body weight gain; no developmental toxicity or abnormalities observed.	HL 1-82 (Staples, et al., 1984)
12 Rats[c]	100 mg/kg by gavage	Maternal deaths, decreased maternal body weight gain; no alterations in postpartum viability, growth rate, or development. No ocular effects observed.	
Rats (# not specified)	0.05, 1.5, 50, 150 mg/kg by gavage	Maternal deaths at 150; C8 was not embryotoxic, no abnormal gross findings, no malformations[d]. Fetal lens findings were observed in all groups. Determined to be a processing artifact. No effect on ovaries, reproductive tract, male/female ratio, implantation sites, corpora lutea, or fetal weights.	3M Report 0681TR0110, 1981
Rats	0.14, 1.2, 9.9, 21 mg/m^3 by inhalation	Maternal deaths at 21; overt maternal toxicity at 9.9; No teratogenic effects were observed in any of the exposed groups; embryo-fetal toxicity was observed at 21; processing artifacts were observed on lens'.	HL 881-81 (Staples, et al., 1984)
18 Rabbits	1.5, 5, 50 mg/kg by gavage	Reduced maternal body weight gain at 50, C8 was not embryotoxic or teratogenic[e]	3M Product Toxicity Sheet, May 24, 1996

a. Pregnant rats were dosed by gavage on days 6-15 of pregnancy. Pregnant rabbits were dosed by gavage on days 6-18 of pregnancy.
b. Sacrificed on Day 21 of gestation.
c. Pups sacrificed on day 35 postpartum.
d. A significantly higher incidence of the skeletal finding "one sternabrae missing", occurred in the high-dose group. This was a minor skeletal aberration and was not considered a malformation in this study. Furthermore, the incidence of this finding did not differ from the control group or the 3 lower-level treatment groups. The incidences of skeletal findings associated with delayed ossification and rib aberrations were not different among the treatment groups and controls.
e. There was a statistically significant increase in the incidence of 13 ribs in the high dose group and 13 ribs spurred in the mid-dose group. While the findings are significantly greater in the treated animals than in the controls, they are not considered to be teratogenic changes or malformations, rather they are considered to represent stress-related changes to compound administration.

MAB000021

EID080819

I.4. Reproductive Toxicity

No information is available on the reproductive toxicity of C8

I.5. Mutagenicity

It has been demonstrated that C8 is not mutagenic in a variety of mutagenicity tests (See Table I-9).

Table I-9

SUMMARY OF MUTAGENICITY STUDIES WITH C8 IN THE RAT

Study Type	Study Description	Results	Reference
Mutagenicity assay	Assayed in *S. Typhimurium* (TA1535, TA1537, TA1538, and TA100) and *S. cerevisiae* D4 yeast, with and without metabolic activation.	Negative	Litton Bionetics; LBI Project 20838, Feb. 1, 1978 (Griffith and Long, 1980)
In vivo mouse micronucleus	3 mice/sex were dosed with 200, 400, 600, 800, and 1000 mg/kg and bone marrow was evaluated at 24, 48 and 72 hours after dosing.	Negative	Corning Hazleton, 17388-0-455, May 16, 1996
Chromosomal aberration	Assayed for ability to induce chromosomal aberrations in CHO cells with and without metabolic activation.	Negative	Corning Hazleton, 17388-0-437, April 25, 1996
Mammalian cell transformation assay	Assayed for cell transformation potential and cytotoxicity in C3H 10T1/2 colony cells.	LD = 50 g/mL; low cytotoxicity No evidence of cell transformation	University of Minnesota Environ. Path Lab, T2942, April 9, 1981

I.6. Chronic Toxicity and Oncogenicity

The chronic toxicity and oncogenicity of C8 has been investigated in two 2-year feeding studies in rats (see Table I-10).

MAB000022

EID080820

Table I-10

SUMMARY OF CHRONIC TOXICITY AND ONCOGENICITY STUDIES WITH C8 IN RATS

#/sex/dose	Concentration (mean daily intake, mg/kg/day)	Results	Reference
50	0, 30, 300 ppm (0, 1.5, and 15 mg/kg/day)	Decreased body weight gain and food consumption, increased ataxia. Decreased RBC counts, hemoglobin, and hematocrit values. Increased liver weights, liver cell hypertrophy, degeneration and necrosis. Not considered to be carcinogenic.	Riker Laboratory 0281CR001 (April 1981–May 1983)
156 Males	0, 0-pair-fed, 300 ppm	Decreased body weight gain and food consumption. Increased estradiol levels. Increased incidence of liver, Leydig cell and pancreatic acinar cell adenomas.	(Cook, et al., 1994) DuPont MR-5686

In the original study, in-life findings consisted of a dose dependent decrease in mean body weight gain and increase in food consumption in males, and a slight treatment-related increase in the incidence of ataxia in females. No increase in mortality was observed. C8-related hematologic alteration included decreased red blood cell counts, hemoglobin and hematocrit values observed at various times throughout the 2-year test period. However, the decreases in erythrocyte counts were observed early in the study and did not progress into generalized anemia. Histopathologically, C8-associated alterations were observed in the liver. These changes were characterized by increased liver weights, hypertrophy, hepatocellular degeneration, and necrosis. As with the erythrocyte counts, the hepatic alterations were observed early in the study and showed little progression over the remainder of the 2-year study. The incidence of tumors was relatively low, and the types of neoplasms found were not different from the tumor profiles commonly observed in geriatric rats. Hepatocellular tumors were slightly increased in the 300 ppm males, however, not to the extent that would be expected considering the morphological evidence of hepatocellular stimulation observed at the 1-year necropsy. The incidence of testicular Leydig cell adenomas (0/50, 3/50, and 7/50 at 0, 30, and 300 ppm, respectively) was suggestive of a compound-related effect. However, because the incidence was within the historical control range, it was not considered to be a compound-related effect. Based on the tumor incidence, types of tumors, time of tumor appearance, and the survival rate at the 2 year time point, the overall conclusion was that C8 was not carcinogenic in the rat (Riker Laboratory, 0281CR0012). However, in this original study, some of the pathological findings were equivocal (liver and Leydig cell tumors), even when evaluated by an outside laboratory, and therefore a second 2-year study was conducted to clarify some of these findings.

