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HAZARD ASSESSMENT OF PERFLUOROOCTANOIC ACID AND ITS SALTS

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PREFACE

This is a preliminary assessment of the potential hazards to human health and the environment associated with exposure to perfluorooctanoic acid (PFOA) and its salts. The majority of the toxicology information is for ammonium perfluorooctanoic acid (APFO). This assessment includes a review of the studies that were available as of July 2001.

A two-generation reproductive toxicity study of APFO is currently being conducted and will be available in the spring of 2002. Effects were observed in a two-generation reproductive toxicity study of a related compound, perfluorooctane sulfonate. The results of the APFO study will be important to determine whether similar effects are observed.

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EXECUTIVE SUMMARY

Introduction

Perfluorooctanoic acid (PFOA) and its salts are fully fluorinated organic compounds that can be produced synthetically or through the degradation or metabolism of other fluorochemical products. PFOA is primarily used as a reactive intermediate, while its salts are used as processing aids in the production of fluoropolymers and fluoroelastomers and in other surfactant uses. In recent years, less than 600 metric tons per year of PFOA and its salts have been manufactured in the United States or imported. Most of the toxicology studies have been conducted with the ammonium salt of perfluorooctanoic acid, which is referred to as APFO in this report.

Environmental Fate and Effects

PFOA is persistent in the environment. It has very low volatility and vapor pressure. It does not hydrolyze, photolyze or biodegrade under environmental conditions.

Several wildlife species have been sampled around the world to determine levels of PFOA. PFOA has rarely been found in fish sampled from the U.S., certain European countries, the North Pacific Ocean and Antarctic locations, or in fish-eating bird samples collected from the U.S., including Midway atoll, the Baltic and Mediterranean Seas, and Japanese and Korean coasts. PFOA was found in a few mink livers from Massachusetts at a concentration range of <18 to 108 ng/g, dry wt., but not found in mink from Louisiana, South Carolina and Illinois. PFOA concentrations in river otter livers from Washington and Oregon States were less than the quantification limit of 36 ng/g, wet wt. PFOA was not detected at quantifiable concentrations in oysters collected in the Chesapeake Bay and Gulf of Mexico of the U.S. coast.

The concentrations of PFOA in surface water, sediments, clams, and fish collected from two locations upstream and five locations downstream of the 3M manufacturing facility at Decatur AL have been determined. Of the five downstream sampling locations, the two closest to the facility had PFOA surface water concentrations significantly greater than the two upstream sites (means of 1900ug/L and 1024 ug/L); the nearest three locations had sediment concentrations significantly greater than the upstream sites (wet wt. means 1855 ug/kg, 892 ug/kg, 238 ug/kg). The average fish whole body PFOA concentration for the two upstream locations was 11.7 ug/kg (wet wt.), while that for the five downstream locations was 106.4 ug/kg. The average PFOA concentration in clams at the two upstream locations was 4.38 ug/kg, while the average for the five downstream locations was 8.42 ug/kg.

Based on available data, APFO does not appear to bioaccumulate in fish. In a study of fathead minnows, the calculated BCF for APFO was 1.8.

Several species were tested to assess the acute toxicity of APFO; these included the fathead minnow (*Pimephales promelas*), bluegill sunfish (*Lepomis machrochirus*), water flea (*Daphnia magna*), and a green algae (*Selenastrum capricornutum*). Comparisons of the different studies are problematic for several reasons. The studies were conducted with different test substances. Generally the ammonium salt or the tetrabutylammonium salt was tested. Purity of the test material is a major concern and was not sufficiently characterized in these tests. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 27 to 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. Finally, only nominal test chemical concentrations were reported; the actual concentrations were not reported.

Twelve tests were conducted with fathead minnows; 96-h LC50 values (based on mortality) ranged from 70 to 843 mg/L. It is unclear why this range is so wide. Assuming these studies are valid, and due to the limitations discussed above, these toxicity values indicate low toxicity. The two acute values for bluegill sunfish also indicate low toxicity (96-h LC50s of >420, and 569 mg/L).

Nine acute tests were conducted with daphnids and 48-h EC50 values (based on immobilization) ranged from 39 to >1000 mg/L. The lower values are indicative of moderate toxicity, but the wide range makes interpretation difficult.

Seven tests were conducted with green algae; 96-h EC50 values (based on growth rate, cell density, cell counts, and dry weights) ranged from 1.2 to >666 mg/L (the Er50 cell density value of 1,000 mg/L is excluded from this discussion). The lower value indicates high to moderate toxicity, based on the acute criteria. The lower value would also be indicative of moderate toxicity, based on the chronic moderate criterion (.0.1≤10 mg/L). A 14-d EC50 value of 43 mg/L, based on cell counts, for green algae was also calculated in one study. This is indicative of low chronic toxicity, based on the chronic criterion (10 mg/L). Green algae appeared to be the most sensitive test species in the 44% APFO test sample, daphnids were the next most sensitive, and fathead minnows were the least sensitive.

Human Health Effects and Biomonitoring

Little information is available concerning the pharmacokinetics of APFO in humans. A preliminary study of retired workers suggests simply that the serum half-life is between 1 and 3.5 years. These data provide evidence of the potential to bioaccumulate PFOA in humans. In addition, this study provides preliminary evidence that the serum half-life may be longer in females than in males.

Animal studies have shown that APFO is well absorbed following oral and inhalation exposure, and to a lesser extent following dermal exposure. In rats and dogs, there are major gender differences in the distribution and elimination of APFO. APFO distributes primarily to the liver,

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plasma, and kidney, and to a lesser extent, other tissues of the body including the testis and ovary. It does not partition to the lipid fraction or adipose tissue. APFO binds to macromolecules in the tissues listed above. APFO is not metabolized and there is evidence of enterohepatic circulation of the compound. The urine is the major route of excretion of APFO in the female rat, while the urine and feces are both major routes of excretion of APFO in male rats. In female rats, the half-life is 24 h in the serum and 60 h in the liver; in male rats, the half-life is 105 h in the serum and 210 h in the liver. In beagle dogs, the plasma half-life is 254 h in females and 507 h in males. In rats, the elimination half-life is one day in females and 15 days in males. Female rats appear to have a secretory mechanism that rapidly eliminates APFO; this secretory mechanism is either lacking or relatively inactive in males. Other studies in rats have shown that testosterone exerts an inhibitory effect on renal excretion of APFO. Hormonal changes during pregnancy do not appear to change the rate of elimination in rats. The gender difference observed in rats and dogs has not been observed in primates and humans.

There are limited data on PFOA serum levels in workers and the general population. Occupational data from plants in the U.S. and Belgium that manufacture or use PFOA indicate that mean serum levels in workers range from 0.84 to 6.4 ppm. The highest level reported in a worker in 1997 was 81.3 ppm. In non-occupational populations, serum PFOA levels were much lower. In both pooled blood bank samples and in individual samples in both adults and children, mean PFOA levels ranged from 3 to 17 ppb. The highest serum PFOA level reported was in a sample from a child (56 ppb).

Epidemiological studies on the effects of PFOA in humans have been conducted on workers. Two mortality studies, as well as studies examining effects on the liver, pancreas, endocrine system, and lipid metabolism, have been conducted to date. In addition, a morbidity study was also recently submitted.

A retrospective cohort mortality study demonstrated a weak association with PFOA exposure and prostate cancer. A statistically significant association was observed in prostate cancer mortality as length of employment increased. This result was not observed in a recent update to the study; however, the results cannot be directly compared because the exposure categories were modified in the update. In a morbidity study, workers with the highest PFOA exposures for the longest durations sought care more often for prostate cancer treatment than workers with lower exposures.

Another study reported an increase in estradiol levels in workers with the highest PFOA serum levels; however, none of the other hormone levels analyzed indicated any adverse effects. Some of the same employees who participated in the hormone study also were included in a study of cholecystokinin (CCK) levels in employees. No positive association was noted between CCK values and PFOA. The other available study examined cholesterol and other serum components in workers. There did not appear to be any significant differences among workers of different exposure levels, except among obese workers (aspartate amino transferase and alanine amino transferase). However, PFOA was not measured directly, but indirectly as total serum fluorine.

There are many limitations to these studies, but most notably the small number of workers with PFOA serum levels greater than 10 ppm. Therefore, all of these results must be interpreted carefully.

In acute toxicity studies in animals, the oral LD50 values for CD rats were >500 mg/kg for males and 250-500 mg/kg for females, and <1000 mg/kg for male and female Wistar rats. There was no mortality following inhalation exposure of 18.6 mg/L for one hour in rats. The dermal LD50 in rabbits was determined to be greater than 2000 mg/kg. APFO is a primary ocular irritant in rabbits, while the data regarding potential skin irritancy are conflicting.

APFO is not mutagenic. APFO did not induce mutation in either S. typhimurium or E. coli when tested either with or without mammalian activation. APFO did not induce chromosomal aberrations *in vitro* in human lymphocytes when tested with and without metabolic activation up to cytotoxic concentrations. APFO was tested twice for its ability to induce chromosomal aberrations in CHO cells *in vitro*. In the first assay, APFO induced both chromosomal aberrations and polyploidy in both the presence and absence of metabolic activation. In the second assay, no significant increases in chromosomal aberrations were observed without activation. However, when tested with metabolic activation, APFO induced significant increases in chromosomal aberrations and in polyploidy. APFO was negative in a cell transformation assay in C_3H $10T_{½}$ mouse embryo fibroblasts and in the *in vivo* mouse micronucleus assay.

Subchronic studies in rats and mice with 28 and 90-days of exposure have demonstrated that the liver is the primary target organ and that males are far more sensitive than females. Dietary exposure to APFO for 90 days resulted in significant increases in liver weight and hepatocellular hypertrophy in female rats at 1000 ppm (76.5 mg/kg/day) and in male rats at doses as low as 100 ppm (5 mg/kg/day). Analyses of serum and liver levels of APFO showed a marked gender difference that accounts for the difference in sensitivity. In a 90-day study with rhesus monkeys, exposure to doses of 30 mg/kg/day or higher resulted in death, lipid depletion in the adrenals, hypocellularity of the bone marrow, and moderate atrophy of the lymphoid follicles in the spleen and lymph nodes. Unlike rodent studies, analyses of the serum and liver levels did not reveal a gender difference in monkeys, but the sample size was very small (N=2). Chronic dietary exposure of rats to 300 ppm APFO (14.2 and 16.1 mg/kg/day for males and females, respectively) for 2 years resulted in increased liver and kidney weights, hematological effects and liver lesions in males and females. In addition, testicular masses were observed in males at 300 ppm and ovarian tubular hyperplasia was observed in females after exposure to 30 ppm (1.6 mg/kg/day), the lowest dose tested.

Prenatal developmental toxicity studies in rats resulted in death and reduced body weight in dams exposed to oral doses of 100 mg/kg/day or by inhalation to 25 mg/m^3 APFO. There was no evidence of developmental toxicity after oral exposure to doses as high as 150 mg/kg/day, while inhalation exposure to 25 mg/m^3 resulted in reduced fetal body weights. In a rabbit oral developmental toxicity study there was a significant increase in skeletal variations after exposure

to 50 mg/kg/day APFO. There was no evidence of maternal toxicity at 50 mg/kg/day, the highest dose tested.

A two-generation reproductive toxicity study is currently being conducted. A two-generation reproductive toxicity study of PFOS showed high mortality of F1 pups at doses as low as 1.6 mg/kg/day. The results of the APFO study will be important to determine whether a similar effect is observed.

Carcinogenicity studies in Sprague-Dawley (CD) rats show that APFO is weakly carcinogenic, inducing Leydig cell adenomas in the male rats and mammary fibroadenomas in the females following dietary exposure to 300 ppm for 2 years (equivalent to 14.2 mg/kg/day in males and 16.1 mg/kg/day in females). The compound (at 300 ppm) has also been reported to be carcinogenic toward the liver and pancreas of male CD rats.

The mechanism(s) of APFO tumorigenesis is not clearly understood. Available data indicate that the induction of tumors by APFO is due to a non-genotoxic mechanism, involving activation of receptors and perturbations of the endocrine system. The liver carcinogenicity/toxicity of APFO appear to be related to induction of peroxisome proliferation following binding to the peroxisome proliferation activation receptor α (PPAR α) in the liver. Available data suggest that the induction of Leydig cell tumors (LCT) and mammary gland neoplasms by APFO may be due to hormonal imbalance resulting from activation of the PPARα and induction of the cytochrome P450 enzyme, aromatase. Preliminary data suggest that the pancreatic acinar cell tumors are related to an increase in serum level of the growth factor, cholecystokinin.

As the mechanisms of carcinogenic action of APFO have not been fully elucidated, it is assumed that the tumors induced in rats are relevant to humans. Review of available mechanistic data of other drugs and chemicals that induce LCT in animals has led a workshop panel to conclude that all but two modes of induction of the luteinizing hormone (LH), "dopamine agonism" and "GnRH agonism", are considered to be relevant to humans, and that the possibility of induction of Leydig cell adenoma in humans by specific agents with other modes of action cannot be ruled out despite the rarity of LCT in humans. At present, there is no evidence that the induction of LCT by APFO is via the "dopamine agonism" or "GnRH agonism" mode of action. It is recognized that there are quantitative differences in certain biological parameters between rats and humans. However, the principal cell control mechanisms appear similar, and the difference in carcinogenic response is probably quantitative. As binding to the PPARα appears to be the critical event leading to hormonal imbalance and APFO tumorigenesis, and the level of PPARα in human livers is lower than that in rodent liver, it appears that humans may be less sensitive than rodents in the development of LCT, mammary gland tumors, or liver neoplasms.

1.0 Chemical Identity

Chemical Name: Perfluorooctanoic Acid
Molecular formula: C8 H F15 O2

Structural formula: F-CF2-CF2-CF2-CF2-CF2-CF2-CF2-C(=O)-X,

The free acid and some common derivatives have the following CAS numbers:
The perfluorooctanoate anion does not have a specific CAS number.

Free Acid	(X = OM+; M = H)	[335-67-1]
Ammonium Salt	(X = OM+; M = NH4)	[3825-26-1]
Sodium Salt	(X = OM+; M = Na)	[335-95-5]
Potassium Salt	(X = OM+; M = K)	[2395-00-8]
Silver Salt	(X = OM+; M = Ag)	[335-93-3]
Acid Fluoride	(X = F)	[335-66-0]
Methyl Ester	(X = CH3)	[376-27-2]
Ethyl Ester	(X = CH2-CH3)	[3108-24-5]

Synonyms: 1-Octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-
PFOA

1.1 Physicochemical Properties

For this report, perfluorooctanoic acid is consistently referred to as PFOA. Most of the toxicology studies have been conducted with the ammonium salt of perfluorooctanoic acid, which will be referred to as APFO in this report. PFOA is a completely fluorinated organic acid. The typical structure has a linear chain of eight carbon atoms produced by the telomerization of tetrafluoroethylene. The physical chemical properties noted below are for the free acid, unless otherwise stated. The data for the free acid, pentadecafluorooctanoic acid [335-67-1], is the most complete. The reported vapor pressure of 10 mm Hg appears high, but is consistent with other perfluorinated compounds with similar boiling points. The free acid is expected to completely dissociate in water.

Determination of the vapor pressure of APFO is problematic. For APFO, the recently reported vapor pressure of < 1 x 10E-5 (3M Environmental Laboratory, 1993) seems too low for a material that sublimes as the ammonium salt. This study measured the water solubility of APFO to be > 10%. It was noted in an earlier study that concentrations of 20 g/L "gelled" (3M

Company, 1979). The partition coefficient was reported in these early studies of 5. Another calculated value, -0.9, might not be accurate due to the method used (Hansch and Leo 1979). The formation of an emulsified layer between the octanol and water surface interface would make determination of log P difficult.

The available physicochemical properties for the PFOA free acid are:

MW: 414 (Beilstein, 1975)
MP: 45 - 50 C (Beilstein, 1975)
BP: 189 - 192 C / 736 mm Hg (Beilstein, 1975)
VP: 10 mm Hg @ 25 C (approx.) (Exfluor MSDS)
Sol. - Water: 3.4 g/L (telomeric [mp = 34 C ref. 0.01 - 0.02 mol/L ~4 - 8 g/L) (MSDS from Merck, Fischer, and Chinameilan Internet sites)
pKa: 2.5 (USEPA AR-226 473)
pH (1g/L): 2.6 (MSDS Merck)

Due to the surface-active properties of PFOA, and the test protocol for the OECD method, PFOA is anticipated to form multiple layers in octanol/water, much like those observed for PFOS. Therefore, an n-octanol/water partition coefficient cannot be determined. Water solubility has been reported for PFOA, but it is unclear whether these values are for a microdispersion of micelles, rather than true solubility. Several reports note that PFOA salts self-associate as micelles at higher concentrations. (Simister, 1992; Calfours, 1985; Edwards, 1997). In aqueous solutions, micelles partition between the air / water interface on the surface.

Decomposition of different salts produces perfluoroheptene (loss of metal fluoride and carbon dioxide). This occurs at 320°C for the sodium salt and at 250-290°C (Beilstein 1975). The ammonium salt sublimes at 130°C (USEPA AR-226 473).

The physicochemical properties of PFOA and its derivatives are summarized in Table 1.

Table 1. Reported Physicochemical Properties

Compound	CAS REG #	MP	BP	VP	Sol.-H2O	Log P
Rf-C(=O)F	335-64-8		131 C			
Rf-CO2H	335-67-1	55 C	189 C	10 mm Hg	3.4 g/L	
Rf-CO2-NH4+	3825-26-1	130 C	sublimes	1 x 10E-5	20 g/L gels	< 5
Rf-C(=O)OMe	376-27-2		159 C			
pH (1 g free acid /L Water) = 2.6			Free acid pKa is approximately 0.6			
Sodium or Silver salts of PFOA decompose above 250 C to generate perfluoroolefins.						

2.0 Production of PFOA and its Salts

PFOA is manufactured by two major alternative processes: 1) the Simons Electro-Chemical Fluorination (ECF) process or 2) the telomerization process.

In the ECF process, an electric current is passed through a solution of anhydrous hydrogen fluoride and an organic feedstock of 1-octanesulfonyl fluoride. The ECF process replaces the carbon-hydrogen bonds on molecules of the organic feedstock with carbon-fluorine bonds. Perfluorination occurs when all the carbon-hydrogen bonds are replaced with carbon-fluorine bonds. The ECF process yields between 30-45 percent straight chain (normal) perfluorooctanesulfonyl fluoride (POSF), along with a variable mixture of byproducts and impurities. The output of the ECF process is not a pure chemical, but is instead a mixture of isomers and homologues including higher and lower straight-chain homologues; branched-chain perfluoroalkyl fluorides of various chain lengths; straight chain, branched, and cyclic perfluoroalkanes and ethers; and other byproducts (3M Company, 2000a). After disposal or recovery of some of the byproducts and impurities, POSF is base hydrolyzed in batch reactors to yield PFOA. The PFOA salts are synthesized by base neutralization of the acid to the salt in a separate reactor (3M Company, 2000b).

In the telomerization process, tetrafluoroethylene is reacted with other fluorine-bearing chemicals to yield fluorinated carboxylic acids. This process yields pure straight-chain acids with an even number of carbon atoms. Distillation can be used to obtain pure components (ECT, 1994). Commercial products manufactured through the telomerization process are generally mixtures of perfluorinated compounds with even carbon numbers (Renner, 2001).

3M Company is the largest manufacturer and importer of PFOA and its salts in the United States. 3M has characterized its manufacture of PFOA and its ammonium and sodium salts in 1997 at less than 500,000 kg per year, and its importation at less than 100,000 kg (3M Company, 2000a). These figures may overstate the total production volume of PFOA since the vast majority of

PFOA is consumed in the manufacture of the ammonium or sodium salts. More precise production volumes of PFOA and the ammonium and sodium salts have been reported to USEPA by 3M, but have been claimed as TSCA confidential business information, preventing disclosure in this report.

Industry participants have characterized 3M as the dominant global producer of PFOA-related chemicals, manufacturing approximately 85 percent or more of total worldwide volumes of the ammonium salt of PFOA (FMG, 2001). USEPA has not located information that would contradict this claim. Current production volume information for manufacturers other than 3M has not been provided by industry, nor is it available in USEPA's Chemical Update System (which contains information on non-polymeric organic chemicals manufactured in the United States or imported in volumes above 4,525 kg). Furthermore, there is no information on the total cumulative production volumes of PFOA since initial commercialization.

Since 1985, USEPA has received a total of approximately 25 notifications for PFOA-related chemicals that were not previously on the TSCA Chemical Inventory. Most of these notifications were from companies other than 3M. In most cases, the notifications qualified for the Low Volume Exemption for new chemicals with a production volume less than 10 metric tons per year.

In terms of on-going production, 3M has not committed publicly to a complete phase-out of PFOA and PFOA-related chemicals as it has for PFOS and PFOS-related chemicals. However, 3M has indicated that it is phasing out certain FLUORAD Brand specialty materials that contain PFOA and its salts such as FC-26, FC-118 and FC-143, FX-1001 and others (3M Company, 2000c).

Aside from the United States, OECD Member countries that reportedly have production capacity include France, Germany, Italy, and Japan. There may also be some production in non-OECD countries such as China. Following are companies that may manufacture PFOA and its salts (3M Company, 2000b; Directory of World Chemical Producers, 1998; Dynax, 2000; Renner, 2001; SEMI, 2001):

OECD

- 3M Company (United States)
- DuPont (United States)
- Exfluor Research Corporation (United States)
- PCR Inc. (United States)
- Atofina (France)
- Ciba Specialty Chemicals (Germany)
- Clariant (Germany)
- Dyneon (Germany)
- Hoechst Aktiengesellschaft (Germany)

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- EniChem Synthesis S.p.A. (Italy)
- Miteni S.p.A. (Italy)
- Asahi Glass (Japan)
- Daikin (Japan)
- Dainippon (Japan)
- Tohkem Products Corporation (Japan)

Non-OECD

- Chenguang Research Institute of the Chemical Industry (China)
- Shanhai 3F New Materials Co., Ltd. (China)

2.1 Uses of PFOA and its Salts

PFOA is used mainly as a chemical intermediate, and its salts are used in emulsifier and surfactant applications.

According to 3M, the vast majority of PFOA is consumed to make the ammonium or sodium salts. 3M also uses PFOA as a reactive intermediate in the industrial synthesis of a fluoroacrylic ester. The fluoroacrylic ester is used in an industrial coating application (3M Company, 2000a).

The salts of PFOA have additional uses, mostly in surfactant and emulsifier applications. These include the following:

- Processing aid in the industrial synthesis of fluoropolymers and fluoroelastomers such as polytetrafluoroethylene and polyvinylidene fluoride with a variety of industrial and consumer uses (3M Company, 2000a; DuPont, 2000; Daikin, 2001).

- Post-polymerization processing aids in the stabilization of suspensions of fluoropolymers and fluoroelastomers prior to further industrial processing (3M Company, 2000a).

- Processing aid for factory-applied fluoropolymer coatings on fabrics, metal surfaces, and fabricated or molded parts (3M Company, 2000a).

- Extraction agent in ion-pair reversed-phased liquid chromatography (Petritis, 1999).

Based on the physicochemical properties of the salts of PFOA, they may also have other related surfactant or emulsifier uses as a photographic chemical or in the manufacture of electronic components such as semiconductors. These same properties may lead industry to explore PFOA as a replacement chemical for PFOS in other applications in which PFOA is not currently used.

2.2 Environmental Fate

2.2.1 Photolysis

Direct photolysis of APFO was examined in two separate studies (Todd, 1979; Hatfield, 2001) and photodegradation was not observed in either study. In the Todd (1979) study, a solution of 50 mg/l APFO in 2.8 liters of distilled water was exposed to simulated sunlight at 22±2 °C. Spectral energy was characterized from 290-600 nm with a max output at ~360 nm. Direct photolysis of the test substance was not detected. However, the author noted that sample purity was not properly characterized which may have contributed to experimental error.

In the Hatfield (2001) study, both direct and indirect photolysis were examined utilizing techniques based on EPA and OECD guidance documents. To determine the potential for direct photolysis, APFO was dissolved in pH 7 buffered water and exposed to simulated sunlight (Scrano, 1999; Nubbe, 1995). For indirect photolysis, APFO was dissolved in 3 separate matrices and exposed to simulated sunlight for periods of time from 69.5 to 164 hours. These exposures tested how each matrix would affect the photodegradation of APFO. One matrix was a pH 7 buffered aqueous solution containing H2O2 as a well-characterized source of OH radicals (Ogata, 1983; Lunak, 1992). This tested the propensity of APFO to undergo indirect photolysis. The second matrix contained Fe2O3 in water that has been shown to generate hydroxyl radicals via a Fenton-type reaction in the presence of natural and artificial sunlight (Kachanova, 1973; Behar, 1966). The third matrix contained a standard solution of humic material. Neither direct nor indirect photolysis of APFO was observed based on loss of starting material. Predicted degradation products were not detected above their limits of quantitation. There was no conclusive evidence of direct or indirect photolysis whose rates of degradation are highly dependent on the experimental conditions. Using the iron oxide (Fe2O3) photoinitiator matrix model, the APFO half-life was estimated to be greater than 349 days.

2.2.2 Volatility

Impinger studies were performed to examine the volatility of APFO and PFOS. Solutions of APFO or PFOS containing ammonium acetate in water/1-propanol (50:50) or phase transfer agents, e.g., n-alkyldimethylbenzylammonium chloride (3M Environmental Laboratory, 1993) were blown with 280 liters of air at a flow rate of 1 L/min. (3M Environmental Laboratory, 1993). The results indicate there is some loss of APFO and PFOS, but most of the solutions retained over 80% or more of the fluorochemicals. The average retention was 92% for both APFO and PFOS. This indicates that there is loss from the solutions. However, some of the solutions, particularly the n-alkyldimethylbenzylammonium chloride solution, appear to retain all the fluorochemicals. These results were reviewed by Dr. Edwin Tucker of the Chemistry Dept. at the University of Oklahoma (3M Environmental Laboratory, 1993). He concluded that

it is very unlikely that these fluorochemicals were removed by bubbling air through water due to their very low vapor pressures. He suggested that a more plausible mechanism for loss from the solution phase is concentration of the surfactants in foam and loss from the bubbled solutions as foam or micro-droplets.

In the second part of the experiment, air was passed over the fluorochemicals and bubbled through a train of impingers containing the ammonium acetate solution. It was expected that if any fluorochemicals were present in the air they would be transferred and retained by the ammonium acetate solution. However, no fluorochemicals were present in either the first or second impinger. The report concludes that the vapor pressure of both compounds is less than 10 E-07.

According to these experiments, APFO and PFOS (potassium salt) have very low volatility and vapor pressure. Quantitative conclusions regarding rates of volatilization from water or Henry's Law constant are not possible. However, APFO and PFOS are capable of transport out of water. Also, the loss of the fluorochemicals may have been as the free acids, not the salt forms. APFO sublimes at 130 C (see Physicochemical Properties Section 1.1). There is no information on the validity of the test method for determining volatility of the test substance. The study also lacks characterization of the purity of the test substance.

2.2.3 Biodegradation

Using an acclimated sludge inoculum, the biodegradation of APFO was investigated using a shake culture study modeled after the Soap and Detergent Association's presumptive test for degradation (Reiner, 1978). Both thin-layer and liquid chromatography did not detect the presence of any metabolic products over the course of 2 1/2 months indicating that PFOA does not readily undergo biodegradation. In a related study, 2.645 mg/L APFO was not measurably degraded in activated sludge inoculum (Pace Analytical, 2001). Test flasks were prepared using a mineral salts medium, 1 mL methanol, and 50 mL settled sludge. Analysis was conducted with a HPLC/MSD system. Several other studies conducted between 1977-1987 also did not observe APFO biodegradation using what probably were standard COD and BOD methods, however, the methods used in these studies were either insufficiently described (i.e. no description of experimental protocols) or there were indications of a high degree of experimental error. The results were, therefore, deemed unreliable by the submitter (3M Company, 1977; 3M Company, 1980; 3M Company, 1985b; Pace Analytical, 1997).

2.2.4 Hydrolysis

The 3M Environmental Laboratory (2001a) performed a study of the hydrolysis of PFOA. The study procedures were based on EPA's OPPTS Guideline Document 835.2110 (EPA 1998); although the procedures do not fulfill all the requirements of the guideline, they were more than

adequate for these studies. Results were based on the observed concentrations of PFOA in buffered aqueous solutions as a function of time. The chosen analytical technique was high performance liquid chromatography with mass spectrometry detection (HPLC/MS).

During the study, samples were prepared and examined at six different pH levels from 1.5 to 11.0 over a period of 109 days. Experiments were performed at 50°C and the results extrapolated to 25°C. Data from two of the pH levels (3.0 and 11) failed to meet the data quality objective and were rejected. Also rejected were the data obtained for pH 1.5 because ion pairing led to artificially low concentrations for all the incubation periods. The results for the remaining pH levels (5.0, 7.0, and 9.0) indicated no clear dependence of the degradation rate of PFOA on pH. From the data pooled over the three pH levels, it was estimated that the hydrolytic half-life of PFOA at 25°C is greater than 92 years, with the most likely value of 235 years. From the mean value and precision of PFOA concentrations, it was estimated the hydrolytic half-life of PFOA to be greater than 97 years.

2.2.5 Bioaccumulation

To determine the potential for bioaccumulation, Fathead minnows were exposed to 25 mg/l APFO for 13 days (Howell et al., 1995). After 13 days exposure, the fish were then removed from APFO contaminated water and analyzed for depuration over 15 days. After 192 and 312 hours exposure to APFO contaminated water, the average concentration of APFO in fish tissue was 44.7 and 46.7 μg/g wet weight (ww), respectively. At this point, APFO appeared to reach steady state. Twenty-four hours after being transferred to clean water, the concentration of APFO decreased to 19.9 μg/g ww and by 96 hours post-exposure, the concentration had decreased to approximately 8 μg/g ww and remained relatively constant until test termination at 360 hours. The calculated BCF for APFO was 1.8. It should be noted that questions have been raised about this study regarding the analytical techniques, high-test chemical concentration, and short test duration.

Vraspir (1979) conducted a study to determine if bluegill sunfish bioaccumulate fluorochemicals from the 3M Decatur plant. Two lots of 30 fish were used. One lot was exposed to Decatur plant effluent for 21 days and the other to river water only for 23 days. Exposed fish, both living and dead, as well as the control fish were homogenized and analyzed for fluorochemicals by GC, TLC, and GC/MS. There were no detectable amounts of APFO in the ethyl acetate or toluene extracts of the tissues. No fluorochemicals were detected in the river water exposed fish. However, interpretation of this study is problematic for several reasons. Effluent concentrations of subject fluorochemicals were not characterized and the specific protocol for exposure of the fish was not found. There was also no information on analysis of the Tennessee River water or effluent used in the study. Additionally, it was not known if there was any opportunity for the depuration of the fish prior to sacrifice. No explanation was attempted as to what was the cause

of the twelve dead fish in the effluent-exposed group. The study also did not differentiate between the bioaccumulation of the test compound and the sorption onto the surface of the fish.

2.2.6 Soil Adsorption

The adsorption-desorption of APFO was studied in 25 ml solutions of 14C-labeled APFO in distilled water with 5 g Brill sandy loam soil for 24 hours at a temperature of 16-19 °C. The study reported a Kd of 0.21 and a Koc of 14 indicating that PFOA has high mobility in Brill sandy loam soil (Welsh 1978). The Koc value, however, is questionable due to the lack of accurate information on the purity of the 14C-labeled test substance (Boyd 1993a,b).

Moody and Field (1999) conducted sampling and analysis of samples taken from groundwater 1 to 3 meters below the soil surface in close proximity to two fire-training areas with a history of aqueous film forming foam use. Perfluorooctanoic acid was detected at maximum concentrations ranging from 116 to 6750 ug/L at the two sites many years after its use at those sites had been discontinued. These results suggest that APFO may have the potential to migrate through soils to relatively shallow groundwater where it persists.

2.3 Environmental Exposure

2.3.1 Combustion

For 1997, 3M estimated 1950 pounds of PFOA-compound (PFOA and related salts) stack releases at its Cottage Grove MN location and another 4500 lbs. from Cottage Grove incinerated offsite (3M Company, 2000a,b). In 1998, 70% of the fluoride-containing wastes at 3M's Decatur location were incinerated off-site; incineration is now the primary disposal method for these materials (3M Company, 2000a,b). For 1999, DuPont estimated stack releases of 24,000 lbs. APFO at its Washington Works WV location, plus another 16,000 lbs. from Washington Works incinerated offsite (DuPont, 2000).

Canadian research has stated that the thermolysis of fluoropolymers, e.g., Teflon, Kel-F, can liberate small quantities of polycarboxylic acids, which include PFOA (Ellis et al., 2001). This information was insufficient to estimate potential yields.

2.3.2 Discharge to Water

By analogy to PFOS, PFOA discharged to water may remain there, become adsorbed to particulate matter and sediment, and/or be assimilated by organisms. For 1999, 3M estimated PFOA-compound water releases of <30,000 lbs. at its Decatur AL location, and <15,000 lbs. at its Cottage Grove MN location (3M Company, 2000a,b). For 1999, DuPont estimated the

following APFO water releases per location: Washington Works WV, 55,000 lbs; Parlin NJ, 300 lbs.; Spruance VA, 150 lbs.; Chambers Works NJ, 9500 lbs. (DuPont, 2000).

DuPont measured and modeled the following APFO concentrations at its sites: Washington Works WV: 0.552 ug/l from a 1999 drinking water sample obtained from GE Plastics immediately downstream on the Ohio River. Modeled 1996 APFO-compound releases indicated an average annual PFOA concentration of 0.423 ug/l, with APFO concentrations likely to exceed 1 ug C-8/l about 50% of the time during the year, and likely to exceed 10 ug APFO/L about 2.2% of the time during the year.

2.3.3 Discharge to Land

3M reported that land treatment of sludge from wastewater treatment at their Decatur AL location ended in mid-1998; less than 500 lbs. were disposed to land at that site in 1997. Sludge from the Decatur site is now transported to an offsite landfill; sludge from 3M's Cottage Grove MN facility is sent to an industrial landfill (3M Company, 2000a,b). DuPont (2000) estimated 3,900 lbs. of APFO sludge landfilled on site in 1999 at their Chambers Works NJ facility. DuPont estimated 2,600 lbs. APFO transferred offsite to a hazardous waste landfill from their Washington Works WV facility.

Prior operations resulted in ground- and surface water concentrations of APFO monitored at three landfills operated by DuPont's Washington Works WV facility. Average surface water concentrations for two landfills were 1392 ug/L and 18.5 ug/L, respectively. A third landfill had a maximum concentration of 33 ug/L in the permitted outfall. Average groundwater concentrations for two landfills were 2537 ug/L and 8.83 ug/L, respectively. A third landfill had a maximum groundwater concentration of 15 ug/L (DuPont, 2000).

DuPont also reported the following APFO concentrations, measured January 2000, in three drinking water wells of the Lubeck Public Service District, downstream of DuPont's Washington Works WV site: 0.8 ug/L, 0.44 ug/L, and 0.313 ug/L (DuPont, 2000). As of August 2000, the Lubeck Public Service District (LPSD) reported APFO concentrations of 0.2 ppb in drinking water at DuPont's Washington Works facility, and 0.2, 0.5, and 0.1 ppb in the three LPSD wells (LPSD, 2000).

2.3.4 Environmental Monitoring

3M's Multi-City Study reported on PFOA concentrations from water, sludge, sediment, POTW effluent, and landfill leachate samples taken in six cities (3M, 2001a). Four of the cities (Decatur AL, Mobile AL, Columbus GA, Pensacola FL) were "supply" cities that have manufacturing or industrial use of fluorochemicals; two of the cities (Cleveland TN, Port St.

Lucie FL) were "control" cities that do not have significant fluorochemical activities. Across all cities, POTW effluent concentrations ranged from 0.040 to 2.42 ppb. The POTW sludge (dry wt.) range was non-detect to 244 ppb; the drinking water range was non-detect to 0.029 ppb; the landfill leachate range was non-detect to 48.1 ppb; the surface water range was non-detect to 0.083; the sediment range was non-detect to 1.75 ppb (dry wt.); and the quiet water range was non-detect to 0.097 ppb. The "control" cities samples generally inhabited the lower end of the above ranges, except for the POTW effluent and sludge findings for Cleveland, which were intermediate in their ranges.

Giesy reported that PFOA was rarely found in fish and fish-eating water birds. Fish were sampled from the U.S., certain European countries, the North Pacific Ocean, and Antarctic locations (Giesy, 2001a). Fish-eating bird samples were collected from the U.S., including Midway atoll, the Baltic and Mediterranean Seas, Japanese and Korean coasts (Giesy, 2001b)

Giesy reported on PFOA in mink and river otter livers from the U.S. (Giesy, 2001c). PFOA was found in a few mink livers from Massachusetts at a concentration range of <18 to 108 ng/g, dry wt., but not found in mink from Louisiana, South Carolina and Illinois. PFOA concentrations in river otter livers from Washington and Oregon States were less than the quantification limit of 36 ng/g, wet wt.

Giesy reported that PFOA was not detected at quantifiable concentrations in oysters collected in the Chesapeake Bay and Gulf of Mexico of the U.S. coast (Giesy, 2001d).

Giesy reported on the concentrations of PFOA in surface water, sediments, clams, and fish collected from locations upstream and downstream of the 3M facility at Decatur AL (Giesy, 2001e). Of the five downstream sampling locations, the two closest to the 3M facility had PFOA surface water concentrations significantly greater than the two upstream sites (means of 1900ug/L and 1024 ug/L, vs. 0.008 (est.) and 0.028 ug/L); the nearest three locations had sediment concentrations significantly greater than the upstream sites (wet wt. means 1855 ug/kg, 892 ug/kg, 238 ug/kg vs. 0.08(est.) and 0.09(est.)). Clam and fish samples were collected at two locations, one upstream and one downstream of the 3M facility. The average fish whole body PFOA concentration for the upstream location was 11.7 ug/kg (wet wt.), while that for the downstream location was 106.4 ug/kg. The average PFOA concentration in clams at the upstream location was 4.38 ug/kg; that for the downstream location was 8.42 ug/kg.

2.4 Human Biomonitoring

Table 1 provides serum PFOA levels in both occupational cohorts and in the general population. The highest levels reported to date in the general population are similar to some of the lowest

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levels in workers exposed to PFOA occupationally. The data are currently limited to those discussed below.

3M has offered voluntary medical surveillance to workers at plants that produce or use perfluorinated compounds since 1976. Serum PFOA levels have been measured and reported since 1993. Prior to this time, only total organic fluorine was measured. The results of biomonitoring for PFOA have been reported for 3 plants: Cottage Grove, Minnesota; Decatur, Alabama; and Antwerp, Belgium. Surveillance years include 1993, 1995, 1997, 1998, and 2000, although not all of the plants offered surveillance in all of these years. The 1998 data reported for the Decatur plant consist of a random sample of employees; however, volunteers participated in all of the other sampling periods for all of the plants.

Mean serum PFOA levels have increased slightly at both the Cottage Grove and Decatur plants since 1993. Workers at the Cottage Grove plant, where PFOA exposures are highest, have the highest PFOA serum levels. The latest sample was in 1997, and the mean serum PFOA level was 6.4 ppm (range = 0.1 – 81.3 ppm) (Olsen et al., 1998). Only 74 employees participated in the 1997 surveillance. The total number of employees working at the plant was not reported.

At the Decatur plant, 263 of 500 employees participated in 2000 (Olsen et al., 2001d). The mean serum PFOA level was 1.78 ppm. This was slightly higher than the mean in 1998 (1.54 ppm). In 2000, 5 employees had serum levels greater than 5 ppm, the Biological Limit Value established by the 3M Exposure Guideline Committee. Cell operators had the largest increase in serum PFOA between 1998 and 2000. The highest level was in a chemical operator on the Scotchgard team (12.70 ppm). The mean level for the rest of the members of the team was 5.06 ppm (range 5 - 9 ppm). Other job categories did not exhibit such a large increase. 3M reports that this is due to increased PFOA production at the Decatur plant beginning in 1999.