The second study included many mechanistic endpoints to help determine the mechanism of tumor formation (DuPont MR-5686, Cook, et al., 1994). In addition to the *ad libitum* control, a second control was pair-fed to the C8 group. Peroxisome proliferation (ß-oxidation activity) and cell proliferation (BrdU, 6-day osmotic pumps)

MAB000023

were measured in the liver and testis. Serum hormone levels (testosterone, estradiol, lutienizing hormone (LH), follicle stimulating hormone (FSH) and prolactin) were also measured. Interim sacrifices were performed at 3-month intervals as well as at 1 month. Increased relative liver weights were observed in the C8-treated rats. Hepatic ß-oxidation activity was also increased in the C8-treated rats at all time points. In contrast, hepatic cell proliferation was not significantly increased in the C8-treated group. C8 did not significantly alter the rate of Leydig cell ß-oxidation or Leydig cell proliferation. Moreover, the rate of ß-oxidation in Leydig cells was approximately 20-times less than the rate of hepatic ß-oxidation, irrespective of treatment. Serum testosterone, FSH, prolactin, and LH levels were unchanged in the C8-treated rats when compared to the controls. There were, however, significant increases in serum estradiol levels in the C8-treated rats at 1, 3, 6, 9, 12, 15, 18 and 21 months. Histopathological evaluation revealed compound-related increases in liver, Leydig cell, and pancreatic acinar cell tumors in C8-treated rats. Based on the data, the Leydig cell tumors appear to be due to the combination of elevated estradiol levels and reduced prolactin levels. The pancreatic acinar cell tumors are related to an increase in serum cholecystokinin (CCK) levels.

II. METABOLISM

Numerous studies have been conducted investigating the excretion and disposition of C8 in various species. Additionally, studies have been conducted with exposed workers at a manufacturing plant which produces C8. Sex and species differences have been noted, whereas reproductive status in females did not have an effect on excretion or disposition in rats. Rabbits (both sexes), female rats, and male hamsters rapidly excrete C8, while male rats and female hamsters excrete C8 more slowly. Mice (both sexes) excrete C8 even more slowly. C8 also has a long ½-life in humans. Measurement of C8 blood levels in an exposed worker showed that the ½-life in men is greater than 1.5 years.

II.1. Animal Studies

The excretion and disposition of C8 has been investigated in rats, mice, hamsters and rabbits. Studies have also investigated the influence of route of exposure. These studies are summarized below.

II.1.a. Male and female rats were administered radiolabeled C8 by intravenous injection. Females excreted essentially 100% of the administered dose by 24 hours, while males had excreted only 20% of the administered dose. Radioactive tissue residues were not detectable after 17 days in the females, while at 36 days males had 2.8% of the ^{14}C in the liver, 1.1% in the plasma and lower but detectable levels in other organs (Riker Laboratory drug Metabolism Report 1-20 (1980)).

II.1.b. Sex differences in the excretion and disposition of radiolabeled C8 were observed in a study of rats, mice, hamsters, and rabbits. Male and female animals of each species were dosed by gavage with 10 mg/kg C8, and urine and feces were collected at 24, 48, 72, 96, and 120 hours post-dosing. Animals were then sacrificed, and blood and

MAB000024

tissues were analyzed. The urine and feces of rabbits was also collected at 144 and 168 hours post-dosing, and rabbits were sacrificed at 168 hours.

The female rat and male hamster had excreted over 99% of the administered dose at the time of sacrifice. The male rat and the female hamster had excreted 39 and 60% of the administered dose, respectively, at the time of sacrifice. Both sexes of rabbits excreted the C8 as rapidly and completely as the female rat and male hamster. The male and female mice retained substantial amounts of the total administered radioactivity in their tissues at the time of sacrifice, only excreting 21% of the administered dose at 120 hours post-dosing (HL 62-82)

II.1.c. Cholestyramine, a non-absorbable anion-exchange resin, was demonstrated to protect rats from the acute lethal effect of C8 when administered within 2 hours of C8 dosing (HL 828-81).

A second study was conducted to investigate the effect of cholestyramine on the elimination of ^{14}C-C8 (10 mg/kg by gavage) from rats and mice (HL 405-82). Adult male rats and mice were given cholestyramine (1000 mg/kg by gavage) 24 hours after dosing with ^{14}C-C8. The cholestyramine did not enhance the elimination of C8 via the feces, urine, or exhaled air. Similarly, Dowex® Ion Exchange Resin was also able to reduce the acute lethal effect of C8. When rats and mice were given Dowex® resin 24 hours after dosing with C8 no signs of enhanced elimination of C8, via the feces, urine or exhaled air, were observed (HL 405-82).

To further investigate the use of cholestyramine to enhance C8 elimination, a third study was conducted in rats. In this study, rats were dosed with ^{14}C-C8 (13.3 mg/kg, by iv.) and then were fed diets containing 4% cholestyramine for 14 days. The cholestyramine increased the elimination of C8 via the feces by 9.8 fold and decreased the concentration of C8 found in the liver, plasma, and red blood cells (Johnson, et al., 1984).

II.1.d. A series of experiments was conducted to evaluate the uptake and clearance of C8 from the blood of male and female (pregnant and non-pregnant) rats following oral exposure, and inhalation exposure.