Serum PFOA levels at the Antwerp plant have been lower than at Decatur or Cottage Grove, and have decreased slightly since 1995 (Olsen et al., 2001e). Participation in medical surveillance at the Antwerp plant was the highest it had ever been in 2000 (258 volunteers out of 340 workers). The mean serum PFOA level was 0.84, and the highest serum level reported was 7.04 ppm. Three employees had levels greater than 5 ppm.

3M's Specialty Materials Manufacturing Division laboratories, where employees perform fluorochemical research (Building 236), conducted voluntary biomonitoring of 45 employees in 2000 (Olsen et al., 2001f). The mean PFOA serum level was 0.106 ppm (range 0.008 – 0.668 ppm).

Data on PFOA levels in the general population are very limited. They are very recent and are only available on small cohorts. The mean serum PFOA levels are much lower in the general population than in workers exposed to PFOA.

Pooled blood samples from U.S. blood banks indicate mean PFOA levels of 3 to 17 ppb (3M Company, 1999a, 1999b). The highest pooled sample reported was 22 ppb. Samples were collected in 1998 and 1999. These data provide a very preliminary view of the PFOA levels that may be present in the U.S. general population. However, it cannot be assumed that these levels are representative of the U.S. population for several reasons: 1) blood donors are not necessarily representative of the U.S. population, 2) many of the blood banks originally contacted for possible inclusion in the study declined to participate, 3) only a small number of samples have actually been analyzed for PFOA, and 4) no other data such as age, sex, or other demographic information are available on the donors.

Preliminary data on individual blood samples have recently been reported (Olsen et al., 2001b, 2001c). Blood samples from 652 U.S. adult blood donors, ages 20-69, were obtained from six American Red Cross blood banks located in: Los Angeles, CA; Minneapolis/St. Paul, MN; Charlotte, NC; Boston, MA; Portland, OR, and Hagerstown, MD. The mean serum PFOA level was 5.6 ppb. The range was <lower limit of quantitation (LLOQ = 1.92 or 2.11) to 52.3 ppb. Blood samples from U.S. children have also been analyzed for serum PFOA. A sample of 599 children, ages 2-12 years old, participating in a study of group A streptococcal infections, revealed a mean PFOA serum level of 5.6 ppb. The range was <LLOQ to 56.1 ppb. The LLOQ was 1.92 or 2.88. The samples were collected from equal numbers of male and female children residing in 23 states. The samples in both of these studies were analyzed using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMSMS). These data are only preliminary and have not completed quality assurance procedures.

In another study, the PFOA concentration was analyzed in human sera and liver samples (Olsen et al., 2001g). Thirty-one donor samples were obtained from 16 males and 15 females over an 18-month period from the International Institute for the Advancement of Medicine (IIAM). The average age of the male donors was 50 years (SD 15.6, range 5-69) and the average age of the female donors was 45 years (SD 18.5, range 13-74). The causes of death were intracranial hemorrhage (n = 16 or 52%), motor vehicle accident (n = 7 or 23%), head trauma (n = 4 or 13%), brain tumor (n = 2 or 6%), drug overdose (n = 1 or 3%) and respiratory arrest (n = 1 or 3%). Both serum and liver tissue were obtained from 23 donors; 7 donors contributed liver tissue only and 1 donor contributed serum only. Serum samples were obtained from 5 ml of blood; liver samples consisted of 10 g of tissue. Samples were frozen at IIAM and shipped frozen to 3M for analysis. Samples were extracted using an ion-pairing extraction procedure and were quantitatively assayed using HPLC-ESMSMS and evaluated versus an unextracted curve. Extensive matrix spike studies were performed to evaluate the precision and accuracy of the extraction procedure. Serum values for PFOA ranged from < LOQ (<3.0) – 7.0 ng/mL. Assuming the midpoint value between zero and LOQ serum value for samples <LOQ, the mean serum PFOA level was 3.1 ng/mL with a geometric mean of 2.5 ng/mL. No liver to serum rations were provided because more than 90% of the individual liver samples were <LOQ.

Serum PFOA levels in corporate staff and managers at a 3M plant in St. Paul, Minnesota, where occupational exposure to PFOA should not have occurred, were reported (3M Company, 1999a).

Four of 31 employees had serum PFOA levels greater than the detection limit of 10 ppb. The mean for these employees was 12.5 ppb.

Table 1. SERUM PFOA LEVELS IN HUMAN POPULATIONS

Occupational Exposures (ppm)				
Plant	**Arithmetic Mean**	**Range**	**Geometric Mean**	**95% Confidence Interval**
Cottage Grove Plant				
1997 (n = 74)	6.4	0.1 – 81.3	*	*
1995 (n = 80)	6.8	0.0 – 114.1	*	*
1993 (n = 111)	5.0	0.0 – 80.0	*	*
Decatur Plant				
2000 (n = 263)	1.78	0.04 – 12.70	1.13	0.99 – 1.30
1998 (n = 126)	1.54	0.02 – 6.76	0.90	0.72 – 1.12
1997 (n = 84)	1.57	not reported	*	*
1995 (n = 90)	1.46	not reported	*	*
Antwerp Plant				
2000 (n = 258)	0.84	0.01 – 7.04	0.33	0.27 – 0.40
1995 (n = 93)	1.13	0.00 – 13.2	*	*
Building 236				
2000 (n = 45)	0.106	0.008 – 0.668	0.053	0.037 – 0.076

General Population Exposures (ppb)		
Source	**Arithmetic Mean**	**Range**
Pooled samples		
Commercial sources of blood, 1999 (n = 35 lots)	3	1 - 13
Blood Banks (n = 18), 1998 ~340-680 donors	17**	12 - 22
Individual samples		
American Red Cross blood banks, 2000 (n = 652)	5.6	4.27 - 52.3
Children, 1995 (n= 599)	5.6	4.27 - 56.1
3M Corporate managers/staff St. Paul, MN, 1998 (n = 31)	12.5***	not reported

*Geometric mean and 95% confidence intervals were not included in the reports.
**PFOA detected in about 1/3 of the pooled samples but quantifiable in only 2
***only 4 employees were above the detection limit of 10 ppb

3.0 Human Health Hazards

3.1. Metabolism and Pharmacokinetics

3.1.1 Half-life in Humans

In order to determine the half-life of PFOA, a group of retirees (n = 20) volunteered to participate in a 5-year half-life study in which serum samples will be drawn every 6 months (Burris et al., 2000). The only other data available on the half-life of PFOA is from a 1980 study in which it was estimated to be approximately 1 year; however, this analysis was based on total organic fluorine in blood serum.

Twenty-seven retirees, age 55 to 74 years, volunteered to participate in this half-life study. PFOA levels in this group ranged from 0.1 to 3.1 ppm. Most of the retirees were employed at the Decatur, Alabama plant for an average of 28 years. The number of years since retirement varied greatly among the participants. The average length of time between retirement and the start of the study was 30 months (2.5 years) but ranged from 5 to 130 months (~ .5 to 10 years). There were 3 collection periods during which serum PFOA samples were collected and analyzed: November 1998, June 1999, and November 1999. Cottage Grove employees, where PFOA exposure was much higher, have only participated in 2 sampling periods; therefore, they were not included in this analysis.

Half-lives were calculated using a one-compartment model. A log-linear relationship (slope = $-k_{el}(2.303)$) was used to estimate the half-life. The half-life was calculated after the elimination constant was determined, using the relationship: $t_{1/2} = 0.693/k_{el}$. Only those retirees who fit the linear one compartmental model (r^2 $ 0.6) for PFOA were included in the analyses. If 3 data points were not available for any of the subjects and if there was a lack of fit to the model, that retiree was not included in the analysis. Twenty participants met these requirements. The median serum half-life of PFOA was 344 days, with a range of 109 to 1308 days. The two highest half-life calculations were for the 2 female retirees who participated in this study (654 and 1308 days). It should be noted that the difference in PFOA serum levels between retirees was quite large (0.1 - 3.1 ppm). It was not specifically stated in the report; however, based on a statement in the report, it is assumed that the 2 female retirees did not have the highest PFOA serum levels.

For most of the participants not included in the analysis, the second measurement was higher than the first. Therefore, the data did not fit the model and they were excluded. Although this may justify not including those participants in the analysis, it is an indication of the many limitations of the data. It is stated in the report that neither age nor number of months retired was associated with the serum PFOA half-life calculations; however, this statement is not supported with any data in the report. In addition, no individual data were provided in the report and the relationship between number of years exposed in the workplace and PFOA levels and half-life were excluded. Also, elimination of PFOA occurs via urine and feces; however, these

measurements were not collected. Therefore, it cannot be determined whether the half-life suggested by the preliminary results reported here represents a true elimination half-life from the body. Finally, the effect of continued non-occupational, low-level exposure on the half-life is unknown.

The data presented above provide a very rough estimate of the plasma half-life of PFOA. It does not provide an elimination rate. In addition, these data do not provide any information about the distribution of PFOA in the body. Without the individual data or supporting information, the statement that time between retirement and entry into the study does not affect the half-life calculation is highly suspect. One would expect age, length of exposure, and time elapsed since occupational exposure to affect PFOA serum levels. Since these data were not provided in the report and since only 3 data points have been calculated to date, one can only estimate that the half-life of PFOA is between 1 and 3.5 years. These data provide evidence of the potential to bioaccumulate PFOA in humans. In addition, these preliminary data suggest that gender plays a role in the half-life.

3.1.2 Absorption Studies in Animals

APFO is well absorbed following oral and inhalation exposure, and to a lesser extent following dermal exposure. In rats, an average of 749 ug or 37% of the fluorine in the administered dose was recovered in the urine within 4.5 hr after PFOA dose (by stomach intubation 2 ml of an aqueous solution containing 2 mg PFOA) (Ophaug and Singer, 1980). The quantity of nonionic fluorine recovered in the urine increased to 61% of the dose at 8 hr, 76% at 24 hr, and 89% at 96 hr.

After a single oral dose of ^{14}C-PFOA (mean dose, 11.0 mg/kg) in solution to groups of three male rats, at least 93% of the total carbon-14 was absorbed at 24 hours (Gibson and Johnson, 1979). The half-life for elimination of total carbon-14 from plasma was 4.8 days.

Following APFO head-only inhalation exposure in male rats (6 hr/day, 5 days/wk for 2 wk to 0, 1, 8 or 84 mg/m3) concentrations of organofluoride in the blood showed a dose relationship with initial levels of 108 ppm in rats treated at 84 mg/m3 (Kennedy et al., 1986). Immediately after the tenth exposure period, the mean organofluoride blood levels were 13 ppm, 47 ppm, and 108 ppm in the 1, 8, and 84 mg/m3 dose groups.

Subchronic dermal APFO treatment in rats and rabbits (10 applications, 5 doses, 2 rest days, 5 doses) with either 0, 20, 200, or 2000 mg/kg resulted in elevated blood organofluorine levels which increased in a dose-related manner (Kennedy, 1985).

O'Malley and Ebbins (1981) conducted a range finding study which indicates significant dermal absorption of PFOA in male and female rabbits. PFOA (100 mg/kg, 1000 mg/kg, and 2000 mg/kg in saline slurry) was applied to approximately 40% of the shaved trunk of the animals, which were then fitted with a plastic collar, and the trunk was wrapped with impervious plastic

sheeting. The exposure period was 24 hr, 5 days/week over 14 days. Mortality was 100% (4/4) in the 2000 mg/kg group, 75% (3/4) in the 1000 mg/kg group and 0% (0/4) in the 100 mg/kg group.

In the past, Chemolite workers have been exposed to large dermal doses of PFOA. It appears that dermal exposure may have played a significant role in the absorption of PFOA in these workers. Upon recognition that PFOA could be absorbed dermally, work practices were changed and engineering controls were adopted that reduced dermal exposures (Gilliland, 1992).

A t-butyl ammonium salt of perfluorooctanoate in the form of treated fabric and as a liquid formulation was applied dermally to rabbits (Johnson, 1995b). Liver samples were analyzed at 28 days post dose for total organic fluorine. The results from treated animals were the same as control values. All total organic values were below the practical quantitation limit. Serum levels were also below the practical quantitation limits of the analysis for samples collected at day 1 and 2 after administration of the mixture or the treated fabric. From the pharmacokinetic study (Johnson, 1995a), it would be unlikely that any extent of absorption could have been detected in this study.

3.1.3 Distribution Studies in Animals

PFOA distributes primarily to the liver, plasma, and kidney, and to a lesser extent, other tissues of the body. It does not partition to the lipid fraction or adipose tissue, but does bind to macromolecules in the tissues. There is evidence of enterohepatic circulation of the compound. Major sex-related differences in the disposition of PFOA have been observed.

Serum and liver concentrations of PFOA were determined in rhesus monkeys in a 90 day oral toxicity study (Griffith and Long, 1980). In monkeys at the 3 mg/kg/day dose, mean serum PFOA was 50 ppm in males and 58 ppm in females. At the same dose, males had 3 ppm and females 7 ppm in liver samples. At 10 mg/kg/day doses, male monkeys had a mean serum PFOA of 63 ppm and females 75 ppm. Liver levels were 9 and 10 ppm for males and females, respectively.

Ophaug and Singer (1980) measured ionic fluoride and total fluorine in the serum of female rats following the administration of PFOA by stomach intubation (2 ml of an aqueous solution containing 2 mg PFOA). Serum from rats 4.5 hr after the administration of PFOA had a nonionic fluorine level 13.6 ppm and virtually all of this was bound to components in the serum and not ultrafilterable. Despite the large increase in nonionic fluorine in the serum, the ionic fluoride level remained very low (0.03 ppm). Prior to intubation of PFOA, the ionic and nonionic fluorine levels in serum were 0.032 and 0.07 ppm, respectively. The nonionic fluorine level in the serum decreased to 11.2 ppm at 8 hr, 0.35 ppm at 24 hr, and 0.08 ppm at 96 hr. The authors conclude that PFOA is rapidly absorbed from the gastrointestinal tract and rapidly cleared from the serum.

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Twenty-four hours after oral administration of APFO (2 mg APFO in 2 ml aqueous solution by stomach intubation), female rats had a mean serum nonionic fluorine level of 0.35 ppm, while male rats had a mean serum nonionic fluorine level of 44.0 ppm (Hanhijarvi et al., 1982). APFO was bound to a similar extent in the plasma of male and female rats (97.5% bound).

In male and female rats administered 14C-PFOA in propylene glycol/water (9.4 umol/kg, i.p.), the concentration of 14C-PFOA-derived radioactivity in the blood was higher and eliminated more slowly in males (t1/2=9 days, males vs 4 hr, females, Vanden Heuvel et al., 1991). In the male rats, the liver had the highest PFOA concentration (21% of dose at 2 hr, 2% of dose at 28 days) followed by the plasma and kidney. Far lower PFOA concentrations were found in the heart, testis, fat, and gastrocnemius muscle. In females at 2 hr post dose, the highest concentrations of PFOA were found in the plasma followed by the kidney, liver and ovaries in that order. The average t1/2 for elimination of PFOA from the liver in male rats was 11 days compared to an average of 9 days for extrahepatic tissues. In females, the average t1/2 for tissue elimination was approximately 3 hr.

Vanden Heuvel et al. (1991) investigated the disposition of PFOA in perfused male rat liver. Approximately 11% of the cumulative dose of ^{14}C-PFOA infused (0.08 umol/min x 48 min, 3.84 umol total) was extracted by the liver during a first pass. In addition, the cumulative percent of PFOA extracted by the liver at 2 min (33%) was substantially greater than that seen after 48 min (11%) indicating that first-pass hepatic uptake of PFOA may be saturable.

Ylinen et al. (1990) studied the difference between male and female Wistar rats in the distribution and accumulation of PFOA after a single and subchronic administration. The single dose of PFOA (50 mg/kg in propylene glycol-water mixture, 1:1, vol. 0.25 ml/100g) was administered intraperitoneally to 10 week old rats (20 male, 20 female). Subchronic administration of PFOA consisted of 3, 10, and 30 mg/kg/day by gavage (in 0.9% NaCl, 0.5 ml/100g) to newly weaned rats (18 male, 18 female). After the single dose, samples were collected for PFOA determination 12, 24-168 (at 24 h intervals), 244 and 336 hours after the administration, and in the subchronic test on the 28th day. The serum was collected by cardiac puncture; after decapitation the brain and at necropsy samples from the liver, kidney, lung, spleen, ovary, testis, and adipose tissue were collected and frozen. The biological half-life of PFOA in the serum and tissues was determined from the linear relationship between time and PFOA concentration in the semilogarithmic plot. In the single-dose study, concentration of PFOA in the serum and tissues was higher in males than females at all time periods. Twelve hours after the administration of PFOA about 10% of the dose was found in the serum of females, whereas about 40% was in the serum of males. After 14 days about 3.5% of the dose remained in the serum. In females, PFOA concentration in the serum, liver, and kidney occurred in a discontinuous fashion, indicating distinct phases. The half-life in the serum was 24 and 105 h in the females and males, respectively. In the females, a half-life of 60 h was estimated in the liver during the first week. In the males, the half-life in liver was 210 h. Although PFOA was retained by the liver, it was not found in the lipid fraction. In the kidney, the half-life was 145 h and 130 h in females and males, respectively. In the spleen, the half-life was 73 h and 170 h in

females and males, respectively. PFOA was also found in brain tissue. PFOA was not detectable in adipose tissue. In the subchronic study, samples taken on the 28th day indicated significantly higher PFOA concentrations in the serum and tissues of males versus females in all three dose levels. After subchronic, as well as single-dose administration, PFOA was mainly distributed in the serum of rats. High concentrations of PFOA were also found in the liver, kidney, and lung of males and females. At the high dose level (30 mg/kg/day), females and males exhibited, respectively, serum concentrations of 13.92 and 51.65 ug/ml, liver concentrations of 6.64 and 49.77 ug/g, kidney concentrations of 12.54 and 39.81 ug/g, spleen concentrations of 1.59 and 4.10 ug/g, lung concentrations of 0.75 and 23.71 ug/g, and brain concentrations of 0.044 and 0.710 ug/g. The ovary contained 1.16 ug/g and the testis contained 7.22 ug/g. A significant positive correlation existed between the administered dose and the concentration of PFOA in the liver, kidney, spleen, and lung of females. On the contrary, no significant correlation between the administered dose and the concentration of PFOA was observed in the males, as 10 mg/kg/day produced higher PFOA concentrations in the serum and organs than 30 mg/kg/day. However, in males, the concentration in the spleen, testis, and brain correlated positively with the concentration in the serum.

Vanden Heuvel et al. (1992) demonstrated that PFOA covalently binds to proteins in the liver, plasma, and testes of rats in vivo. Carbon-14-labeled PFOA was administered to six-week old male Harlan Sprague-Dawley rats in propylene glycol/water (1:1, v/v; 1 ml/kg) at a dose of 9.4 umol/kg, i.p. No time-dependent changes in either absolute or relative concentrations of covalently bound PFOA-derived 14C were found at 2 h, 1 and 4 days post-treatment. Covalently bound PFOA was represented by 0.1 to 0.3% of the tissue 14C content. The absolute concentration of covalently bound PFOA was significantly higher in the plasma than in the liver. The testes had the highest relative concentration of PFOA-derived radioactivity covalently bound. In *in vitro* tests, covalent binding of 14C-PFOA to a constant concentration of albumin (8 uM) increased in a linear fashion with increasing PFOA concentration. The covalent binding of PFOA to hemoglobin in vitro was diminished by the addition of cysteine but not methionine, suggesting that protein sulfhydryl groups may be involved.