The uptake and clearance of C8 from the blood of female rats following a single oral dose was rapid, with peak reached 1-2 hours post-treatment and virtual total clearance by 24 hours. A dose-response was demonstrated with no apparent changes in blood C8 levels following multiple oral dosing. The slower clearance rate in male rats was demonstrated following a single oral dose. The same general statements apply following inhalation exposure. A single 6-hour inhalation exposure resulted in: peak blood levels within 1 hour after cessation of exposure; the material rapidly cleared from the blood; the number of exposures did not affect blood levels; and male rats cleared the compound much more slowly. Pregnant and non-pregnant rats showed similar C8 blood

MAB000025

levels following either oral or inhalation exposure (HL 593-91). Specifics of the experiments are summarized below.

II.1.e.1. Oral administration-Blood levels of C8 as a function of time post-dosing (female rats)

C8 levels of 14 ppm were seen 15 minutes following administration of C8. These levels peaked at 30 ppm at 1-2 hours, dropped to 26 ppm by 8 hours, and to 0.7 and 0.045 ppm at 24 and 168 hours, respectively. C8 is absorbed and rapidly cleared from the blood of female rats given a single oral dose.

II.1.e.2. Oral administration-Blood levels of C8 as a function of dose (female rats)

C8 levels 30 minutes following administration of 2.5 – 150 mg/kg ranged from 3 –162 ppm. The same dose response was observed at 24 hours with blood levels ranging from 0.12 - 18 ppm. The response was linear. The level of C8 in the blood is directly related to the amount of C8 administered.

II.1.e.3. Oral administration-Blood levels of C8 as a function of number of doses (female rats)

Blood levels in female rats given 1 versus 11 doses of C8 were not considerably different. Concentrations at 15 minutes following administration were 14 and 17 ppm for 1 and 11 doses, respectively. At 30 minutes C8 concentrations were 16 and 25 ppm; at 8 hours 26 and 13 ppm; at 24 hours, 0.7 and 0.8 ppm; and at 168 hours, 0.045 and 0.10 ppm for 1 and 11 doses, respectively. C8 does not appear to accumulate in the blood of female rats following repeated oral administration. The number of treatments does not appear to influence the C8 blood level.

II.1.e.4. Oral administration-Blood levels of C8 following a single 25 mg/kg dose (male and female rats)

Time following single oral dose (hours)	Blood Levels of C8 (ppm)	
	Male Rats	Female Rats
½	23	16
8	63	26
24	50	0.7
168	23	0.045

C8 is retained in the blood of male rats to a greater extent than female rats.

II.1.e.5. Inhalation exposure-Blood levels of C8 as a function of time post-exposure (female rats)

C8 levels of 96 ppm were observed 15 minutes following a single 6-hour exposure to 10 mg C8/m^3. The level was maintained through 1 hour, fell to approximately 70 ppm at 8 hours, 52 ppm at 24 hours, and dropped to 0.39 ppm at 168

EID080824

MAB000026

hours post-exposure. This same general pattern was observed in rats exposed to either 0.1 or 1 mg/m^3. The lag phase seen following oral exposure was not observed here due to blood sampling following a 6-hour inhalation exposure (rather than a single dose at a given time). C8 is absorbed and rapidly cleared from the blood of female rats following a single inhalation exposure.

II.1.e.6. Inhalation exposure -Blood levels of C8 as a function of dose (female rats)

Time following single inhalation exposure dose (hours)	Blood Levels of C8 (ppm) Following exposure to C8 at		
	0.1 mg/m^3	1 mg/m^3	10 mg/m^3
½	2	7	109
2	2	17	69
8	0.85	4	71
24	0.14	0.56	52

The response is linear at 30 minutes. C8 blood levels are directly related to the amount of C8 inhaled. At the high concentration used, the clearance rate is somewhat slower than observed at the lower levels. This suggests massive overloads in the clearance system.

II.1.e.7. Inhalation exposure -Blood levels of C8 following a single 6 hour exposure to 10 mg/m^3 (male and female rats)

Time following single oral dose (hours)	Blood Levels of C8 (ppm)	
	Male Rats	Female Rats
½	137	109
2	157	69
8	182	71
24	147	52

C8 is retained in the blood of male rats to a greater extent than female rats following inhalation exposure.

II.1.e.8. Oral administration-Blood levels of C8 following a single 25 mg/kg dose (pregnant and non-pregnant female rats)

Time following single oral dose (hours)	Blood Levels of C8 (ppm)	
	Pregnant Rats	Non-pregnant Rats
½	16	10
2	33	39
8	26	31

C8 clearance following oral dosing is similar in pregnant and non-pregnant female rats.

MAB000027

EID080825

II.1e.9. Oral and Inhalation exposure -Blood levels of C8 as a function of number of exposure concentration (pregnant female rats)

Time following single oral dose of 25 mg/kg	Blood Levels of C8 (ppm) Following		
(hours)	1 exposure	6 exposures	10 exposures
½	18	12	12
2	39	37	15
8	31	nd[a]	11
24	2	nd	1

Time following single inhalation exposure to 10 mg/m^3	Blood Levels of C8 (ppm) Following	
(hours)	1 exposure	10 exposures
½	77	53
2	90	nd

a. nd = not done

When comparing the C8 levels seen at 2 and 8 hours, following 10 consecutive oral doses, there appears to be a lowering of the C8 blood levels. Blood levels following 1 or 10 consecutive inhalation exposures (6 hours/day) were not different. C8 does not appear to accumulate in the blood of pregnant rats following repeated oral or inhalation exposures.

III.f. The ability of ^{14}C-C8 to transfer through the placenta was investigated in rats (HL 61-82). A single dose of 10 mg/kg ^{14}C-C8 was administered to pregnant rats on the 19th day of pregnancy. Maternal blood and placental levels of ^{14}C-C8 increased between 2 and 4 hours post-dosing, and decreased between 4 and 8 hours post-dosing.