Hanhijarvi et al. (1987) compared the disposition of PFOA between male and female Wistar rats during subchronic administration. PFOA was administered by gavage to 48 newly-weaned animals at 0, 3, 10, and 30 mg/kg (in 0.9% NaCl, 0.5ml/100g) for 28 consecutive days. Urine was collected on the 7th and 28th day of the study (discussed below). At the end of the study, blood was collected via cardiac puncture. At each dose level, the mean PFOA concentrations in the plasma of the male rats were significantly higher than those of the female rats. The mean plasma PFOA concentrations for the male rats were 48.6+-26.5 ug/ml (dosed at 3 mg/kg), 83.1+-24.7 ug/ml (10 mg/kg), and 53.4+-11.2 ug/ml (30 mg/kg). The corresponding figures for female rats were 2.43+-5.96 ug/ml, 11.3+-8.59 ug/ml, and 9.06+-8.80 ug/ml in the same order. The PFOA concentrations in the plasma of the male animals suggested that the binding sites of PFOA may become saturated at the chronic daily dose level of 30 mg/kg. Although the plasma PFOA concentrations were significantly higher in the male rats, no significant histopathological differences between the sexes were observed at necropsy.

The disposition of PFOA was studied in male Wistar rats after castration and estradiol administration as well as in intact males and females (Ylinen et al., 1989). The male rats (N=20) were castrated at the age of 28 days and after 5 weeks were used in the tests. Half of the operated and 10 intact males were administered estradiol valerate subcutaneously 500 ug/kg every second day during 14 days before the test. Blood samples were collected by cardiac puncture. At the end of the test (96 hr), the concentration of PFOA in the serum of intact males was considerably higher (17-40 times) than in the serum of other groups. There was no statistically significant difference in the serum concentrations between the other groups. PFOA was similarly bound to the proteins in the serum of males and females.

Johnson et al. (1984) investigated the effect of feeding cholestyramine to rats on the fecal elimination of APFO. Since APFO exists as an anion at physiologic pH, it would be expected to complex with cholestyramine in vivo. Ten Male Charles River CD rats (12 weeks old, 300-342 g) were administered ammonium 14C-perfluorooctanoate (2.1 mg/ml) dissolved in 0.9% NaCl as a single intravenous dose (2 ml/rat, average APFO dose 13 mg/kg). Five rats were given 4% cholestyramine in feed. Urine and feces samples were collected at intervals for 14 days, at which time the animals were sacrificed and liver samples were collected. At 14 days post dose, the mean percentage of APFO dose eliminated in the feces of cholestyramine-treated rats (43.2+-5.5) was 9.8-fold the mean percentage of dose eliminated in feces by untreated rats (4.4+-1.0). Excretion in urine was 41% for treated rats and 67% for untreated rats. Carbon-14 present in the liver represented 12.1+-2.1 ug eq/g and 22.3+-6.2 ug eq/g in treated and untreated rats, respectively (4% and 8% of dose, respectively). In plasma, the levels were 5.1+-1.7 ug eq/ml and 14.7+-6.8 ug eq/ml in treated and untreated rats, respectively. In red blood cells, the levels were 1.8+-0.7 ug eq/ml and 4.2+-2.4 ug eq/ml in treated and untreated rats, respectively. The high concentration of 14C-APFO in liver at 2 weeks after dosing and the fact that cholestyramine treatment enhances fecal elimination of carbon-14 nearly 10-fold suggests that there is enterohepatic circulation of APFO.

The disposition of PFOA (tetrabutyl ammonium salt perfluorooctanoic acid) in female rabbits has been reported (Johnson, 1995a). Individual rabbits were given intravenous doses at 0, 4, 16, and 24 mg/kg and appeared normal throughout the study (the animal treated at the 40 mg/kg dose level died within 5 minutes of dosing). Serum samples were analyzed for total organic fluorine at 2, 4, 6, 8, 12, 24, and 48 hours post dose. At 2 hrs, serum organic fluorine levels in the 0, 4, 16, and 24 mg/kg dosed rabbits were 1.25 ppm, 4.09 ppm, 14.9 ppm, and 41.0 ppm, respectively. There was a rapid decrease in serum level of total organic fluorine with time, non-detectable at 48 hr. The biological half-life was on the order of 4 hours. The total organic fluorine in whole liver at 48 hr post dose for control animals, 4 mg/kg, 16 mg/kg, and 24 mg/kg intravenous doses were 20 ug, 43 ug, 66 ug, and 54 ug.

3.1.4 Metabolism Studies in Animals

Vanden Heuvel et al. (1991) investigated the in vivo metabolism of PFOA in rats administered ^{14}C-PFOA (9.4 umol/kg, i.p.). Pooled daily urine samples (0-4 days post-treatment) and bile

extracts analyzed by HPLC contained a single radioactive peak eluting identically to the parent compound. Tissues were taken from rats treated 4, 14, and 28 days previously with 14C-PFOA to determine the presence of PFOA-containing lipid conjugates. Only the parent compound was present in rat tissues; no PFOA-containing hybrid lipids were detected. Fluoride concentrations in plasma and urine before and after PFOA treatment were unchanged, indicating that PFOA does not undergo defluorination in vivo.

Ophaug and Singer (1980) also found no change in ionic fluoride level in the serum or urine following oral administration of PFOA to female rats. Ylinen et al. (1989) found no evidence of phase II metabolism of PFOA following a single intraperotoneal PFOA dose (50 mg/kg) in male and female rats.

3.1.5 Elimination Studies in Animals

The urine is the major route of excretion of PFOA in the female rat, while the urine and feces are both major routes of excretion of PFOA in male rats (Vanden Heuvel et al., 1991). Male and female rats were administered ^{14}C-PFOA in propylene glycol/water (9.4 umol/kg, i.p.). Female rats eliminated PFOA-derived radioactivity rapidly in the urine with 91% of the dose being excreted in the first 24 hr, while male rats excreted only 6% of the dose in that time period. Negligible radioactivity was recovered in the feces of female rats. In male rats during the 28-day collection period the cumulative excretion of PFOA-derived 14C in urine and feces was 36.4% and 35.1%, respectively. The female rat retained less than 10% of the administered dose after 24 hr, while the male rats retained 30% of the administered dose after 28 days. The whole-body elimination half-life in females was less than one day, and in males it was 15 days. In renal-ligated rats injected i.p. with 14C-PFOA, approximately 0.3% of the PFOA-derived radioactivity was excreted in the bile after 6 hr (Vanden Heuvel et al., 1991). No sex-related difference in the biliary excretion of PFOA was observed when the kidneys were ligated.

Johnson and Gibson (1980) observed a sex difference in extent and rate of excretion of total carbon-14 between male and female rats after a single iv dose (mean dose: female, 16.7 mg/kg; male 13.1 mg/kg) of 14C-PFOA. Female rats excreted essentially all of the dose via urine in 24 hours while at the same time period male rats excreted only 20 percent of the dose; male rats excreted 83% via urine and 5.4% via feces by 36 days post dose. No radioactivity was detected in tissues of female rats at 17 days post dose; male rats had 2.8% of the dose in liver and 1.1% in plasma at 36 days post dose with lower levels (< 0.5% of the dose) in other organs.

Ophaug and Singer (1980) investigated the metabolic fate of PFOA in female Holtzman rats. Animals weighing approximately 250 g were administered by stomach intubation 2 ml of an aqueous solution containing 2 mg PFOA. The animals were then placed in metabolism cages and provided rat chow and tap water for 4.5, 8, 24, or 52.5 hr. In addition, four rats were placed in metabolism cages and fed a low fluoride (<0.5 ppm) diet and distilled water for a period of 96 hr. At the end of the experimental period the urine, feces and serum were collected. Within 4.5 hr after PFOA dose, an average of 749 ug or 37% of the fluorine in the administered dose was

recovered in the urine. The quantity of nonionic fluorine recovered in the urine increased to 61% of the dose at 8 hr, 76% at 24 hr, and 89% at 96 hr. Urinary excretion of ionic fluoride in the PFOA dosed animals was not significantly different than that of the control animals. Fecal excretion of nonionic fluorine was 4.5% of the administered dose at 52.5 hr and 14.3% at 96 hr. The urine from undosed animals contained no detectable nonionic fluorine.

The urinary excretion of APFO in rats was investigated by Hanhijarvi et al. (1982). Four male and six female Holtsman rats were administered 2 mg APFO in 2 ml aqueous solution by stomach intubation. Seven female rats were administered 2 ml distilled water as controls. The animals were then placed in metabolism cages with rat chow and tap water. Urine was collected until animals were sacrificed at 24 h by cardiac puncture. Serum was collected. Ionic fluoride and total fluorine content of serum and urine was determined, and nonionic fluorine was calculated as the difference. For clearance studies of APFO and inulin, the rats were anesthetized with Inactin. The femoral artery was cannulated for continuous infusion of 5% mannitol in isotonic saline and the femoral artery was cannulated for drawing blood samples. The urinary bladder was also cannulated for serial collections of urine. Intravenous priming doses of 5.2-5.6 mg [1-14C] ammonium perfluorooctanoate (sp act 0.5 uCi/mg) and 8.8 ug tritiated inulin (methoxy-3H, sp act 114 uCi/mg) were given to each animal. The radiolabled inulin and APFO in 5% mannitol in isotonic saline was then infused at a rate of 0.21 ml/min. An additional 0.42-0.63 mg/hr 14C-APFO and 9.6 ug/hr tritiated inulin was infused during the experiments. When the urine and serum collections for the clearance study were complete, probenecid was administered (65-68 mg/kg, ip) and additional clearance tests were performed. In the cumulative excretion study, rats were dosed iv with a mixture of radiolabeled APFO (10-20%) and unlabeled APFO (80-90%). Five percent mannitol in isotonic saline was infused at a rate of 0.081 ml/min and urine specimens were collected over 30-min intervals. The effect of probenecid was assessed by administering 65-68 mg/kg ip at least 30 min prior to the administration of APFO. Twenty-four hours after oral administration of APFO, female rats had excreted 76+-2.7% of the dose in the urine and had a mean serum nonionic fluorine level of 0.35+-0.11 ppm, while male rats had excreted only 9.2+-3.5% of the dose and had a mean serum nonionic fluorine level of 44.0+-1.7 ppm. APFO was bound to a similar extent in the plasma of male and female rats (97.5+-0.25% bound). The clearance studies demonstrated major differences between the sexes in rats. The APFO clearance in female rats was several times greater than the inulin clearance. Administration of probenecid, which strongly inhibits the renal active secretion of organic acids, reduced APFO/inulin clearance ratio in females from 14.5 to 0.46. APFO clearance was reduced from 5.8 to 0.11 ml/min/100g. Net APFO excretion was reduced from 4.6 to 0.13 ug/min/100g. In male rats, however, the APFO/inulin clearance ratio and the net excretion of APFO were virtually unaffected by probenecid. In the males, APFO clearance was 0.17 ml/min/100g, APFO/inulin clearance ratio was 0.22, and net APFO excretion was 0.17 ug/min/mg. In the cumulative excretion studies, female rats excreted 76% of the APFO dose, while males excreted only 7.8% of the dose over a 7-hr period. Probenecid administration modified the cumulative excretion curve for males only slightly. However, in females probenecid markedly reduced PFO elimination to 11.8%. It is concluded that the female rat possesses an active secretory mechanism which rapidly eliminates APFO from the body. This

secretory mechanism is lacking or is relatively inactive in male rats and accounts for the greater toxicity of APFO in male rats.

Hanhijarvi et al. (1987) compared the urinary elimination of PFOA between male and female Wistar rats during subchronic administration. PFOA was administered by gavage to 48 newly-weaned animals at 0, 3, 10, and 30 mg/kg (in 0.9% NaCl, 0.5ml/100g) for 28 consecutive days. Urine was collected on the 7th and 28th day of the study. At the end of the study, blood was collected via cardiac puncture. At necropsy, tissue specimens for histopathologic examination were collected from the controls and from the group receiving 30 mg/kg/day PFOA. On the seventh day of the study period, the female rats in lowest dose group (3 mg/kg/day) exhibited significantly greater urinary PFOA excretion than the males (3.12+-0.30 vs 1.50+-0.57 mg/24hr/kg). Unlike the female rats, on the 7th day of the study all three groups of male rats excreted significantly less PFOA than their daily dose of PFOA, which suggested that the males had not reached a steady state by seven days. On the 28th day, the males excreted an amount of PFOA equal to their daily dose.

Hanhijarvi et al. (1988) investigated the excretion kinetics of PFOA in the beagle dog. Six laboratory bred beagle dogs (3 male, 3 female) were anesthetized with methoxyflurane and catheters were placed in both ureters after laparototomy and cystotomy. The animals were given an intravenous dose of 30 mg/kg of PFOA followed by continuous infusion with 5% mannitol solution at 1.7 ml/min. Urine was collected at 10 minute intervals for 60 min. A 5 ml blood sample was collected in the middle of each urine sampling period. Probenicid (30 mg/kg i.v.) was then administered, and urine and blood samples were again collected as before. Renal clearance of PFOA was calculated for the before and after probenecid injection periods. Four additional dogs (2male, 2 female) were given 30 mg/kg PFOA intravenously. These dogs were kept in metabolism cages, and blood samples were collected intermittently for 30 days. From these dogs, plasma PFOA half-lives were determined. There was no difference between the renal clearances of the male and female dogs either before or after probenecid. Renal clearance rate was approximately 0.03 ml/min/kg. Probenecid significantly reduced the PFOA clearance in both sexes, indicating an active secretion mechanism for PFOA. The plasma half-lives of PFOA were longer in the male dogs (473 h and 541 h) than in the female dogs (202 h and 305 h).

The urinary excretion of PFOA was studied in male Wistar rats after castration and estradiol administration as well as in intact males and females (Ylinen et al., 1989). The male rats (N=20) were castrated at the age of 28 days and after 5 weeks were used in the tests. Half of the operated and 10 intact males were administered estradiol valerate subcutaneously 500 ug/kg every second day during 14 days before the test. Urine was collected in metabolism cages during 96 hr after a single intraperotoneal PFOA dose (50 mg/kg). Blood samples were collected by cardiac puncture. Castration and administration of estradiol to the male rats had a significant stimulatory effect on the urinary excretion of PFOA. During the first 24 hours, female rats excreted 72+-5% (N=6) of the dose, whereas the intact males excreted only 9+-4% (N=6). After the estradiol treatment, both the intact and castrated males excreted PFOA in amounts similar to females (61+-19% and 68+-14%, respectively). The castrated males without

estradiol treatment excreted PFOA in urine faster than the intact males (50+-13%), but less than the females and the estrogen treated males. At the end of the test (96 hr), the concentration of PFOA in the serum of intact males was considerably higher (17-40 times) than in the serum of other groups. There was no statistically significant difference in the serum concentrations between the other groups. PFOA was similarly bound by the proteins in the serum of males and females.

Vanden Heuvel et al. (1992a) investigated whether androgens or estrogens are involved in the marked sex-differences in the urinary excretion of PFOA. Castration of males greatly increased (> 1-fold) the elimination of ^{14C}-PFOA (9.4 umol/kg, i.p.) in urine, demonstrating that a factor produced by the testis is responsible for the slow elimination of PFOA in male rats. Castration plus 17B-estradiol had no further effect on PFOA elimination whereas castration plus testosterone replacement at the physiological level reduced PFOA elimination to the same level as rats with intact testis. Thus, in male rats, testosterone exerts an inhibitory effect on renal excretion of PFOA. In female rats, neither ovariectomy nor ovariectomy plus testosterone affected the urinary excretion of PFOA, demonstrating that the inhibitory effect of testosterone on PFOA renal excretion is a male-specific response. Probenecid, which inhibits the renal transport system, decreased the high rate of PFOA renal excretion in castrated males but had no effect on male rats with intact testis.

Hormonal changes during pregnancy do not appear to cause a change in the rate of elimination of carbon-14 after oral administration of a single dose of ammonium ^{14C}-PFOA (Gibson and Johnson, 1983). At 8 or 9 days after conception, four pregnant rats and 2 nonpregnant female rats were dosed (mean dose, 15 mg/kg) and individual urine samples were collected at 12, 24, 36, and 48 hours post dose and analyzed for carbon-14 content. Essentially all of the carbon-14 was eliminated via urine within 24 hours for both groups of rats.

Feeding of cholestyramine to rats enhanced the fecal elimination of APFO (Johnson et al. (1984). Male rats were administered ammonium [14]perfluorooctanoate (2.1 mg/ml) dissolved in 0.9% NaCl as a single intravenous dose (2 ml/rat, average APFO dose 13 mg/kg). At 14 days post dose, the mean percentage of APFO dose eliminated in the feces of cholestyramine-treated rats (43.2+-5.5) was 9.8-fold the mean percentage of dose eliminated in feces by untreated rats (4.4+-1.0). Excretion in urine was 41% for treated rats and 67% for untreated rats.

3.2 Epidemiology Studies

3.2.1 Mortality Study

A retrospective cohort mortality study was performed on employees at the Cottage Grove, Minnesota plant which produces APFO (Gilliland and Mandel, 1993). At this plant, APFO production was limited to the Chemical Division. The cohort consisted of workers who had been employed at the plant for at least 6 months between January 1947 and December 1983. Death certificates of all of the workers were obtained to determine cause of death. There was almost

complete follow-up (99.5%) of all of the study participants. The exposure status of the workers was categorized based on their job histories. If they had been employed for at least 1 month in the Chemical Division, they were considered exposed. All others were considered to be not exposed to PFOA. The number of months employed in the Chemical Division provided the cumulative exposure measurements. Of the 3537 (2788 men and 749 women) employees who participated in this study, 398 (348 men and 50 women) were deceased. Eleven of the 50 women and 148 of the 348 men worked in the Chemical Division, and therefore, were considered exposed to PFOA.

Standardized Mortality Ratios (SMRs), adjusted for age, sex, and race were calculated and compared to U.S. and Minnesota white death rates for men. For women, only state rates were available. The SMRs for males were stratified for 3 latency periods (10, 15, and 20 years) and 3 periods of duration of employment (5, 10, and 20 years).

For all female employees, the SMRs for all causes and for all cancers were less than 1. The only elevated (although not significant) SMR was for lymphopoietic cancer, and was based on only 3 deaths. When exposure status was considered, SMRs for all causes of death and for all cancers were significantly lower than expected, based on the U.S. rates, for both the Chemical Division workers and the other employees of the plant.

In all male workers at the plant, the SMRs were close to 1 for most of the causes of death when compared to both the U.S. and the Minnesota death rates. When latency and duration of employment were considered, there were no elevated SMRs. When employee deaths in the Chemical Division were compared to Minnesota death rates, the SMR for prostate cancer for workers in the Chemical Division was 2.03 (95% CI .55 - 4.59). This was based on 4 deaths (1.97 expected). There was also a statistically significant association with length of employment in the Chemical Division and prostate cancer mortality. Based on the results of proportional hazard models, the relative risk for a 1-year increase in employment in the Chemical Division was 1.13 (95% CI 1.01 to 1.27). It rose to 3.3 (95% CI 1.02 -10.6) for workers employed in the Chemical Division for 10 years when compared to the other employees in the plant. The SMR for workers not employed in the Chemical Division was less than expected for prostate cancer (.58).

An update of this study was conducted to include the death experience of employees through 1997 (Alexander, 2001a). The cohort consisted of 3992 workers. The eligibility requirement was increased to 1 year of employment at the Cottage Grove plant, and the exposure categories were changed to be more specific. Workers were placed into 3 exposure groups based on job history information: definite PFOA exposure (n = 492, jobs where cell generation, drying, shipping and packaging of PFOA occurred throughout the history of the plant); probable PFOA exposure (n = 1685, other chemical division jobs where exposure to PFOA was possible but with lower or transient exposures); and not exposed to fluorochemicals (n = 1815, primarily non-chemical division jobs).

In this new cohort, 607 deaths were identified: 46 of these deaths were in the PFOA exposure group, 267 in the probable exposure group, and 294 in the non-exposed group. When all employees were compared to the state mortality rates, SMRs were less than 1 or only slightly higher for all of the causes of death analyzed. None of the SMRs were statistically significant at $p = .05$. The highest SMR reported was for bladder cancer (SMR = 1.31, 95% CI = 0.42 – 3.05). Five deaths were observed (3.83 expected).

A few SMRs were elevated for employees in the definite PFOA exposure group: 2 deaths from cancer of the large intestine (SMR = 1.67), 1 from pancreatic cancer (SMR = 1.34), and 1 from prostate cancer (SMR = 1.30). In addition, employees in the definite PFOA exposure group were 2.5 times more likely to die from cerebrovascular disease (5 deaths observed, 1.94 expected; 95% CI = 0.84 – 6.03).

In the probable exposure group, 3 SMRs should be noted: cancer of the testis and other male genital organs (SMR = 2.75, 95% CI = 0.07 – 15.3); pancreatic cancer (SMR = 1.24, 95% CI = 0.45 – 2.70); and malignant melanoma of the skin (SMR = 1.42, 95% CI = 0.17 – 5.11). Only 1, 6, and 2 cases were observed, respectively. The SMR for prostate cancer in this group was 0.86 (n = 5).

There were no notable excesses in SMRs in the non-exposed group, except for cancer of the bladder and other urinary organs. Four cases were observed and only 1.89 were expected (95% CI = 0.58 – 5.40).

It is difficult to interpret the results of the prostate cancer deaths between the first study and the update because the exposure categories were modified in the update. Only 1 death was reported in the definite exposure group and 5 were observed in the probable exposure group. All of these deaths would have been placed in the chemical plant employees exposure group in the first study. The number of years that these employees worked at the plant and/or were exposed to PFOA was not reported. This is important because even 1 prostate cancer death in the definite PFOA exposure group resulted in an elevated SMR for the group. Therefore, if any of the employees' exposures were misclassified, the results of the analysis could be altered significantly.

The excess mortality in cerebrovascular disease noted in employees in the definite exposure group was further analyzed based on number of years of employment at the plant. Three of the 5 deaths occurred in workers who were employed in jobs with definite PFOA exposure for more than 5 years but < 10 years (SMR = 15.03, 95% CI = 3.02 – 43.91). The other 2 occurred in employees with less than 1 year of definite exposure. The SMR was 6.9 (95% CI = 1.39 – 20.24) for employees with greater than 5 years of definite PFOA exposure. In order to confirm that the results regarding cerebrovascular disease were not an artifact of death certificate coding, regional mortality rates were used for the reference population. The results did not change. When these deaths were further analyzed by cumulative exposure (time-weighted according to exposure category), workers with 27 years of exposure in probable PFOA exposed jobs or those with 9 years of definite PFOA exposure were 3.3 times more likely to die of cerebrovascular

disease than the general population. A dose-response relationship was not observed with years of exposure.

The slight excess in bladder cancer in the cohort as a whole should be noted, especially given the bladder cancer mortality experience at 3M's Decatur plant, which produces mostly PFOS. Bladder cancer mortality was 4 times higher in workers with high PFOS exposure jobs at 3M's Decatur, Alabama plant than the general population (SMR = 4.81, 95% CI = 0.99 - 14.06) (Alexander, 2001b). Three deaths were reported, and all of them occurred in the high exposure group. Serum PFOA levels in workers are lower at the Decatur plant, where PFOA is used as an elastomer in fluoropolymer production or is produced as a by-product, than at Cottage Grove; however, the manufacture of PFOA began at Decatur in 1999. Therefore, PFOA exposures will likely increase at this plant. It is not clear whether PFOA, PFOS or some other chemical may be responsible for the bladder cancer deaths observed at these plants; however, follow up should continue in an effort to shed some light on this observation.

It is difficult to compare the results of the first and second mortality studies at the Cottage Grove plant since the exposure categories were modified. Although the authors claim that the newer exposure categories are more accurate, it is still likely that exposure misclassification occurred. Without measured exposures (serum PFOA levels), it is difficult to judge the reliability of the exposure categories that were defined. In the second study, the chemical plant employees were sub-divided into PFOA-exposed groups, and the film plant employees essentially remained in the "non-exposed" group. This was an effort to more accurately classify exposures; however, these new categories do not take into account duration of exposure or length of employment. Another limitation to this study is that 17 death certificates were not located for deceased employees and therefore were not included in the study. The inclusion or exclusion of these deaths could greatly change the analyses for the causes of death that had a small number of cases. Follow up of worker mortality at Cottage Grove (and Decatur) needs to continue. Although there were more than 200 additional deaths included in this analysis, it is a small number and the cohort is still relatively young. Given the results of studies on fluorochemicals in both animals and humans, further analysis is warranted.

3.2.2 Hormone Study

Endocrine effects have been associated with PFOA exposure in animals; therefore, 2 cross-sectional studies were conducted on employees of a plant producing PFOA (Olsen, et al., 1998a). Medical surveillance, hormone testing and PFOA serum levels were obtained for volunteer workers in 1993 (n = 111) and 1995 (n = 80). Sixty-eight employees were common to both sampling periods. In 1993, the range of PFOA was 0-80 ppm (although 80 ppm was the limit of detection that year, so it could have been higher) and 0-115 ppm in 1995 using thermospray mass spectrophotometry assay. Eleven hormones were assayed from the serum samples. They were: cortisol, dehydroepiandrosterone sulfate (DHEAS), estradiol, FSH, 17 gamma-hydroxyprogesterone (17-HP), free testosterone, total testosterone, LH, prolactin, thyroid-stimulating hormone (TSH) and sex hormone-binding globulin (SHBG).

Employees were placed into 4 exposure categories based on their serum PFOA levels: 0-1 ppm, 1- < 10 ppm, 10- < 30 ppm, and >30 ppm. Statistical methods used to compare PFOA levels and hormone values included: multivariable regression analysis, ANOVA, and Pearson correlation coefficients.

PFOA was not highly correlated with any of the hormones or with the following covariates: age, alcohol consumption, BMI, or cigarettes. Most of the employees had PFOA serum levels less than 10 ppm. In 1993, only 12 employees had serum levels > 10 ppm, and 15 in 1995. However, these levels ranged from approximately 10 ppm to over 114 ppm. There were only 4 employees in the >30 ppm PFOA group in 1993 and only 5 in 1995. Therefore, it is likely that there was not enough power to detect differences in either of the highest categories. The mean age of the employees in the highest exposure category was the lowest in both 1993 and 1995 (33.3 years and 38.2 years, respectively). Although not significantly different from the other categories, BMI was slightly higher in the highest PFOA category.

Estradiol was highly correlated with BMI ($r = .41$, $p < .001$ in 1993, and $r = .30$, $p < .01$ in 1995). In 1995, all 5 employees with PFOA levels > 30 ppm had BMIs > 28, although this effect was not observed in 1993. Estradiol levels in the >30 ppm group in both years were 10% higher than the other PFOA groups; however, the difference was not statistically significant. The authors postulate that the study may not have been sensitive enough to detect an association between PFOA and estradiol because measured serum PFOA levels were likely below the observable effect levels suggested in animal studies (55 ppm PFOA in the CD rat). Only 3 employees in this study had PFOA serum levels this high. They also suggest that the higher estradiol levels in the highest exposure category could suggest a threshold relationship between PFOA and estradiol.

Free testosterone was highly correlated with age in both 1993 and 1995. The authors did not report a negative association between PFOA serum levels and testosterone. There were no statistically significant trends noted for PFOA and either bound or free testosterone. However, 17-HP, a precursor of testosterone, was highest in the >30 ppm PFOA group in both 1993 and 1995. In 1995, PFOA was significantly associated with 17-HP in regression models adjusted for possible confounders. However, the authors state that this association was based on the results of one employee (data were not provided in the report). There were no significant associations between PFOA and cortisol, DHEAS, FSH, LH, and SHBG.

There are several design issues that should be noted when evaluating the results of this study. First, although there were 2 study years (1993 and 1995), the populations were not independent. Sixty-eight employees participated in both years. Second, there were 31 fewer employees who participated in the study in 1995, thus reducing the power of the study. There were also very few employees in either year with serum PFOA levels greater than 10 ppm. Third, the cross-sectional design of the study does not allow for analysis of temporality of an association. Since the half-life of PFOA is at least 1 year, the authors suggest that it is possible that there may be some biological accommodation to the effects of PFOA. Fourth, only one sample was taken for

each hormone for each of the study years. In order to get more accurate measurements for some of the hormones, pooled blood taken in a short time period should have been used for each participant. Fifth, some of the associations that were measured in this study were done based on the results of an earlier paper that linked PFOA with increased estradiol and decreased testosterone levels. However, total serum organic fluorine was measured in that study instead of PFOA, making it difficult to compare the results. Finally, there may have been some measurement error of some of the confounding variables.

In 1997, voluntary medical surveillance was again offered to employees (Olsen, et al., 1998b). In this sampling period, cholecystokinin (CCK) levels were analyzed in 74 employees to determine if they were positively associated with serum PFOA levels. CCK levels were observed because research has suggested that pancreas acinar cell adenomas seen in rats exposed to PFOA may be the result of increased CCK levels. Seventeen of the subjects were common to all three sampling periods (1993, 1995, and 1997). The same statistical methods were used in this study period as used in 1993 and 1995, and the four PFOA exposure categories were also the same.

The mean PFOA serum level in employees participating in the 1997 study period was 6.4 ppm (range 0.1 - 81.3 ppm). The mean CCK value was 28.5 pg/ml (range 8.8 - 86.7 pg/ml). The highest CCK values were reported in the 2 exposure categories less than 10 ppm. The means were 50% higher in these 2 categories than in the categories greater than 10 ppm ($p = .06$). When adjusted for potential confounders, multivariable regression models indicated a weak negative association between CCK and PFOA; however, the data were not included in the report.

The following explanations may indicate why this study failed to find a positive association between PFOA and CCK values:

- It is possible that the hepatocarcinogenic effects of peroxisome proliferators in rodents do not act the same biochemically in humans.
- The serum PFOA levels observed in workers may have been too low to detect an effect. Effects in animals were observed at higher doses than most of the serum levels found in workers.
- CCK receptors may be different between rats and humans. Therefore, the monkey may be a more appropriate animal model to study the pancreatic effects of PFOA in humans.
- The involvement of CCK in the initiation or promotion of pancreatic cancer is controversial.
- The rat may not be an appropriate model in the study of pancreatic cancer in humans, since acinar cell malignancies, induced by carcinogens in rats, are rare in humans.

The same methodological issues that applied to the study in 1993 and 1995 apply to this portion of the study as well.

3.2.3 Cholesterol Study

Based on animal testing which reported that animals exposed to PFOA develop hepatomegaly and alterations in lipid metabolism, a cross-sectional, occupational study was performed to determine if similar effects are present in workers exposed to PFOA. In a PFOA production facility, 115 workers were studied to determine whether serum PFOA affected their cholesterol, lipoproteins, and hepatic enzymes (Gilliland and Mandel, 1996). Forty-eight workers who were exposed to PFOA from 1985-1989 were included in the study (96% participation rate). Sixty-five employees who either volunteered or were asked to participate, were included in the unexposed group. These employees were assumed to have little or no PFOA exposure based on their job description. However, when serum levels were analyzed, it was noted that this group of workers had PFOA levels much greater than the general population. Therefore, instead of job categories, total serum fluorine was used to classify workers into exposure groups.

Total serum fluorine was used as a surrogate measure for PFOA. Serum PFOA was not measured, due to the cost of analyzing the samples. Blood samples were analyzed for total serum fluorine, serum glutamyl oxaloacetic transaminase (SGOT), serum glutamyl pyruvic transaminase (SGPT), gamma glutamyl transferase (GGT), cholesterol, low-density lipoproteins (LDL), and high-density lipoproteins (HDL). All of the participants were placed into five categories of total serum fluorine levels: <1 ppm, 1-3 ppm, >3 - 10 ppm, >10 - 15 ppm, and > 15 ppm. The range of the serum fluorine values was 0 to 26 ppm (mean 3.3 ppm). Approximately half of the workers fell into the > 1 - 3 ppm category, while 23 had serum levels < 1 ppm and 11 had levels > 10 ppm.

There were no significant differences between exposure categories when analyzed using univariate analyses for cholesterol, LDL, and HDL. In the multivariate analysis, there was not a significant association between total serum fluorine and cholesterol or LDL after adjusting for alcohol consumption, age, BMI, and cigarette smoking. There were no statistically significant differences among the exposure categories of total serum fluorine for SGOT, SGPT, and GGT. However, increases in SGOT and SGPT occurred with increasing total serum fluorine levels in obese workers (BMI = 35 kg/m^2).

Since PFOA was not measured directly and there is no exposure information provided on the employees (eg. length of employment/exposure), the results of the study provide limited information. The authors state that no adverse clinical outcomes related to PFOA exposure have been observed in these employees; however, it is not clear that there has been follow-up of former employees. In addition, the range of results reported for the liver enzymes were fairly wide for many of the exposure categories, indicating variability in the results. Given that only one sample was taken from each employee, this is not surprising. It would be much more helpful to have several samples taken over time to ensure their reliability. It also would have been interesting to compare the results of the workers who were known to be exposed to PFOA to those who were originally thought not to be exposed to see if there were any differences among

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the employees in these groups. There were more of the "unexposed" employees (n = 65) participating in the study than those who worked in PFOA production (n = 48).

3.2.4 Study on Episodes of Care (Morbidity)

In order to gain additional insight into the effects of fluorochemical exposure on workers' health, an "episode of care" analysis was undertaken at the Decatur plant to screen for morbidity outcomes that may be associated with long-term, high exposure to fluorochemicals. An "episode of care" is a series of health care services provided from the start of a particular disease or condition until solution or resolution of that problem. Episodes of care were identified in employees' health claims records using Clinical Care Groups (CCG) software. All inpatient and outpatient visits to health care providers, procedures, ancillary services and prescription drugs used in the diagnosis, treatment, and management of over 400 diseases or conditions were tracked.

Episodes of care were analyzed for 652 chemical employees and 659 film plant employees who worked at the Decatur plant for at least 1 year between January 1, 1993 and December 31, 1998. Based on work history records, employees were placed into different comparison groups: Group A consisted of all film and chemical plant workers; Group B had employees who only worked in either the film or chemical plant; Group C consisted of employees who worked in jobs with high POSF exposures; and Group D had employees who worked in high exposures in the chemical plant for 10 years or more prior to the onset of the study. Film plant employees were considered to have little or no fluorochemical exposure, while chemical plant employees were assumed to have the highest exposures.

Ratios of observed to expected episodes of care were calculated for each plant. Expected numbers were based on 3M's employee population experience using indirect standardization techniques. A ratio of the chemical plant's observed to expected experience divided by the film plant's observed to expected experience was calculated to provide a relative risk ratio for each episode of care (RREpC). 95% confidence intervals were calculated for each RREpC. Episodes of care that were of greatest interest were those which had been reported in animal or epidemiologic literature on PFOS and PFOA: liver and bladder cancer, endocrine disorders involving the thyroid gland and lipid metabolism, disorders of the liver and biliary tract, and reproductive disorders.

The only increased risk of episodes for these conditions of a priori interest were for neoplasms of the male reproductive system and for the overall category of cancers and benign growths (which included cancer of the male reproductive system). There was an increased risk of episodes for the overall cancer category for all 4 comparison groups. The risk ratio was greatest in the group of employees with the highest and longest exposures to fluorochemicals (RREpC = 1.6, 95% CI = 1.2 – 2.1). Increased risk of episodes in long-time, high-exposure employees also was reported for male reproductive cancers (RREpC = 9.7, 95% CI = 1.1 - 458). It should be noted that the confidence interval is very wide for male reproductive cancers and the sub-category of prostate

cancer. Five episodes of care were observed for reproductive cancers in chemical plant employees (1.8 expected), of which 4 were prostate cancers. One episode of prostate cancer was observed in film plant employees (3.4 expected). This finding should be noted because an excess in prostate cancer mortality was observed in the Cottage Grove plant mortality study when there were only 2 exposure categories (chemical plant employees and film plant employees). The update of the study sub-divided the chemical plant employees and did not confirm this finding when exposures were divided into definitely exposed and probably exposed employees.

There was an increased risk of episodes for neoplasms of the gastrointestinal tract in the high exposure group (RREpC = 1.8, 95% CI = 1.2- 3.0) and the long-term employment, high exposure group (RREpC = 2.9, 95% CI = 1.7 – 5.2). Most of the episodes were attributable to benign colonic polyps. Similar numbers of episodes were reported in film and chemical plant employees.

In the entire cohort, only 1 episode of care was reported for liver cancer (0.6 expected) and 1 for bladder cancer (1.5 expected). Both occurred in film plant employees. Only 2 cases of cirrhosis of the liver were observed (0.9 expected), both in the chemical plant. There was a greater risk of lower urinary tract infections in chemical plant employees, but they were mostly due to recurring episodes of care by the same employees. It is difficult to draw any conclusions about these observations, given the small number of episodes reported.

Chemical plant employees in the high exposure, long-term employment group were 2 ½ times more likely to seek care for disorders of the biliary tract than their counterparts in the film plant (RREpC = 2.6, 95% CI = 1.2 - 5.5). Eighteen episodes of care were observed in chemical plant employees and 14 in film plant workers. The sub-categories that influenced this observation were episodes of cholelithiasis with acute cholecystitis and cholelithiasis with chronic or unspecified cholecystitis. Most of the observed cases occurred in chemical plant employees.

Risk ratios of episodes of care for endocrine disorders, which included sub-categories of thyroid disease, diabetes, hyperlipidemia, and other endocrine or nutritional disorders, were not elevated in the comparison groups. Conditions which were not identified a priori but which excluded the null hypothesis in the 95% confidence interval for the high exposure, long-term employment group included: disorders of the pancreas, cystitis, and lower urinary tract infections.

The results of this study only should be used for hypothesis generation. Although the episode of care design allowed for a direct comparison of workers with similar demographics but different exposures, there are many limitations to this design. The limitations include: 1) episodes of care are reported, not disease incidence, 2) the data are difficult to interpret because a large RREpC may not necessarily indicate high risk of incidence of disease, 3) many of the risk ratios for episodes of care had very wide confidence intervals, thereby providing unstable results, 4) the analysis was limited to 6 years, 5) the utilization of health care services may reflect local medical practice patterns, 6) individuals may be counted more than once in the database because they can

be categorized under larger or smaller disease classifications, 7) episodes of care may include the same individual several times, 8) not all employees were included in the database, such as those on long-term disability 9) the analysis may be limited by the software used, which may misclassify episodes of care, 10) the software may assign 2 different diagnoses to the same episode, and 11) certain services, such as lab procedures may not have been reported in the database.

3.3 Acute Toxicity Studies in Animals

3.3.1 Oral Studies

The acute oral toxicity of APFO was tested in male and female rats in three studies. Death occurred at concentrations ≥464 mg/kg (Internat'l Res and Dev Corp., 1978). Abnormal findings upon necropsy (kidney, stomach, uterus) were observed (Glaza,1997) at 500 mg/kg (higher concentrations were not tested). Clinical signs of toxicity observed in these three studies included the following: red-stained face, stained urogenital area, wet urogenital area, hypoactivity, hunched posture, staggered gait, excessive salivation, ptosis, piloerection, decreased limb tone, ataxia, corneal opacity, and hypothermic to touch.

In one study (Internat'l Res and Dev Corp., 1978), the oral LD50 values for Charles River CD rats were 680 mg/kg (399 – 1157 mg/kg 95% confidence limit) for males; 430 mg/kg (295 – 626 mg/kg 95% confidence limit) for females; and 540 mg/kg (389 – 749 mg/kg 95% confidence limit) for males and females. The remaining two studies provided LD50 values of (1) >500 mg/kg for male Crl:CD(SD)BR rats, and 250-500 mg/kg for female Crl:CD(SD)BR rats (Glaza,1997); and (2) <1000 mg/kg for male and female Sherman-Wistar rats (3M Company, 1976b).

3.3.2 Inhalation Studies

The acute inhalation toxicity of APFO was tested in male and female Sprague-Dawley rats, at a dose level of 18.6 mg/L (nominal concentration), and exposure duration of one hour. Signs of toxicity, during, and up to 14 days after the exposure period, included the following: excessive salivation, excessive lacrimation, decreased activity, labored breathing, gasping, closed eyes, mucoid nasal discharge, irregular breathing, red nasal discharge, yellow staining of the anogenital fur, dry and moist rales, red material around the eyes, and body tremors. Upon necropsy, lung discoloration was observed in a higher than normal incidence of rats (8/10). Based on the study results, the test substance was not fatal to rats at a nominal exposure concentration of 18.6 mg/L and exposure duration of one hour (Bio/dynamics, Inc. 1979).