Time following single oral dose of 10 mg/kg	Levels of C8	
	Maternal	Fetal
(hours)	(µg equivalents/mL blood)	(µg equivalents/mL tissue)
2	12	0.7
4	20	3
8	12	3

II.2. Human Exposure

Determinations of organic fluorine blood levels in workers exposed to C8 in an industrial environment were performed. Approximately 90% of the organic fluorine was composed of the C8 anion. The highest levels were found in workers with the longest work history in fluorochemical production. The majority of the values remained at approximately the same level throughout the 2 ½ year monitoring period. Monitoring of C8 blood levels of a worker who was removed from the fluorochemical production site due to high C8 blood levels (70 ppm) suggests that fluorochemicals are very slowly

MAB000028

EID080826

eliminated. From this limited data it is hypothesized that the ½-life of C8 is 1.5 years in men (Ubel, et al., 1980).

Group	Number analyzed	Blood Organic Fluorine Levels (ppm)
Normal human sera	from published literature	0.01-0.13
Industrial controls	4	0.01-0.08
Laboratory personnel (>20 years exposure)	8	0.04-2.00
Plant workers	49	1.00-71.00

III. MECHANISMS OF ACTION

C8 is not metabolized in rats. C8 produces hepatomegaly, induces hepatic peroxisomes in mice and rats, and has been shown to produce hepatic, Leydig cell, and pancreatic acinar tumors in a 2-year feeding study in rats. The male rat is more susceptible to the toxic effects of C8 than the female rat, presumably due to the longer ½-life in males. Short-term studies have been conducted investigating the mechanisms of action responsible for the various effects.

III.1. Investigation of C8s' Effect on the Liver.

III.1.a. Because C8 had been shown to induce a striking hepatomegaly in rats, a study was conducted to investigate the hepatic biochemical and morphological changes associated with C8-induced hepatomegaly in rats (Pastoor, et al., 1987). In this study male rats were dosed daily for 1, 3, or 7 days with 50 mg C8/kg body weight by intragastric intubation. The total cytochrome P450 content and activity of benzphetamine *N*-demethylase was increased in the livers of C8-treated rats, indicating the proliferation of smooth endoplasmic reticulum. In contrast, the soluble, cytoplasmic enzymes, glutathione *S*-transferase and UDP-glucuronyltransferase, were unaffected. Carnitine acetyltransferase activity was disproportionately increased relative to carnitine palmitoyl transferase activity, confirming the predominant proliferation of peroxisomes versus mitochondria. Electron microscopy confirmed the proliferative response of the endoplasmic reticulum, peroxisomes, and microsomes in the livers of the C8-treated rats. This study also demonstrated that C8 does not possess hypolipidemic activity.

III.1.b. C8 increased serum estradiol concentrations in 2-week gavage studies, and feeding studies at various time points up to 2 years. This was accompanied by increases in liver weights, and hepatic β-oxidation activity (Cook, et al., 1992; Cook, et al., 1994). Since peroxisome proliferators induce both β-oxidation activity and cytochrome P450 enzymes, an investigation was conducted to determine if C8 increases serum estradiol levels by stimulating aromatase activity (Liu, et al., 1996a). Fourteen days of treatment with up to 40 mg C8/kg/day produced dose-dependent increases in liver weights, serum estradiol, and hepatic aromatase activity. A significant linear correlation was established between estradiol and hepatic aromatase activity. *In vitro* experiments using cultured hepatocytes suggest that the increase in serum estradiol is at least partly

EID080827

MAB000029

due to a direct effect on the liver to increase synthesis of estradiol through induction of aromatase cytochrome P450 in the endoplasmic reticulum.

III.2. Investigation of C8s' Effect on Testicular Leydig Cells

Because C8 produced an increased incidence of testicular Leydig cell tumors in a 2-year feeding study in rats, and because C8 was negative in short-term tests for genotoxicity, a non-genotoxic (hormonal-mediated) mechanism for tumor formation was investigated. The studies summarized below support a hormonally-mediated mechanism of Leydig cell tumorigenesis: C8 produces an increase in hepatic aromatase activity, which elevates serum estradiol concentrations, which in turn modulates growth factors in the testis, which results in tumor formation.

III.2.a. Fourteen days of treatment with up to 50 mg C8/kg/day produced dose-dependent increases in hepatic β-oxidation activity, and serum concentrations of estradiol, and decreases in serum testosterone concentrations, body weights, and relative accessory sex organ weights in male rats (Cook, et al., 1992). Challenge experiments, using human chorionic gonadotropin (hCG), gondaotropin-releasing hormone (GnrH), or naloxone challenges, suggest that the decrease in testosterone may be due to a lesion at the level of the testes, due to a decrease in the conversion of 17α-hydroxyprogesterone to androstenedione.

III.2.b. Using *in vitro, in vivo* and *ex vivo* studies, C8 was examined for its ability to directly affect Leydig cells *in vitro* using isolated Leydig cells from untreated rats, and *ex vivo* using Leydig cells isolated from C8-treated rats. Additionally, the ability of C8 to affect testicular interstitial fluid hormone levels and induce aromatase activity was investigated (Biegel, et al., 1995). The *in vitro* studies demonstrated that C8 directly inhibits testosterone production, while the *ex vivo* studies demonstrated that this inhibition is reversible. In the *in vivo* study, serum and testicular interstitial fluid estradiol were increased and testicular interstitial fluid transforming growth factor α were increased. Additionally, hepatic aromatase activity was increased while aromatase activity levels were not affected in the testis, muscle, or fat. These data suggest that the increases in estradiol levels are primarily due to increases in aromatase activity.

III.2.c. Previous studies with C8 showed a direct effect on Leydig cells to alter steriodogenesis. It was therefore proposed that peroxisome proliferators, in general, may directly affect Leydig cell function to produce Leydig cell tumors. A study investigating whether several peroxisome proliferators (including C8), directly affect Leydig cell function *in vitro* was conducted. This study showed that peroxisome proliferators, as a class of compounds, directly modify the steroidogenic function of Leydig cells *in vitro*. This also suggests that compounds which directly affect Leydig cell function *in vitro* may also induce Leydig cell tumors *in vivo* (Liu, et al., 1996b).