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3.3.3 Dermal Studies

The acute dermal toxicity of APFO was tested in male and female Hra(NZW)SPF rabbits, at a dose level of 2000 mg/kg, and a 24-hour exposure period. All animals appeared normal and exhibited body weight gain throughout the study, with the exception of one male that lost weight during the first week. Dermal irritation consisted of slight to moderate erythema, edema, and atonia; slight desquamation; coriaceousness; and fissuring. No visible lesions were observed upon necropsy. The dermal LD50 in rabbits was determined to be greater than 2000 mg/kg (Glaza, 1995).

3.3.4 Eye Irritation Studies

The eye irritation potential of APFO was tested in albino rabbits, at a dose level of 0.1 gram. In two of three studies, APFO was determined to be a primary ocular irritant. In the studies in which APFO was found to be a primary ocular irritant, APFO was left in contact with the eye for 7 days, then rinsed, or not rinsed. Irritation scores varied during the observation period. Irritation scores of the conjunctivae, iris, and cornea ranged from 2 – 4 in one study (Biosearch, Inc. 1976) and from 2 –10 in the other study (3M Company, 1976a). In both studies, irritation remained evident for the duration of the observation period (7-days post-exposure). In the study in which APFO was determined to be a non-irritant (Gabriel), the test substance was left in contact with the eye for 5 or 30 seconds, and then the eyes were rinsed. In this study, positive scores were reported for conjunctivae irritation for up to 7-days post-exposure, so the author's negative conclusion for ocular irritancy is problematic.

3.3.5 Skin Irritation Studies

The skin irritation potential of APFO was tested in albino rabbits in two studies, at a dose level of 0.5 grams, under occluded test conditions. In one study (Riker Laboratories, Inc. 1983), APFO produced irreversible tissue damage in female rabbits, following a 3-minute, 1-hour, and 4-hour contact period. Moderate erythema and edema, as well as chemical burn, eschar, and necrosis, were observed following all three contact periods. An endpoint was not achieved in this study due to extreme irritation following each contact period. In the second study (Gabriel), APFO was reported as a non-irritant of skin after an exposure period of 24 or 72 hours, based on primary irritation scores of zero.

3.4 Mutagenicity Studies

APFO was tested twice (Lawlor, 1995; 1996) for its ability to induce mutation in the *Salmonella – E. coli*/mammalian-microsome reverse mutation assay. The tests were performed both with and without metabolic activation. A single positive response seen at one dose level in S. typhimurium TA1537 when tested without metabolic activation was not reproducible. APFO did not induce mutation in either S. typhimurium or E. coli when tested either with or without mammalian activation.

APFO did not induce chromosomal aberrations *in vitro* in human lymphocytes when tested with and without metabolic activation up to cytotoxic concentrations (Murli, 1996c; NOTOX, 2000).

APFO was tested twice for its ability to induce chromosomal aberrations in CHO cells in vitro. In the first assay, APFO induced both chromosomal aberrations and polyploidy in both the presence and absence of metabolic activation. In the second assay, no significant increases in chromosomal aberrations were observed without activation. However, when tested with metabolic activation, APFO induced significant increases in chromosomal aberrations and in polyploidy (Murli, 1996b).

APFO was tested in a cell transformation and cytotoxicity assay conducted in C_3H $10T_{½}$ mouse embryo fibroblasts. The cell transformation was determined as both colony transformation and foci transformation potential. There was no evidence of transformation at any of the dose levels tested in either the colony or foci assay methods (Garry & Nelson, 1981).

APFO was tested twice in the *in vivo* mouse micronucleus assay. APFO did not induce any significant increases in micronuclei and was considered negative under the conditions of this assay (Murli, 1996a).

3.5 Subchronic Toxicity Studies in Animals

Two unpublished 28-day feeding studies were performed at Industrial Bio-Test Laboratories, Inc. (Metrick and Marias, 1977 and Christopher and Marias, 1977). In both rats and mice the liver was the target organ. In rats, males had more pronounced hepatotoxicity and histopathologic effects than females.

In a 28-day study of ChR-CD albino rats, eight randomly assigned groups of five males and five females were studied (Metrick and Marias, 1977). After rats were allowed to acclimate for a week in individual cages they then received similar feed containing 0, 30, 100, 300, 1000, 3000, 10,000, or 30,000 ppm APFO for 28 days. At the beginning of the study the animals averaged 88 grams for males and 76 grams for females. The animals were observed daily and body weights and food consumption were recorded weekly. Animals that died during the study were examined for gross pathology, as were surviving animals at 28 days. It is stated that the study included a complete examination of gross pathology and a complete set of tissues and organs were examined, but the specific list is not supplied. Livers were weighed to determine relative organ weight then stained for histopathologic examination.

All animals in the 10,000 and 30,000-ppm groups died before the end of the first week. There were no premature deaths or other clinical signs of toxicity in the other groups. Body weight gains were reduced in the groups receiving 1000 or more ppm. Slight reductions in body weight gain were also observed in males exposed to 300 ppm and males and females fed 100 ppm. Reduced food intake was observed in rats fed 1000 ppm or higher in a dose-related manner.

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Relative liver weights were increased in males fed 30 ppm or more and females fed 300 ppm or more. Gross pathological exam did not reveal treatment-related effects in kidneys or other organs besides livers. Focal to multifocal cytoplasmic enlargement of hepatocytes was noted in animals fed 300 ppm, and multifocal to diffuse enlargement of hepatocytes was noted in animals fed 1000 ppm or higher. These effects were more pronounced in males (Metrick and Marias. 1977).

In a 28-day study of Charles River-CD albino mice, eight randomly assigned groups of five males and five females were studied (Christopher and Marisa, 1977). After mice were allowed to acclimate for 8 days in individual cages they then received similar feed containing 0, 30, 100, 300, 1000, 3000, 10,000, or 30,000 ppm of APFO for 28 days. At the beginning of the study the animals averaged 88 grams for males and 76 grams for females. The animals were observed daily and body weights and food consumption were recorded weekly. Animals that died during the study were examined for gross pathology, as were surviving animals at 28 days. It is stated the study included a complete examination of gross pathology and a representative set of tissues and organs were examined, but the specific list is not supplied. Livers were weighed to determine relative organ weight then stained for histopathologic examination.

All animals in the 1000-ppm and higher groups died before the end of day 9. The entire 300-ppm group died within 26 days except 1 male. One animal in each of the 30 and 100-ppm groups died prematurely. Clinical signs were observed in mice exposed to 100 ppm and higher doses of PFOA. At 100 ppm some animals exhibited cyanosis on days 10 and 11 of testing, but appeared normal throughout the rest of the study. Animals feed 300 ppm exhibited roughed fur and muscular weakness as well as signs of cyanosis after 9 days of treatment. Animals fed 1000 ppm exhibited similar effects after 6 days and those receiving 3000 ppm or greater doses exhibited effects after 4 days.

All mice fed APFO lost weight. Reductions in body weight gain were followed by weight losses in mice fed 30, 100, or 300 ppm. A dose-related pattern was seen in the depressed body weights.

Relative and absolute liver weights were increased in mice fed 30 ppm or more APFO. Gross pathological examination of kidneys or other organs besides livers is not discussed. Treatment-related changes were observed in the livers among all APFO treated animals including enlargement and/or discoloration of 1 or more liver lobes. Histopathologic examination of all APFO treated mice revealed diffuse cytoplasmic enlargement of hepatocytes throughout the liver (pan lobular hypertrophy) accompanied by focal to multifocal cytoplasmic vacuoles. Degeneration and /or necrosis of hepatocytes and focal bile duct proliferation were also noted in mice within all groups (Christopher and Marias, 1977).

Three 90-day subchronic toxicity studies have been conducted. One was conducted in rats (Goldenthal, 1978a), one was conducted in rhesus monkeys (Goldenthal, 1978b) and the third was conducted in male rats (Palazzolo, 1993).

In the monkey study, Goldenthal (1978b) administered rhesus monkeys (2/sex/group) doses of 0, 3, 10, 30 or 100 mg/kg/day perfluorooctanoic acid (FC-143) in 0.5% Methocel7 by gavage for 7 days/week for 90 days. All doses were given in a constant volume; individual daily doses were based upon the weekly body weights. Animals were observed twice daily for general physical appearance and behavior and pharmacotoxic signs. General physical examinations were performed during the control period and monthly during the study period. Individual body weights were recorded weekly. Blood and urine samples were collected once during the control period and at 1 and 3 months of the study for hematology, clinical chemistry and urinalysis. Monkeys were fasted overnight prior to the collection of blood and urine samples. Organs and tissues from animals that were sacrificed at the end of the study and from animals that died during the treatment period were weighed, examined for gross pathology and samples taken for histopathology. Histopathology was performed on the following organs from all monkeys in the control and treatment groups: adrenals, aorta, bone, brain, esophagus, eyes, gallbladder, heart (with coronary vessels), duodenum, ileum, jejunum, cecum, colon, rectum, kidneys, liver, lung, skin, mesenteric lymph node, retropharyngeal lymph node, mammary gland, nerve (with muscle), spleen, pancreas, prostate/uterus, rib junction (bone marrow), salivary gland, lumbar spinal cord, pituitary, stomach, testes/ovaries, thyroid, parathyroid, thymus, trachea, tonsil, tongue, urinary bladder, vagina, identifying tattoo, and any tissues(s) with lesions.

All monkeys in the 100-mg/kg/day groups died during the study. The first death occurred during week 2; all animals were dead by week 5. Signs and symptoms which first appeared during week 1 included anorexia, frothy emesis which was sometimes brown in color, pale face and gums, swollen face and eyes, slight to severe decreased activity, prostration and body trembling. Three monkeys from the 30-mg/kg/day group died during the study; one male died during week 7 and the two females died during weeks 12 and 13. Beginning in week 4, all four animals showed slight to moderate and sometimes-severe decreased activity. One monkey had emesis and ataxia, swollen face, eyes and vulva, as well as pallor of the face and gums. Beginning in week 6, two monkeys had black stools and one monkey had slight to moderate dehydration and ptosis of the eyelids.

No monkeys in the 3 or 10 mg/kg/day groups died during the study. Animals in the 3-mg/kg/day-dose group occasionally had soft stools or moderate to marked diarrhea; frothy emesis was also occasionally noted in this group. One monkey in the 10 mg/kg/day group was anorexic during week 4, had a pale and swollen face in week 7 and had black stools for several days in week 12. The other animals in the 10-mg/kg/day groups did not show any unusual signs or symptoms.

Changes in body weight were similar to the controls for animals from the 3 and 10 mg/kg/day dose groups. Monkeys from the 30 and 100 mg/kg/day groups lost body weight after week 1. At the end of the study, this loss was statistically significant for the one surviving male in the 30-mg/kg/day group (2.30 kg vs 3.78 kg for the control).

Hematology values at the end of the 1 and 3 months of treatment were similar for the control and the 3 and 10 mg/kg/day groups. At 30 mg/kg/day, the surviving male had decreased numbers of erythrocytes, decreased hemoglobin, decreased hematocrit, and increased platelets. Prothrombin time and activated prothrombin time were also increased. These increases were apparent at 1 month but were much more marked at three months.

Following one month of treatment, glucose was significantly elevated in the 3-mg/kg/day group (117 vs 89 mg/100 ml in the control). The authors of the report attribute this to a single high value for male #7366 who had a value of 131. The other three monkeys in the 3-mg/kg/day groups had levels of 112, 105, and 120-mg/100 ml. Glucose levels in the 10 and 30 mg/kg/day groups were 104 and 122-mg/100 ml, respectively, after one month of treatment. At three months of treatment, glucose levels were 81, 96, 88, and 66-mg/100 ml in the control, 3, 10 and 30 mg/kg/day groups respectively.

There was a decrease in alkaline phosphatase levels in the 30-mg/kg/day group (365 vs 597 IU/l in the control) at one month, which persisted in the one surviving male (360 vs 851 IU/l in the control) at 3 months. Alkaline phosphatase levels in the 3- and 10 mg/kg/day groups at three months were 783 and 743 IU/l showing a dose-related trend toward decreased levels.

SGOT levels were reduced in the 30-mg/kg/day groups at one month (59 vs 29 IU/l in the control) and in the one surviving male at 3 months (88 vs 45 IU/l in the control). SGPT was elevated in both the 10 and 30 mg/kg/day dose groups at 1 month; the levels were 15, 34, and 44 IU/l in the control, 10 and 30 mg/kg/day groups, respectively. SGOT levels in the 10-mg/kg/day group were comparable to the controls at 3 months (34 vs 31 IU/l in the control) but were still elevated in the one surviving male in the 30-mg/kg/day dose group (46 IU/l).

Cholesterol in the one surviving male in the 30 mg/kg/day group was elevated (240 vs 165 mg/100ml) and total protein and albumin in this animal were reduced. Total protein was 5.52 vs a control level of 8.21 g/100 ml and total albumin was 2.00 vs a control level of 4.82 g/100 ml.

There were no treatment related changes in urinalysis studies at any time period studied.

There were no macroscopic lesions noted at gross necropsy of any animals which died during the study or which were sacrificed at the end of the treatment period.

The following changes in absolute and relative organ weight changes were noted: absolute and relative weight of the hearts in females from the 10 mg/kg/day group were decreased; absolute brain weight of females from this same group were also decreased and relative group mean weight of the pituitary in males from the 3 mg/kg/day group was increased. The significance of these weight changes is difficult to assess, as they were not accompanied by morphologic changes.

One male and two females from the 30 mg/kg/day group and all animals from the 100 mg/kg/day group had marked diffuse lipid depletion in the adrenals. All males and females from the 30 and 100 mg/kg/day groups also had slight to moderate hypocellularity of the bone marrow and moderate atrophy of lymphoid follicles in the spleen. One female from the 30-mg/kg/day group and all animals in the 100-mg/kg/day group had moderate atrophy of the lymphoid follicles in the lymph nodes. No other compound related lesions were seen in at the 30 and 100 mg/kg/day groups. No treatment related lesions were seen in the organs of animals from the 3 and 10 mg/kg/day groups.

The levels of PFOA in the serum and liver are presented below.

Dose	Serum (ppm)		Liver (ppm)		Liver total (ug)	
	Males	Females	Males	Females	Males	Females
0	ND	1	0.05	0.07	3	5
3	53	65	3	7	250	350
3	48	50	ND	ND	ND	ND
10	45	79	9	ND	600	ND
10	71	71	ND	10	ND	750
30	ND	ND	125	80	8000	7500
30	145	ND	60	125	4000	9000
100	ND	ND	100	325	6000	20000

In the first rat study, Goldenthal (1978a) administered CD rats (5/sex/group) dietary levels of 0, 10, 30, 100, 300, and 1000 ppm perfluorooctanoic acid. These dose levels are approximately equivalent to 0.56, 1.72, 5.64, 17.9, and 63.5 mg/kg/day in males, and 0.74, 2.3, 7.7, 22.36 and 76.47 mg/kg/day in females. Animals were housed individually in wire mesh cages and had free access to food and water. Animals were observed twice daily for signs of toxicity and for mortality. Detailed examinations were performed once a week. Individual body weight and food consumption were recorded weekly during the pretest and treatment periods. Blood and urine samples were collected during the pretest period and at 1 and 3 months of the study for hematology and clinical chemistry and urinalysis. At week 13, sex and group, frozen and shipped to the sponsor for analysis, pooled serum samples. Organs and tissues from animals that were sacrificed at the end of the study and from two females that died during the treatment period were weighed, examined for gross pathology and samples taken for histopathology. Histopathology was performed on the following organs from rats from the control, 100, 300, and 1000 ppm dose groups: brain with cervical cord, lumbar spinal cord, peripheral nerve, eyes, pituitary, thyroid with parathyroid, adrenals, lung, heart with coronary vessels, aorta, spleen, mesenteric lymph node, thymus, bone with marrow (sternum), salivary gland, small intestines (duodenum, jejunum, ileum) colon, pancreas, liver, kidneys, urinary bladder, testes, ovaries, prostate, uterus, skin (mammary gland), any tissue(s) with gross lesions. Livers from rats from the 10 and 30-ppm dose groups were also examined microscopically and liver samples from all dose groups were frozen and sent to the sponsor for analysis.

One female in the 100 and one female in the 300-ppm group died during collection of blood. These deaths were not considered to be treatment related. All other animals survived until scheduled sacrifice.

There was a significant reduction in mean body weight in males in the 1000-ppm group (362 g vs 466 g in the control group). Food consumption was reduced in males in the 100, 300 and 1000-ppm groups, but the differences were not statistically significant.

Males in the 30, 100, 300 and 1000-ppm groups had significantly reduced numbers of erythrocytes at the end of the treatment period. The values were 7.95, 7.05, 7.16, 6.72, and 6.94 in the control, 30, 100, 300 and 1000-ppm groups, respectively. Males had reduced leukocyte values compared to the controls in all dose groups, but were statistically significant at the 300 ppm group only; leukocyte values were 10.64, 8.88, 9.33, 9.35, 7.63, and 8.06 in the control, 10, 30, 100, 300 and 1000 ppm groups, respectively. A similar phenomenon was seen with hemoglobin values, which were reduced at all, dose levels but were significant at the 10-ppm dose level only. Hemoglobin values were 16.2, 14.7, 15.0, 15.4, 14.9, 13.1 in the control, 10, 30, 100, 300 and 1000 ppm groups, respectively. There was no similar effect upon the hematological parameters of female rats in the study.

Males at the 30, 100, 300, and 1000-ppm dose levels had increased glucose levels (mg/100 ml), which were statistically significant at all but the 100-ppm dose level. Reported glucose levels were 121, 120, 136, 134, 143 and 135 mg/100 ml for the 0, 10, 30 100, 300 and 1000 ppm groups, respectively. B.U.N. levels were elevated in males at the 100, 300, and 1000 ppm dose levels; mean values at 90 days were 20.4, 23.9 and 35.1 mg/100 ml for the three dose groups, respectively, compared to 16.2 mg/100 ml for the controls. Alkaline phosphatase was elevated in males in the 100, 300, and 1000-ppm groups; the levels were 147, 204 and 212 IU/l for the three groups, respectively, compared to 104 IU/l for the controls. Females showed no similar changes in biochemical measurements.

Neither males nor females showed any treatment related changes in urinalysis parameters although females from all groups showed a higher frequency of occult blood in the urine than did males.

The only gross necropsy observation was noted in males at the 1000-ppm dose level. These animals had enlarged livers that showed varying degrees of surface discoloration. Neither females from the 1000-ppm dose level nor males or females from the lower dose levels showed such effects.

Both absolute and relative liver weights were significantly increased in males in the 30, 300 and 1000-ppm groups and in one female in the 1000-ppm group. Compound-related liver lesions occurred in all male rats in the 100, 300 and 1000-ppm groups. These lesions consisted of focal to multifocal, very slight-to-slight hypertrophy of hepatocytes in centrilobular to midzonal regions of the affected liver lobules. In some instances these lesions were accompanied by an

increased amount of yellowish-brown pigment resembling lipofuscin in the cytoplasm of hepatocytes and occasionally in sinusoidal lining cells. The incidence and severity of the lesions was more pronounced among male rats at the 1000-ppm dietary level.

A comparison of the serum levels of PFOA is shown below. The greater toxicity observed in the males than in the females is due to the gender difference in elimination as demonstrated by the differences in serum PFOA levels.

Dose	PFOA in Serum (ppm)	
	Males	Females
0	0	0
10	21	ND
30	34	0.15
100	36	ND
300	38	0.25
1000	49	0.65

ND = Not Determined.