MAB000030

EID080828

III.3. Investigation of C8s' Effect on the Pancreas

Several peroxisome proliferators have been shown to produce pancreatic acinar cell hyperplasia/adenocarcinomas in 2-year feeding studies, including C8. Therefore, *in vitro* and *in vivo* investigations of C8's (*in vitro* only) and Wyeth-14, 643's (a model peroxisome proliferator) mechanism of tumorigenesis in the pancreas were conducted. These mechanisms include cholecystokinin receptor agonism (CCK_A) trypsin inhibition, alterations in gut fat content, cholestasis and altered bile flow/composition. All of these mechanisms enhance pancreatic growth either by binding to the CCK_A receptor or by increasing plasma CCK levels. C8 did not bind directly to the CCK_A receptor and it failed to inhibit trypsin, a common mechanism for increasing plasma CCK levels. *In vivo* studies with Wyeth-14, 643 suggest that these peroxisome proliferators produce pancreatic tumors by cholestasis, which may be responsible for the decrease in bile acid output which contributes to the increase in plasma CCK levels. Therefore, for Wyeth-14, 643 (and perhaps C8), the pancreatic tumors may be secondary to hepatic cholestasis (Obourn, et al., 1997).

IV. CLINICAL REPORTS OF HUMAN EXPOSURE

IV.1.a. Health screening examinations were offered to employees of a 3M plant that produced C8, as well as other fluorochemicals. No health problems related to exposure were encountered among those examined. Additionally, no relationship was observed between deviations from normal laboratory test results and blood levels of organic fluorine (the liver enzyme SGGT was the most frequently encountered test result exceeding the normal range. C8 exposure levels ranged from 0.03 to 7.6 mg/m^3 (Ubel, et al., 1980).

IV.1.b. A study was made of Washington Works employees potentially exposed to C8. Results of blood chemistry testing (SGOT, LDH, AP, and bilirubin) indicated no conclusive evidence of an occupationally related health problem among workers exposed to C8 (Fayerweather, 1981).

IV.1.c. Although C8 is the major organofluorine compound found in humans, little information is available concerning human responses to C8 exposure. Therefore, a study was conducted among 115 workers exposed to C8 occupationally (serum fluorine levels varied between 0 and 26 ppm, with a mean of 3.3). In an examination of the cross-sectional associations between C8 and hepatic enzymes, lipoproteins, and cholesterol, there was no significant clinical hepatic toxicity of the C8 levels observed in this study (Gilliland and Mandel, 1996). Serum C8 levels were positively associated with estradiol and negatively associated with free testosterone and not associated with luteinizing hormone. The negative association between testosterone and C8 was stronger in older men. Thyroid stimulating hormone and C8 were positively associated. Prolactin and C8 were positively associated in moderate drinkers. The effect of adiposity on serum glutamyl oxaloacetic acid and glutamyl pyruvic transaminase decreased as C8 increased. The induction of gamma glutamyl transferase by alcohol was decreased as C8 increased. The effect of alcohol on HDL was reduced as C8 increased. A positive association

MAB000051

between hemoglobin, mean cellular volume, and leukocyte counts with C8 was observed. These results suggest that C8 affects male reproductive hormones and that the liver is not a significant site of toxicity in humans at the C8 levels observed in this study. However, C8 appears to modify hepatic and immune responses to xenobiotics (Gilliland and Mandel, 1993).

V. EPIDEMIOLOGY

V.1.a. A retrospective cohort mortality study was made of employees at a 3M plant where C8 and other fluorocompounds are manufactured. Records on 4218 employees were reviewed. Only those who worked for 6 months or more (3688 workers) were included in the mortality follow-up. Of the 180 known deaths, 177 death certificates were obtained. Overall the number of deaths was significantly less than expected. The observed-to-expected ratio for cancer deaths was 1.0 (Ubel, et al., 1980).

V.1.b. In a retrospective cohort mortality study, a relationship between mortality and employment at a plant where C8 and other fluorocompounds are manufactured were investigated (Gilliland and Mandel, 1993). The cohort consisted of 2788 male and 749 female workers employed between 1947 and 1983. The all-causes standardized mortality rate (SMR) was 0.75 for males and 0.77 for females. There was no significantly increased cause-specific SMR for men or women. The SMRs for prostate cancer were 2.03 in the exposed group and 0.58 in the not-exposed group. In the exposed group there were 4 observed and 2 expected deaths from prostate cancer. Among men, 10 years of employment in C8 production was associated with a significant 3-fold increase in prostate cancer mortality when compared to no employment in production. Given the small number of prostate cancer deaths and the natural history of the disease, the association between production work and prostate cancer must be viewed as hypothesis generating and not over interpreted. If the prostate cancer mortality excess is related to C8, the results of this study and other clinical studies suggest that C8 may increase prostate cancer mortality through endocrine alterations.

VI. DISCUSSION OF ENDPOINTS

VI.1. Discussion of Target Organs

The primary target organ for C8-induced toxicity is the liver in mice, rats, and dogs, regardless of route of exposure. The hepatotoxicity manifests as increased liver weights, hepatocellular hypertrophy, liver degeneration, increases in liver enzymes, necrosis of the liver, and induction of peroxisomes (rats and mice only). Many of these effects were demonstrated to be reversible when animals were provided with a recovery period. Evidence of hepatotoxicity was not evident in studies in monkeys or humans.

In contrast with the rodent, the target organs in the monkey were the

MAB000032

gastrointestinal tract and the reticuloendothelial system (Griffith and Long, 1980). While the liver does not appear to be a primary target organ in humans, exposure to C8 appears to modify the hepatic and immune response to xenobiotics (Gilliland and Mandel, 1996).

VI.2. Discussion of Differences in Species-Specific Sensitivities

The induction of peroxisome proliferation by xenobiotics is generally determined as an increase in the activities of certain peroxisome-specific enzymes, or as an increase in the numerical or volume density of peroxisomes in the affected organ. Peroxisome proliferation is associated with: increases in number and volume of peroxisomes; an increase in DNA synthesis and liver growth; and liver, Leydig cell, and pancreatic acinar cell tumors. The phenomenon of peroxisome proliferation is not uniform across all species. While rats and mice are particularly sensitive to this phenomenon, guinea pigs, cats, dogs and primates (including man), are predominantly non-responsive.

VI.3. Tumors Associated with C8 in the Rat

C8 has been demonstrated to be a peroxisome proliferator in the rat. C8 exposure in the rat was found to be associated with tumors in the liver, Leydig cell, and pancreatic acinar cell. Peroxisome proliferators, in general, were initially recognized to be associated with hepatocarcinogenesis in rats. However, more recently peroxisome proliferators have been associated with the induction of a triad of tumors in rats: liver, Leydig cell, and pancreatic acinar cell. Hyperplasia of these cell types is typically observed prior to, and along with, the occurrence of neoplasia. Several known peroxisome proliferators (clofibrate, HCFC-123, methylclofenapate, and Wyeth-14,643) are reported to induce this triad of tumors in rats. Hence, this tumor profile appears to be common phenomenon for at least a subset of compounds that are peroxisome proliferators.

VI.3.a. Significance of C8-Induced Rodent Tumor to Human Risk

VI.3.a.1. Liver Tumors

The abundance of data indicates that there is a hepatocarcinogenic hazard of peroxisome proliferators to responsive species (rats and mice) in chronic studies, whereas the carcinogenic hazard to non-responding species, such as humans, is clearly questionable. The epidemiology data, albeit limited, strongly support that the relevance of the hepatocarcinogenic effects of C8 and other peroxisome proliferators for human hazard assessment should be considered negligible.

VI.3.a.2. Leydig Cell Tumors

Leydig cell hyperplasia and adenomas are commonly observed in laboratory rats. The incidence of spontaneous Leydig cell adenomas in Crl:CD®BR rats ranges from approximately 0-12% by 2 years of age, and ranges from approximately 64 -100 % in F344 rats. In contrast, the rate in humans has been reported to be approximately 0.4 per

EID080831

MAB000033

million (0.00004%). Although a direct comparison is somewhat tenuous, the data suggest a substantial difference in the susceptibility of rodents and humans to Leydig cell tumorigenesis. This is supported by epidemiology data from compounds that clearly produce Leydig cell tumors in rodent studies but are commonly ingested by humans and are not associated with Leydig cell tumorigenesis in humans.

C8 and other peroxisome proliferators do not produce increases in peroxisomes in Leydig cells and are hypothesized to produce these tumors via a different mechanism than the liver tumors. The mechanism of tumorigenesis is not completely understood, and therefore relevance to humans can not be completely ruled out. However, it is known that non-genotoxic compounds (such as C8) produce Leydig cell tumors by altering the endocrine system. Therefore, a threshold for tumorigenesis is expected. If this is the case, use of a margin of safety approach is appropriate for the quantitative dose-response assessment. It is important to consider the slope of the dose-response at the low end of the observed range in determining an acceptable margin of safety.

VI.3.a.3. Pancreatic Acinar Cell Tumors

C8 and other peroxisome proliferators do not produce increases in peroxisomes in the pancreas and are hypothesized to produce these tumors via a different mechanism than the liver tumors. The mechanism of tumorigenesis is not understood, and therefore relevance to humans can not be completely ruled out. However there is a growing weight of evidence that the pancreatic acinar cell tumors are hormonally mediated, therefore they should be treated similarly to peroxisome-proliferator-induced Leydig cell tumors.

VII. SUMMARY

C8 has moderate acute oral toxicity with LD_{50}'s ranging from 178 mg/kg in male guinea pigs to 680mg/kg in adult male rats. An aqueous paste of C8 produced mild to moderate dermal irritation in rabbits and clinical signs of toxicity were observed at doses as low as 1000 mg/kg. Instillation of solid C8 into the rabbit eye produced moderate corneal opacity, iritis, and conjunctivitis. These ocular effects gradually receded. C8 has high acute inhalation toxicity with a 4-hour ALC of 0.8 mg/L in the rat. Subchronic inhalation exposure to C8 produced reversible liver effects at concentrations as low as 8 mg/m^3 (measured as 7.6 mg/m^3). Oral and skin absorption subchronic studies confirmed the hepatotoxicity of C8 in the rat. In chronic feeding studies in rats, C8 produced an increased incidence of tumors in the liver, pancreas, and testis. C8 was found not to be a developmental toxic or mutagenic in several tests for mutagenicity.

The relevance to human health of tumors induced by peroxisome proliferators in rodents has been the focus of several investigators. Regarding the liver, there is a strong association and probable link between peroxisome-proliferator-induced liver growth and the subsequent development of rodent liver tumors. A combination of *in vivo* and *in vitro* studies as well as epidemiology data, has led several investigators to conclude that humans appear to be insensitive or unresponsive to peroxisome-proliferator-induced hepatic effects, and therefore these nongenotoxic agents pose little or no

MAB000034

EID080832

hepatocarcinogenic hazard to humans. Evidence is also accumulating that the initiating events, which lead to the development of Leydig cell and pancreatic acinar cell tumors are from changes in the liver. These hepatic changes appear to alter the hormonal control of the testis and pancreas. Although these relationships need to be confirmed, it is likely that these extrahepatic tumors pose little or no carcinogenic hazard to humans. Additionally, programs monitoring the health of C8-exposed workers and retrospective cohort studies of workers exposed to C8 provide no evidence of an association between C8 exposure and adverse human health effects.