In the second rat study, Palazzolo (1993) administered 45-55 male Sprague-Dawley rats per group, doses of 1, 10, 30, or 100 ppm (approximate mean compound consumption at week 13 of 0.05, 0.47, 1.44, and 4.97 mg/kg/day) APFO *ad libitum* in the diet for 13 weeks. Two control groups (a nonpair-fed control group and a control group pair-fed to the 100 ppm dose group) were also exposed during that period. Following the 13-week exposure period, 10 animals per group were fed basal diet for an additional 8-weeks post-treatment and observed for any signs of recovery. All test diets were assayed and evaluated for test material homogeneity and stability. All animals were observed twice daily for mortality, moribundity, and general clinical signs of toxicity. Body weights were recorded once before exposures began, weekly during the treatment period, and then on the day of necropsy. Food consumption was recorded weekly for all dosed-groups, including the nonpair-fed control group; daily for the pair-fed animals, and then weekly for all of the animals retained for the recovery phase of the study. A total of 15 animals per dosed-group were sacrificed following 4, 7, or 13 weeks of treatment; 10 animals per dosed-group were sacrificed after 13 weeks of treatment and following 8 weeks of non-treatment. Serum samples collected from 10 animals per dosed-group at each scheduled sacrifice during treatment and from 5 animals per dosed-group during recovery were analyzed for estradiol, total testosterone, luteinizing hormones, and for test material residue. The level of palmitoyl CoA oxidase, an indicator of peroxisome proliferation, was analyzed from a section of liver that was obtained from 5 animals per dosed-group at each scheduled sacrifice. The following organs from all animals at each scheduled sacrifice were weighed: brain, liver, lungs, testis (one), seminal vesicle, prostate, coagulating gland, and urethra. The following tissues in these same animals were preserved in 10% phosphate-buffered formalin and examined macroscopically: external surface of the body, all orifices, the cranial cavity, the external surfaces of the brain and spinal cord, the nasal cavity and paranasal sinuses; the thoracic, abdominal, and pelvic cavities

and viscera; and also examined microscopically: any observed lesions, brain, liver, lungs, testes (one), seminal vesicle, prostate, coagulating gland, and urethra. In addition, the following tissues were preserved in glutaraldehyde for electron microscopic examination: brain, liver, lungs, testes (one), seminal vesicle, and prostate.

In the analysis of the data, animals in groups exposed to 1, 10, 30, and 100 ppm APFO were compared to the control animals in the nonpair-fed group, while the data from the pair-fed control animals were compared to animals exposed to100 ppm APFO. All test diets were considered to be homogeneous and stable under the experimental conditions. All animals survived to scheduled sacrifice, with the exception of one animal in the 100-ppm dosed-group that was sacrificed on week 4 due to severe neck sores unrelated to treatment. Twice-daily examinations of all animals were unremarkable. At 100 ppm, significant reductions in body weights were seen compared to the pair-fed control group during week 1 and the nonpair-fed control group during weeks 1-13 (i.e., throughout treatment). During recovery, however, no reductions in body weights were apparent. Body weight data in the other dosed-groups were comparable to controls. At 100 ppm, mean body weight gains were significantly higher than the pair-fed control group during week 1 and significantly lower than the nonpair-fed control group during weeks 1-13. At 10 and 30 ppm, mean body weight gains were significantly lower than the nonpair-fed control group at week 2. These differences in body weight gains were not observed during the recovery period. Significant differences in food consumption were observed at 100 ppm during weeks 1 and 2 only, when compared to the nonpair-fed control group; no other significant differences in food consumption were noted. There were no significant differences among the groups for any of the hormones evaluated in the serum. Likewise, serum analysis of test material residue showed no increase in serum APFO levels over the course of treatment. Statistically significant higher hepatic palmistry CoA oxidase activity was observed at 30 and 100 ppm; however, this effect returned to control levels by the end of the recovery period. At 10 ppm, statistically significant higher levels of hepatic palmitoyl CoA oxidase activity were observed at week 5 only. Mean enzyme activities were highest during week 8 for animals exposed to 10, 30, and 100 ppm. All dosed groups exhibited significant increases in absolute and relative liver weights and hepatocellular hypertrophy were observed at weeks 4, 7, and 13, compared to the pair-fed control group. The authors suggested that these changes might be associated with peroxisome proliferation, especially since increases in hepatic palmitoyl CoA oxidase activity were also observed at this dose level during treatment. During recovery, however, none of the liver effects were observed, indicating that these treatment-related liver effects were reversible.

Therefore, under the conditions of this study, a NOAEL of 1.0 ppm (0.05 mg/kg/day) and a LOAEL of 10 ppm (0.47 mg/kg/day) are indicated based on reductions in body weight and body weight gain, and on increases in absolute and relative liver weights with hepatocellular hypertrophy.

3.6 Developmental Toxicity Studies in Animals

Three prenatal developmental toxicity studies of APFO have been conducted, one inhalation and two oral studies.

The first of these studies was an oral developmental toxicity study in rats (Gortner, 1981). Based on the results of a range-finding study, an upper dose level of 150 mg/kg/day was set for the definitive study in which five groups of 22 time-mated Sprague-Dawley rats were administered 0, 0.05, 1.5, 5, and 150 mg/kg/day APFO in distilled water by gavage on gestation days (GD) 6-15. Doses were adjusted according to body weight. Dams were monitored on GD 3-20 for clinical signs of toxicity. Individual body weights were recorded on GD 3, 6, 9, 12, 15, and 20. Animals were sacrificed on GD 20 by cervical dislocation and the ovaries, uteri, and contents were examined for the number of corpora lutea, number of viable and non-viable fetuses, number of resorption sites, and number of implantation sites. Fetuses were weighed and sexed and subjected to external gross necropsy. Approximately one-third of the fetuses were fixed in Bouin's solution and examined for visceral abnormalities by free-hand sectioning. The remaining fetuses were subjected to skeletal examination using alizarin red.

Signs of maternal toxicity consisted of statistically significant reductions in mean maternal body weights on GD 9, 12, and 15 at the high-dose group of 150 mg/kg/day. Mean maternal body weight on GD 20 continued to remain lower than controls, although the difference was not statistically significant. Other signs of maternal toxicity that occurred only at the high-dose group included ataxia and death in three rat dams. No other effects were reported. Administration of APFO during gestation did not appear to affect the ovaries or reproductive tract of the dams. Under the conditions of the study, a NOAEL of 5 mg/kg/day and a LOAEL of 150 mg/kg/day for maternal toxicity were indicated.

A significantly higher incidence in fetuses with one missing sternebrae was observed at the high-dose group of 150 mg/kg/day; however this skeletal variation also occurred in the controls and the other three dose groups (at similar incidence but lower than the high-dose group) and therefore was not considered to be treatment-related. No significant differences between treated and control groups were noted for other developmental parameters that included the mean number of males and females, total and dead fetuses, the mean number of resorption sites, implantation sites, corpora lutea and mean fetus weights. Likewise, a fetal lens finding initially described as a variety of abnormal morphological changes localized to the area of the embryonal nucleus, was later determined to be an artifact of the free-hand sectioning technique and therefore not considered to be treatment-related. Under the conditions of the study, a NOAEL for developmental toxicity of 150 mg/kg/day (highest dose group) was indicated.

A second oral prenatal developmental toxicity study was conducted in rabbits (Gortner, 1982). Based on the results of a range-finding study, an upper dose level of 50 mg/kg/day was set for the definitive study in which four groups of 18 pregnant New Zealand White rabbits were administered 0, 1.5, 5, and 50 mg/kg/day APFO in distilled water by gavage on gestation days

(GD) 6-18. Pregnancy was established in each sexually mature female by i.v. injection of pituitary lutenizing hormone in order to induce ovulation, followed by artificial insemination with 0.5 ml of pooled semen collected from male rabbits; the day of insemination was designated as day 0 of gestation. A constant dose volume of 1 ml/kg was administered. Individual body weights were measured on GD 3, 6, 9, 12, 15, 18, and 29. The does were observed daily on GD 3-29 for abnormal clinical signs. On GD 29, the does were euthanized and the ovaries, uterus and contents examined for the number of corpora lutea, live and dead fetuses, resorptions and implantation sites. Fetuses were examined for gross abnormalities and placed in a 37^0 C incubator for a 24-hour survival check. Pups were subsequently euthanized and examined for visceral and skeletal abnormalities. A blood sample was taken from six does prior to dosing and then on GD 18 and 29; a liver sample was taken from the same animals on GD 29. All samples were sent to the sponsor for analysis. This information was unavailable at the time of this review.

Signs of maternal toxicity consisted of statistically significant transient reductions in body weight gain on GD 6-9 when compared to controls; body weight gains returned to control levels on GD 12-29. Administration of APFO during gestation did not appear to affect the ovaries or reproductive tract contents of the does. No clinical or other treatment-related signs were reported. Under the conditions of the study, a NOAEL of 50 mg/kg/day, the highest dose tested, for maternal toxicity was indicated.

No significant differences were noted between controls and treated groups for the number of males and females, dead or live fetuses, and fetal weights. Likewise, there were no significant differences reported for the number of resorption and implantation sites, corpora lutea, the conception incidence, abortion rate, or the 24-hour mortality incidence of the fetuses. Gross necropsy and skeletal/visceral examinations were unremarkable. The only sign of developmental toxicity consisted of a dose-related increase in a skeletal variation, extra ribs or 13^{th} rib, with statistical significance at the high-dose group (38% at 50 mg/kg/day, 30% at 5 mg/kg/day, 20% at 1.5 mg/kg/day, and 16 % at 0 mg/kg/day). A statistically significant increase in 13^{th} ribs-spurred occurred in the mid-dose group of 5 mg/kg/day; however, the biological significance of this effect is uncertain since in both the high- and low-dose groups, this effect occurred at the same rate and was not statistically significantly different from controls. Therefore, under the conditions of the study, a LOAEL for developmental toxicity of 50 mg/kg/day (highest dose group) was indicated.

Staples et al. (1984) also conducted a developmental toxicity study of APFO. The study design consisted of an inhalation and an oral portion, each with two trials or experiments. The first trial was the teratology portion of the study, in which the dams were sacrificed on GD 21; while in the second trial, the dams were allowed to litter and the pups were sacrificed on day 35-post partum.

For the inhalation portion of the study, the two trials consisted of 12 pregnant Sprague-Dawley rats per group exposed to APFO by whole-body vapor inhalation to 0, 0.1, 1, 10, and 25 mg/m^3 6 hours/day, on GD 6-15. In the oral portion of the study, 25 and 12 Sprague-Dawley rats for the first and second trials, respectively, were administered 0 and 100 mg/kg/day APFO in corn oil by gavage on GD 6-15. For both routes of administration, females were mated on an as-needed basis and when the number of mated females was bred, they were ranked within breeding days by body weight and assigned to groups by rotation in order of rank. Finally, two additional groups (six dams per group) were added to each trial that was pair-fed to the 10 and 25 mg/m^3 groups.

For the teratology portion of the study (trial one), dams were weighed on GD 1, 6, 9, 13, 16, and 21 and observed daily for abnormal clinical signs. On GD 21, the dams were sacrificed by cervical dislocation and examined for any gross abnormalities, liver weights were recorded and the reproductive status of each animal was evaluated. The ovaries, uterus and contents were examined for the number of corpora lutea, live and dead fetuses, resorptions and implantation sites. Pups (live and dead) were counted, weighed and sexed and examined for external, visceral, and skeletal alterations. The heads of all control and high-dosed group fetuses were examined for visceral alterations as well as macro- and microscopic evaluation of the eyes.

For trial two, in which the dams were allowed to litter, the procedure was the same as that for trial one up to GD 21. Two days before the expected day of parturition, each dam was housed in an individual cage. The date of parturition was noted and designated Day 1 PP. Dams were weighed and examined for clinical signs on Days 1, 7, 14, and 22 PP. On Day 23 PP all dams were sacrificed. Pups were counted, weighed, and examined for external alterations. Each pup was subsequently weighed and inspected for adverse clinical signs on Days 4, 7, 14, and 22 PP. The eyes of the pups were also examined on Days 15 and 17 PP for the inhalation portion and on Days 27 and 31 PP for the gavage portion of the study. Pups were sacrificed on Day 35 PP and examined for visceral and skeletal alterations.

Inhalation Exposure

Trial One:

Treatment-related clinical signs of maternal toxicity for trial one (teratology) occurred at 10 and 25 mg/m^3 and consisted of wet abdomens, chromodacryorrhea, chromorhinorrhea, a general unkempt appearance, and lethargy in four dams at the end of the exposure period (high-concentration group only). Three out of 12 dams died during treatment at 25 mg/m^3 (on GD 12, 13, and 17). Food consumption was significantly reduced at both 10 and 25 mg/m^3; however, no significant differences were noted between treated and pair-fed groups. Significant reductions in body weight were also observed at these concentrations, with statistical significance at the high-concentration only. Likewise, statistically significant increases in mean liver weights were seen at the high-concentration group. Under the conditions of the study, a NOAEL and LOAEL for maternal toxicity of 1 and 10 mg/m^3, respectively, was indicated.

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No effects were observed on the maintenance of pregnancy or the incidence of resorptions. Mean fetal body weights were significantly decreased in the 25-mg/m^3 groups and in the control group pair-fed 25 mg/m^3. A detailed microscopic visceral and eye examination of the fetuses did not reveal any treatment-related effects; however in the control group that was pair-fed 25 mg/m^3, a statistically significant increased incidence of fetuses with partially ossified sternebrae was observed. Under the conditions of the study, a NOAEL and LOAEL for developmental toxicity of 10 and 25 mg/m^3, respectively, was indicated.

Trial Two:

Clinical signs of maternal toxicity seen at 10 and 25 mg/m^3 were similar in type and incidence as those described for trial one. Maternal body weight gain during treatment at 25 mg/m^3 was less than controls, although the difference was not statistically significant. In addition, 2 out of 12 dams died during treatment at 25 mg/m^3. No other treatment-related effects were reported, nor were any adverse effects noted for any of the measurements of reproductive performance. Under the conditions of the study, a NOAEL and LOAEL for maternal toxicity of 1 and 10 mg/m^3, respectively, were indicated.

Signs of developmental toxicity in this group consisted of statistically significant reductions in pup body weight on Day 1 PP (6.1 g at 25 mg/m^3 vs. 6.8 g in controls). On Days 4 and 22 PP, pup body weights continued to remain lower than controls, although the difference was not statistically significant (Day 4 PP: 9.7 g at 25 mg/m^3 vs.10.3 in controls; Day 22 PP: 49.0 g at 25 mg/m^3 vs. 50.1 in controls). No significant effects were reported following external examination of the pups or with ophthalmoscopic examination of the eyes. Under the conditions of the study, a NOAEL and LOAEL for developmental toxicity of 10 and 25 mg/m^3, respectively, were indicated.

Oral Exposure

Trial One:

Three out of 25 dams died during treatment of 100 mg/kg APFO during gestation (one death on GD 11; two on GD 12). Clinical signs of maternal toxicity in the dams that died were similar to those seen with inhalation exposure. Food consumption and body weights were reduced in treated animals compared to controls. No adverse signs of toxicity were noted for any of the reproductive parameters such as maintenance of pregnancy or incidence of resorptions. Likewise, no significant differences between treated and control groups were noted for fetal weights, or in the incidences of malformations and variations; nor were there any effects noted following microscopic examination of the eyes.

Trial Two:

Similar observations for clinical signs were noted for the dams as in trial one. Likewise, no adverse effects on reproductive performance or in any of the fetal observations were noted.

3.7 Carcinogenicity Studies in Animals

3.7.1 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 FC-143 for two years. Groups of 15 additional rats per sex were fed 0, or 300 ppm FC-143 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 (a clinical sign) and ovarian tubular hyperplasia (which is reversible), the LOAEL for female rats is 30 ppm.]

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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%, 4% and 14%, respectively; the respective incidences of mammary fibroadenoma in the female groups were 22%, 42% and 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.

In a follow-up 2-year dietary study (300 ppm) in male Sprague-Dawley (CD) rats, APFO was found to induce liver tumors and pancreatic acinar cell tumors in addition to Leydig cell tumors; however, details on the study design and tumor incidence were not reported (Cook et al., 1994). APFO has also been shown to promote liver carcinogenesis in rodents (Abdellatif et al.,1991; Nilsson et al., 1991).

3.7.2 Mode of Action Studies

The mechanisms of toxicological/carcinogenic action of APFO are not clearly understood. Short-term genotoxicity assays suggest that APFO is not a DNA-reactive compound; it is non-mutagenic in the Ames test using five strains of *Salmonella typhimurium*, or in an assay with *Saccharomyces cerevisiae* (Griffith and Long, 1980). Available data indicate that the induction of tumors by APFO is due to a non-genotoxic mechanism, involving activation of receptors and perturbations of the endocrine system.

3.7.2.1 Liver Tumors

It has been well documented that APFO is a potent peroxisome proliferator, inducing peroxisome proliferation in the liver of rats and mice (*e.g.*, Ikeda et al., 1985; Pastoor et al., 1987; Sohlenius et al., 1992). A sex-related difference in the induction of liver peroxisome proliferation exists in rats (Kawashima et al., 1989), but not in mice (Sohlenius et al., 1992). The higher induction of liver peroxisome proliferation in male rats was shown to be strongly dependent on the sex hormone testosterone (Kawashima et al., 1989). Like many other peroxisome proliferators, APFO has also been shown to cause hepatomegaly (an early biomarker of peroxisome proliferator hepatocarcinogenesis) in rats (Takagi, et al., 1992; Cook, 1994) and mice (Kennedy, 1987), and induce oxidative DNA damage in liver of rats (Takagi et al., 1991). The totality of these data appears to suggest that the liver toxicity and carcinogenicity of APFO may be related to induction of peroxisome proliferation. Meanwhile, estrogen has been shown to

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promote hepatocarcinogenesis in rats (Yager and Yager, 1980; Cameron et al., 1982); an increase in estrogen levels after APFO exposure (discussed below) may also play a role in hepatocarcinogenesis in rats.

3.7.2.2 Leydig Cell Tumors

A large number of non-genotoxic compounds of diverse chemical structures have been reported to induce Leydig cell tumors (LCT) in rats, mice, or dogs. A review of the available information on LCT induction in animals led a workshop panel to classify these compounds into seven groups based on their modes of action (Clegg et al., 1997). The common theme in the mode of action for most compounds is that these compounds affect the hormonal control of Leydig cell growth by disrupting the hypothalamic-pituitary-testicular axis at various points that result in increasing the serum levels of luteinizing hormone (LH). It has been postulated that in addition to stimulating the production of testosterone, LH may also play a mitogenic role in the Leydig cells; a sustained increase in circulating LH levels and chronic stimulation of Leydig cells by growth-stimulating mediators such as IGF-1, TGF-β, leukotrienes and various free radicals can lead to LCT development (*rev. in*: Clegg et al., 1997).

A series of studies have been conducted to investigate the mechanism of tumor formation in male Sprague-Dawley (CD) rats exposed to APFO (Cook et al., 1992; Biegel et al., 1995; Liu et al., 1996). No significant increases in LH were seen in the rats after treatment of APFO at various dose levels for 14 days. However, serum and testicular levels of estradiol were significantly increased and testosterone levels were significantly decreased. It was postulated that the elevated estradiol levels may cause Leydig cell hyperplasia and tumor formation by acting as a mitogen and/or enhancing growth factor secretion; the transforming growth factor α (TGF α), which binds to the epidermal growth factor (EGF) receptor and stimulated cell proliferation, for instance, has been detected in Leydig cells (Teerds et al., 1990). Subsequent experiments have shown that APFO increased the levels of estradiol by inducing cytochrome P450 XIX (aromatase), which converts testosterone to estradiol. Peroxisome proliferators are known to induce β-oxidation and cytochrome P-450 monooxygenases by binding to the peroxisome proliferation activation receptor α (PPAR α; a subfamily of steroid hormone receptors). It is believed that APFO induces cytochrome P450 XIX (aromatase) by binding to and activating the PPARα.

Although significant increases in LH were not seen in Sprague-Dawley rats after treatment of APFO in the 14 day-studies, it appears that increase in LH levels cannot be ruled out to be involved (in addition to increased estradiol level) in the induction of LCT by APFO. In these studies, significant increase in hepatic aromatase (which converts testosterone to estradiol) activities associated with decreased serum testosterone levels and increased estradiol levels were observed in the treated rats. Testosterone, which is synthesized and secreted by the Leydig cells, is regulated by LH; testosterone and LH form a closed-loop feedback system in the HPT axis. In order to maintain adequate testosterone plasma levels, reduced testosterone levels (caused by increased aromatase activity) are expected to lead to increased LH levels through the negative

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feedback mechanism. It has been pointed out that increases in LH may not always be seen in all studies of chemicals for which the proposed mode of action calls for elevated LH, and that compensation may have occurred to restore homeostasis and inappropriate timing of sampling are some of the explanations for failing to detect changes in LH levels (Clegg et al., 1997).