Of primary concern in humans is the slow clearance of C8 from human blood, the opportunity for exposure in the work place, and the moderate-high acute toxicity, regardless of route of exposure.

MAB000035

EID080833

REFERENCES

DuPont Co., Haskell Laboratory Report Numbers(HL):

55-61, Acute Oral Test.1961, H. Sherman.

56-61, Subacute Feeding Study.1961, H. Sherman.

123-65, Effects of Fluorocarbon Disbursing Agents on the Livers of Rats and Dogs.1965, J. R. Barnes and H. Sherman.

124-65, Two-Week Feeding Study.1965, H. Sherman.

128-68, Acute Oral Test.1963, S. B. Fretz.

160-69, Acute Inhalation Dust Toxicity.1969, F. O. Tayfun and R. S. Waritz.

253-79, Inhalation Subacute.1979, G. T. Hall.

635-79, Eye Irritation Test in Rabbits.1979, D. F. Edwards.

636-79, Skin Irritation Test on Rabbits.1979, D. F. Edwards.

659-79, Skin Absorption LD_{50} - Rabbit and Rat.1979, D. F. Edwards.

589-80, Rat Skin Absorption Subacute Study with FC-143.1980, L. S. Silber.

682-80, Skin Absorption LD_{50}-Female Rats.1980, J. E. Henry.

205-81, Subacute Inhalation Toxicity of Pentadecafluorooctanoic Acid, Ammonium Salt.1981, S. D. Nash and M. A. Britelli.

291-81, Oral LD_{50} Test in Guinea Pigs.1981, J. A. Hall.

295-81, Oral LD_{50} Test in Rats.1981, J. A. Hall.

329-81, Oral LD_{50} Test in Mice.1981, J. A. Hall.

560-81, Mouse Feeding Study - 14 Day.1981, L. S. Ford.

565-81, Acute Oral Toxicity Comparison Test in Rats.1981, L. Hinckle.

567-81, Oral LD_{50} Test in Rats.1981, J. E. Henry.

593-81, Ammonium Perfluorooctanoate Blood Levels in the Female Rat.1981, Kennedy, G. L.

MAB000036

600-81, Liver Weight Comparison in Castrated and Ovariectomized Rats vs. Normal Rats.1981, L. Hinckle.

537-82, Fourteen-Day Feeding Study in Male and Female Mice.1982, J. E. Henry.

788-82, Acute Oral Toxicity of FC-143 as a Function of Age.1983, L. Hinckle.

138-83, C-8 Liver Weights in Rats and Mice: Oral Administration to Males and Females.1983, C. N. Wyle.

DuPont MR 5686, Investigation of Hormonally-Mediated Mechanisms for Xenobiotic-Induced Leydig Cell Tumors in Male Rats. (1989).

3M Co. Reports:

Personal communication, J. Long to P.J. Gort, January 8, 1979.

3M Report M-601 (1981).

3M Report 0681TR0110, 1981.

3M Product Toxicity Sheet, May 24, 1996.

Biosearch, Inc. Report No. T1395, March 4, 1976.

Corning Hazleton, 17388-0-455, May 16, 1996.

Corning Hazleton, 17388-0-437, April 25, 1996.

Hazleton Laboratory America, Inc. 2-6-87 (Submitted to EPA, TSCA 8eCAP Submission, Fiche 536984).

Litton Bionetics; LBI Project 20838, February 1, 1978.
Riker Laboratory Drug Metabolism Report 1-20 (1980).

Riker Laboratories, Report 09790AB0485, March 15, 1981.

Riker Laboratory 0281CR0012, April 1981–May 1983.

University of Minnesota Environ. Path Lab, T2942, April 9, 1981.

Published References:

Biegel, L. B., R. C. M. Liu, M. E. Hurtt, and J. C. Cook (1995). Effects of ammonium perfluorooctanoate on Leydig cell function: *In vitro*, *in vivo*, and *ex vivo* studies. Toxicol. Appl. Pharmacol. **134**: 18-25.

MAB000037

Cook, J. C., M. E. Hurtt, S. R. Frame, and L. B. Biegel (1994). Mechanisms of extrahepatic tumor induction by peroxisome proliferators in Crl:CD®BR (CD) rats. Toxicologist (abstract) **14**.

Cook, J. C., S. M. Murray, S. R. Frame, and M. E. Hurtt (1992). Induction of Leydig cell adenomas by ammonium perfluorooctanoate: A possible endocrine-related mechanism. Toxicol. Appl. Pharmacol. **113**: 209-217.

Fayerweather, W. E., Liver study of Washington Works employees exposed to C8: Results of blood biochemistry testing. DuPont, Epidemiology Division of Corporate Medical, January 15, 1981.

Gilliland, F. D. and J. S. Mandel (1993). Mortality among employees of a perfluorooctanoic acid production plant. J. Occup. Med. **35**: 950-954.

Gilliland, F. D. and J. S. Mandel (1996). Serum perfluorooctanoic acid and hepatic enzymes, lipoproteins, and cholesterol: A study of occupationally exposed men. American Journal of Industrial Medicine **29**: 560-568.

Griffith, F. D. and J. E. Long (1980). Animal toxicity studies with ammonium perfluorooctanoate. Am. Ind. Hyg. Assoc. J. **41**: 576-583.

Johnson, J. D., S. J. Gibson, and R. E. Ober (1984). Cholestyramine-enhanced fecal elimination of carbon-14 in rats after administration of ammonium [14C]perfluorooctanoate or potassium [14C]perfluorooctanesulfonate. Fundam. Appl. Toxicol. **4**: 972-976.

Kennedy, Jr., G.L. (1985). Dermal toxicity of ammonium perfluorooctanoate. Toxicol. Appl. Pharmacol. **81**: 348-355.

Kennedy, Jr., G.L., G. T. Hall, M. R. Brittelli, J. R. Barnes, and H. C. Chen (1986). Inhalation Toxicity of Ammonium Perfluorooctanoate. Fd. Chem. Toxic. **24**(12): 1325-1329.

Kennedy Jr., G. L. (1987). Increase in mouse liver weight following feeding of ammonium perfluorooctanoate and related fluorochemicals. Toxicol. Letters **39**: 295-300.

Liu, R. C. M., C. Hahn, and M. E. Hurtt (1996a). The direct effect of hepatic peroxisome proliferators on rat Leydig cell function *in vitro*. Fundam. Appl. Toxicol. **30**: 102-108.

Liu, R. C. M., M. E. Hurtt, J. C. Cook, and L. B. Biegel (1996b). Effect of the peroxisome proliferator, ammonium perfluorooctanoate (C8), on hepatic aromatase activity in adult male Crl:CDBR (CD) rats. Fundam. Appl. Toxicol. **30**: 220-228.

EID080836

MAB000038

Obourn, J. D., S. R. Frame, R. H. Bell Jr., D. S. Longnecker, G. S. Elliott, and J. C. Cook (1997). Mechanisms for the pancreatic oncogenic effects of the peroxisome proliferator Wyeth-14, 643. Toxicology and Applied Pharmacology 145: 425-436.

Pastoor, T. P., K. P. Lee, M. A. Perri, and P. J. Gillies (1987). Biochemical and morphological studies of ammonium perfluorooctanoate-induced hepatomegaly and peroxisome proliferation. Experimental and Molecular Pathology 47: 98-109.

Perkins, R. G. (1992). Investigation of ammonium perfluorooctanoate effect on hormone levels and peroxisomal proliferation in the rat. The Toxicologist 12(1): 52.

Staples, R. E., B. A. Burgess, and W. D. Kerns (1984). The embryo-fetal toxicity and teratogenic potential of ammonium perfluorooctanoate (APFO) in the rat. Fundamental and Applied Toxicology 4: 429-440.

Ubel, F. A., S. D. Sorenson, and D. E. Roach (1980). Health status of plant workers exposed to fluorochemicals-a preliminary report. Am. Ind. Hyg. Assoc. J. 41(8): 584-589.

MAB000039

EID080837

BOGDANMS

\\server\name

PSCRIPT Page Separator

MAB000040

EID080838

DRAFT

Proposal to Conduct a
General Human Health and Environmental Effects
Risk Analysis on C-8

Purpose: The purpose of this project is to evaluate the risks to human health and the environment from exposure to C-8 during manufacture, transport, product use, and disposal of C-8. The analysis will be conducted in a fashion that will provide semi-quantitative estimates of risks so that exposures yielding the highest risks can be identified and recommendations on reducing these risks can be developed. Risks from manufacture, transport and product use will developed in a way that will facilitate future comparisons of risks estimated for potential C-8 alternatives. The project will be conducted in three parts. The first two will be conducted in parallel, in which human health and ecological risks will be characterized. The final part will develop conclusions on exposures that contribute the highest risks so that recommendations for risk management strategies and alternatives can be developed. The project is estimated to take 12 months to complete from the time of initiation. The Exposure analyses listed below will require collaboration with appropriate plant personnel. *(The dates presented assume a Feb. 1, 1997 SBU approval date.)*

Scope:

		Time Line	Est. Cost($)
I.	Human Health Risk		
A.	Hazard Identification	4/18/97	8000

Hazards to human health will be reviewed and summarized in this section. The critical toxicity endpoints of relevance to human health risk will be identified and potential dosimeters to be used for interspecies extrapolation of risk will be discussed. The Haskell toxicity summary will be updated as part of this task.

B. Dose-Response Analysis — 9/30/97 — 43,200

The dose-response characteristics of C-8 will be evaluated. This may include conducting benchmark dose analyses to identify no-observed adverse effect levels where necessary. Appropriate dosimeters for interspecies extrapolation will also be developed based on the likely mode of action. The pharmacokinetics of C-8 will also be reviewed. If possible, rudimentary physiologically-based pharmacokinetics approaches will be developed to facilitate interspecies extrapolation of risk. Risks vs. dose relationships will be developed in this phase

C. Exposure Analysis — 9/30/97 — 16,000

Reasonable exposure scenarios for C-8 will be developed. This are likely to include airborne, drinking water, dermal, and other oral ingestion pathways. Intake rates and durations of exposure will be developed. Haskell will work with an assigned person(s) from the plant site to help characterize these exposure pathways for manufacturing, transport, product use, and waste disposal operations. The business will provide Haskell with data on concentrations of C-8 in the affected media (air, water, soil). These data will be tabulated. Monte Carlo techniques may be used to calculate expected upper confidence limits for these exposures, depending the availability of data. The cost associated with this task include only Haskell personnel time.

D. Risk Characterization — 12/15/97 — 16,000

Risks will be summarized according to the major routes of exposure (air, water, dermal, other oral) for each C-8 application (manufacture, transport, product use, disposal). The risks will be characterized by comparing the likely exposure concentrations to the dose-response relationship. This method is generally referred

MAB000041

to as a Margin of Exposure. The characterization will provide the risk manager with information that will help identify the operations and exposure pathways that present the highest risk. The characterizations will also enable future comparisons to be made of potential risks posed by C-8 alternatives.

II Ecological Effects
- A. Hazard Identification
- B. Dose-Response Analysis
- C. Exposure Analysis
- D. Risk Characterization

III. Recommendations on Risk Management Strategies and Alternatives

12/15/97 4800

This section will evaluate collectively the risks identified to human health and ecological receptors. Based on these analyses, recommendations will be made as to which operations could be targeted to reduce the largest risks for the least cost. This will be a very subjective exercise (narrative) and will require some input from the plant people.

MAB000042