3.7.2.3 Mammary Gland Tumors

Estradiol has also been shown to stimulate the secretion of TGF α by mammary epithelial cells and the overexpression of TGF α has been suggested as one possible factor in producing sustained cell proliferation of mammary tumor cells and the subsequent development of neoplasia (Liu et al., 1987). Hence, it is possible that the APFO-induced elevation of estradiol levels may also be responsible for the development of mammary fibroadenomas in Sprague Dawley rats in addition to LCT (discussed above). In fact, this is consistent with the mechanism by which spontaneous mammary neoplasms were developed in aging female Sprague Dawley rats. It has been demonstrated that the early appearance and high spontaneous incidence of mammary gland tumors in untreated, aging female Sprague-Dawley rats is due to increased exposure to endogenous estrogen and prolactin as a result of an accelerating effect on normal, age-related perturbations of the estrous cycle in this strain of rat (Cutts and Noble, 1964; Chapin et al., 1996).

3.7.2.4 Pancreatic Tumors

The mechanism by which APFO induced pancreatic acinar cell tumors is unknown. A number of other peroxisome proliferators also produce pancreatic acinar cell tumors in rats. Available data suggest that the pancreatic acinar cell tumors are related to an increase in serum cholecystokinin (CCK) level secondary to hepatic cholestasis (Cook et al., 1994; Obourn et al., 1997). CCK is a growth factor that has been shown to stimulate normal, adaptive, and neoplastic growth of pancreatic acinar cells in rats (Longnecker, 1987). However, data on the role of CCK in pancreatic tumor formation are conflicting.

4.0 Hazards to the Environment

4.1 Introduction

The aquatic toxicity and hazard of APFO to aquatic organisms was assessed. This task was made more difficult by several problems discussed below. These problems complicated the task of determining if the ecotoxicity tests were valid and could be used in the assessment. Furthermore, these problems limited the confidence that could be placed on the toxicity test values, and thus in turn lowered the confidence of conclusions that could be drawn in assessing the inherent toxicity and hazard of APFO to aquatic organisms.

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1) A variety of different APFOs with varying designations and lot numbers were tested. Generally, the ammonium salt or the tetrabutylammonium salt was tested. The exact composition and identification of impurities, which may affect toxicity, in each lot number used is not known.

2) A variety of testing laboratories conducted the APFO toxicity studies over a period of time from approximately 1974-1996. This situation served to increase overall test variability and thus made inter-laboratory comparisons more difficult.

3) Purity of the tested material, or percent test material and percent other material(s), was a major concern. Purity was not sufficiently characterized in these tests. In some tests it appeared that 100% test chemical was used; in others a chemical of lesser purity (approximately 85%) was used. Purity of test material does affect toxicity and should be taken into account when possible, by expressing toxicity on the same purity basis.

4) Water, an isopropanol solvent, or a combination of both were used with the test material in many of the toxicity tests, for no obvious indicated reason. Solvents are mixed with the test material to make it miscible with the test dilution water before the test is begun. Solvents are used in tests where the concentrations of the test material are extremely low and a very small amount of test material must be added to the test chambers. It was not clear from the summaries of these studies why a solvent was used or was even found to be necessary. In fact, 3M summarized each test and stated "Data may not accurately relate toxicity of the test sample with that of the test substance." Thus, in those tests where 100% test material was not used, the toxicity values had to be adjusted to take into account the percent solvent(s), and to express the values on a 100% test chemical basis, so that the tests could be compared.

5) In all these toxicity tests only nominal test chemical concentrations were used. Measured test chemical concentrations are instead always recommended so that one can accurately determine the actual test chemical concentration to which the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values will have to accordingly be adjusted by 50%. Analytical measurements of chemical concentration should have been taken or made available. Then, recovery rates could have been determined, and physicochemical processes (e.g., hydrolysis, volatility) that might lower the actual concentrations to which the test organisms were exposed could have been taken into account. Nominals may be used when measured concentrations are taken and the relationship of both is known.

In order to proceed with any sort of environmental hazard review it was necessary to ignore these test limitations and to assume that the nominal concentrations were an "adequate" expression of the measured test chemical concentrations. Criteria for assessing degree of acute toxicity are based on well-established values (low is >100 mg/L; medium or moderate is >1≤100 mg/L; high is ≤1 mg/L).

4.2 Acute Toxicity to Freshwater Species

Several species were tested to assess the acute toxicity of APFO; these included the fathead minnow (*Pimephales promelas*), bluegill sunfish (*Lepomis machrochirus*), water flea (*Daphnia magna*), and a green alga (*Selenastrum capricornutum*). The toxicity test endpoints have been adjusted to 100% test chemical and test results are presented in Tables 2 (organized by test substance) and 3 (organized by test species). Each value is related to a testing facility and reference.

Twelve tests were conducted with fathead minnows; 96-h LC50 values (based on mortality) ranged from 70 to 843 mg/L. It is unclear why this range is so wide. Assuming these studies are valid, and due to the limitations discussed above, these toxicity values indicate low toxicity. The two acute values for bluegill sunfish also indicate low toxicity (96-h LC50s of >420, and 569 mg/L).

Nine acute tests were conducted with daphnids and 48-h EC50 values (based on immobilization) ranged from 39 to >1000 mg/L. The lower values are indicative of moderate toxicity, but the wide range makes interpretation difficult.

Seven tests were conducted with green algae; 96-h EC50 values (based on growth rate, cell density, cell counts, and dry weights) ranged from 1.2 to >666 mg/L (the Er50 cell density value of 1,000 mg/L is excluded from this discussion). The lower value indicates high to moderate toxicity, based on the acute criteria. The lower value would also be indicative of moderate toxicity, based on the chronic moderate criterion (.0.1≤10 mg/L). A 14-d EC50 value of 43 mg/L, based on cell counts, for green algae was also calculated in one study. This is indicative of low chronic toxicity, based on the chronic criterion (10 mg/L). Green algae appeared to be the most sensitive test species in the 44% APFO test sample, daphnids were the next most sensitive, and fathead minnows were the least sensitive.

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Table 2 Summary of Acute Ecological Toxicity Data for APFO (grouped by test substance)			
Test Organism	**Duration**	**Value (mg/L)***	**Reference**
Test Sample: APFO ammonium salt			
Fathead minnow (*Pimephales promelas*)	96-h LC50	70	3M Company, 1974a
	96-h LC50	766	3M Company, 1980a
	96-h LC50	301	3M Company, 1987c
	96-h LC50	740	Ward et al.,, 1995
Bluegill sunfish (*Lepomis machrochirus*)	96-h LC50	> 420	3M Company, 1978
	96-h LC50	569	3M Company, 1978
Water flea (*Daphnia magna*)	48-h EC50	126	3M Environmental Laboratory, 1982
	48-h EC50	> 1000	3M Environmental Laboratory, 1982
	48-h EC50	221	3M Company, 1987b
	48-h EC50	720	Ward et al., 1995
Green algae (*Selenastrum capricornutum*)	96-h EC50	310	Ward et al., 1995
	96-h EC50	1000	Ward et al., 1995
Bacteria (*Photobacterium phosphoreum*)	30-min EC50	870	3M Company, 1987a
	30-min EC50	730	3M Environmental Laboratory, 1996a
Activated sludge	7-min NOEC	1000	3M Company, 1980b
	30-min EC50	> 1000	3M Company, 1987d
Test Sample: APFO			
Fathead minnow (*Pimephales promelas*)	96-h LC50	440	3M Company, 1974b
	96-h LC50	843	3M Company, 1985
Test Sample: APFO ammonium salt in 50% water			
Fathead minnow (*Pimephales promelas*)	96-h LC50	>500	EnviroSystems, Inc., 1990a
	96-h NOEC	500	EnviroSystems, Inc., 1990a
Water flea (*Daphnia magna*)	48-h EC50	292	EnviroSystems, Inc., 1990b
Bacteria (*Photobacterium phosphoreum*)	30-min EC50	> 500	3M Environmental Laboratory, 1990a
Test Sample: APFO ammonium salt in 50% water, continued			
Activated sludge	30-min EC50	> 500	3M Environmental Laboratory, 1990b

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Test Sample: APFO ammonium salt in 80% water			
Fathead minnow (*Pimephales promelas*)	96-h LC50	494	Ward et al., 1996a
Water flea (*Daphnia magna*)	48-h EC50	240	Ward et al., 1996c
Green algae (*Selenastrum capricornutum*)	96-h EC50	396	Ward et al., 1996b
	96-h EC50	> 666	Ward et al., 1996b
Bacteria (*Photobacterium phosphoreum*)	30 min EC50	630	3M Environmental Laboratory, 1996b
	30 min EC50	390	3M Environmental Laboratory, 1996c
Activated sludge	30-min EC50	>664	3M Environmental Laboratory, 1996d
Test Sample: APFO in 50% isopropanol			
Fathead minnow (*Pimephales promelas*)	96h LC50	140	T.R. Wilbury Laboratories, Inc., 1996a
Water flea (*Daphnia magna*)	48-h EC50	360	T.R. Wilbury Laboratories, Inc., 1996b
Green algae (*Selenastrum capricornutum*)	96-h EC50	90	T.R. Wilbury Laboratories, Inc., 1995
Test Sample: APFO (44%) in 27.9% water and 27.2% isopropanol			
Fathead minnow (*Pimephales promelas*)	96-h EC50	391	T.R. Wilbury Laboratories, Inc., 1995
Fathead minnow (*Pimephales promelas*)	96-h EC50	422	T.R. Wilbury Laboratories, Inc., 1995
Test Sample: APFO (44%) in 27.9% water and 27.2% isopropanol			
Water flea (*Daphnia magna*)	48-h EC50	41	Ward et al., 1995
Water flea (*Daphnia magna*)	48-h EC50	39	Ward et al., 1995
Green algae (*Selenastrum capricornutum*)	96-h EC50	2.1	Ward et al., 1995
Green algae (*Selenastrum capricornutum*)	96-h EC50	3.6	Ward et al., 1995
Green algae (*Selenastrum capricornutum*)	96-h EC50	1.2	Ward et al., 1995

*Values were adjusted to represent 100% active ingredient.
[A]These values may be inconsistent due to different diets tested.

Table 3 Summary of Ecological Toxicity Data for APFO (grouped by species)			
Test Organism	**Duration**	**Value (mg/L)**	**Reference**
Fathead minnow (*Pimephales promelas*)	96-h LC50	70[B]	3M Company, 1974a
	96-h LC50	766[B]	3M Company, 1980a
	96-h LC50	301[B]	3M Company, 1987c
	96-h LC50	440[C]	3M Company, 1974b
	96-h LC50	843[C]	3M Company, 1985
	96-h LC50	> 500[D]	EnviroSystems, Inc., 1990a
	96-h NOEC	500[D]	EnviroSystems, Inc., 1990a
	96-h LC50	494	Ward et al., 1996a
	96h LC50	140[F]	T.R. Wilbury Laboratories, Inc., 1996a
	30-day NOAEL	> 100[B]	EG&G Bionomics Aquatic Toxicology Laboratory, 1978
	96-h EC50	391[G]	T.R. Wilbury Laboratories, Inc., 1995
	96-h EC50	422[G]	T.R. Wilbury Laboratories, Inc., 1995
Bluegill sunfish (*Lepomis machrochirus*)	96-h LC50	> 420[B]	3M Company, 1978
	96-h LC50	569[B]	3M Company, 1978
Water flea (*Daphnia magna*)	48-h EC50	126[AB]	3M Environmental Laboratory, 1982
	48-h EC50	> 1000[AB]	3M Environmental Laboratory, 1982
	48-h EC50	221[B]	3M Company, 1987b
	48-h EC50	292[D]	EnviroSystems, Inc., 1990b
	48-h EC50	240	Ward et al., 1996c
	48-h EC50	360[F]	T.R. Wilbury Laboratories, Inc., 1996b
	21-day IC50	43[B]	3M Company, 1984
	21-day NOEC	22[B]	3M Company, 1984
	21-day NOEC	22[B]	3M Company, 1984
	48-h EC50	41[G]	Ward et al., 1995
	48-h EC50	39[G]	Ward et al., 1995
Green algae (*Selenastrum capricornutum*)	96-h EC50	396	Ward et al., 1996b
	96-h EC50	>666[E]	Ward et al., 1996b
	96-h EC50	90[F]	T.R. Wilbury Laboratories, Inc., 1995
	14-day EC50	43[B]	Elnabarawy, 1981
	96-h EC50	2.1[G]	Ward et al., 1995
	96-h EC50	3.6[G]	Ward et al., 1995
	96-h EC50	1.2[G]	Ward et al., 1995

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Bacteria (*Photobacterium phosphoreum*)	30-min EC50	870[B]	3M Company, 1987a
	30-min EC50	730[B]	3M Environmental Laboratory, 1996a
	30-min EC50	>500[D]	3M Environmental Laboratory, 1990a
	30 min EC50	630	3M Environmental Laboratory, 1996b
	30 min EC50	390	3M Environmental Laboratory, 1996c
Activated sludge	7-min NOEC	1000[B]	3M Company, 1980b
	30-min EC50	>1000[B]	3M Company, 1987d
	30-min EC50	> 500[D]	3M Environmental Laboratory, 1990b
	30-min EC50	> 664[E]	3M Environmental Laboratory, 1996d

*Values were adjusted to represent 100% active ingredient.
[A]These values may be inconsistent due to different diets tested.
[B]Tested substance was APFO ammonium salt.
[C]Tested substance was APFO
[D]Tested substance was APFO ammonium salt in 50% water.
[E]Tested substance was APFO ammonium salt in 80% water.
[F]Tested substance was APFO in 50% isopropanol.
[G]Test Sample: APFO (44%) in 27.9% water and 27.2% isopropanol

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ANNEX 1

ROBUST SUMMARIES

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HUMAN BIOMONITORING DATA

Title: Identification of Fluorochemicals in Sera of American Red Cross Adult Blood Donors

TEST SUBSTANCE

Identity: PFOA and 6 other fluorochemicals

Remarks: The results reported are preliminary. The final report is expected November 2001.

METHOD

Study design: Cross-sectional

Manufacturing/Processing/Use: N/A

Hypothesis tested: To determine levels of PFOA in the serum of American Red Cross blood banks in 6 regions of the U.S.

Study period: 2000

Setting: N/A

Total population: Serum pooled from 6 ARC blood banks in various geographic regions in the US: Los Angeles, CA; Minneapolis/St. Paul, MN; Charlotte, NC; Boston, MA; Portland, OR, and Hagerstown, MD.

Subject selection criteria: Unknown

Total # of subjects in study: 652 donors, age 20-69 years

Comparison population: N/A

Participation rate: N/A

Subject description: No information was provided on the individuals from whom the sera samples were taken.

Health effects studied: 7 fluorochemicals in human blood serum, including PFOA

Data collection methods: Blood sera samples were analyzed using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMSMS).

Details on data collection: No information was provided as to how the blood was drawn, stored, etc.

Exposure period: Unknown--PFOA serum levels used as surrogate for exposure.

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Description/delineation of exposure groups/categories: N/A

Measured or estimated exposure: N/A

Exposure levels: N/A

Statistical methods: Arithmetic means, ranges, geometric means and 95% confidence intervals were calculated. Central tendency and distribution of the data by age, gender, location and their respective interaction terms will be done in the final report. A reliability assessment is also being analyzed.

Other methodological information: N/A

RESULTS

Describe results: The mean serum PFOA level was 5.6 ppb. The range was was <lower limit of quantitation (LLOQ = 1.92 or 2.11) to 52.3 ppb. Analyses stratified by age, gender, and geographic location will be forthcoming in the final report.

Study strengths and weaknesses: These data are cross-sectional data used to determine PFOS levels in the general population. No other descriptive information about the subjects is available in this preliminary report. The sample size is relatively small.
Blood donors cannot be considered representative of the general population of the US.

Research sponsors: 3M Medical Department, Corporate Occupational Medicine

Consistency of results:

CONCLUSIONS
N/A

REFERENCE

Olsen, GW, Burris, JM, Lundberg, JK, Hansen, KJ, Mandel, JH, Zobel, LR. Identification of fluorochemicals in sera of American Red Cross adult blood donors. Interim report. June 25, 2001.

HUMAN BIOMONITORING DATA

Title: Identification of Fluorochemicals in Sera of Children in the United States

TEST SUBSTANCE

Identity: PFOA and 6 other fluorochemicals

Remarks: The results reported are preliminary. The final report is expected November 2001.

METHOD

Study design: Cross-sectional.

Manufacturing/Processing/Use: N/A

Hypothesis tested: To determine the serum concentrations of selected fluorochemicals in a sample of children to provide a more specific understanding of the distribution of these compounds in children.

Study period: Child sera samples were collected from January 1994 to March 1995. The sera samples were analyzed in Spring 1999.

Setting: N/A

Total population: Not reported

Subject selection criteria: The sera samples were provided to 3M by the University of Minnesota Department of Pediatrics. They were obtained from a large clinical trial on Group A streptococcal infections in children. The children were residents of 23 states in the US. These children presented with signs and symptoms of acute-onset pharyngitis. All of the children had positive throat cultures at the initial visit.

Total # of subjects in study: n = 599 children, age 2-12 years

Comparison population: N/A

Participation rate: N/A

Subject description: No information was provided on the children from whom the sera samples were taken.

Health effects studied: PFOA levels in blood, as well as 6 other fluorochemicals.

Data collection methods: Blood sera samples were collected using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMSMS). The samples were collected from equal numbers of male and female children residing in 23 states.

Details on data collection: No information was provided as to how the blood was drawn, stored, etc.

Exposure period: N/A

Description/delineation of exposure groups/categories: Blood sera samples were collected from children 2 – 12 years old.

Measured or estimated exposure: N/A

Exposure levels: N/A

Statistical methods: Arithmetic means, ranges, geometric means and 95% confidence intervals were calculated. Central tendency and distribution of the data by age, gender, location and their respective interaction terms will be done in the final report. A reliability assessment is also being analyzed.

Other methodological information: N/A

RESULTS

Describe results: The mean PFOA serum level was 5.6 ppb. The range was <LLOQ to 56.1 ppb. The LLOQ was 1.92 or 2.88. Analyses stratified by age, gender, and geographic location will be forthcoming in the final report.

Study strengths and weaknesses: These data are cross-sectional data used to determine PFOA levels in U.S. children. No other descriptive information about the subjects is available in this preliminary report. The sample size is relatively small.

Research sponsors: 3M Medical Department, Corporate Occupational Medicine

Consistency of results: To date, no other data have been collected on PFOA serum levels in children.

CONCLUSIONS
N/A

REFERENCE
Olsen, GW, Burris, JM, Lundberg, JK, Hansen, KJ, Mandel, JH, Zobel, LR. Identification of fluorochemicals in sera of children in the United States. Interim Report. June 25, 2001.

PHARMACOKINETIC STUDY

Title: The sex-related difference in perfluorooctanoate excretion in the rat

TEST SUBSTANCE

Identity: Perfluorooctanoic acid (PFOA); also referred to as [1-^{14}C]Perfluorooctanoic acid (ammonium salt, APFO)

Remarks: Purity was not specified

METHOD

Method/guideline followed: Guideline not noted

Test type: Mammalian Excretion

Species/strain/cell type or line: Holtzman rats

Sex: Male and female

Age and body weight range of animals used: Not noted

Number of animals/sex/dose: Test group: 4 males, 6 females
Control group: 7 females

Route of administration: Oral gavage

Vehicle: Distilled water

Doses: 2 mg of nonionic fluorine as APFO in a volume of 2 mL

Excretion routes, body fluids, and tissues monitored and/or sampled during study: Blood and urine

Statistical methods used: See methods

Method remarks: Animals were placed in individual metabolic cages and sacrificed by cardiac puncture 24 hours after gavage treatment with APFO. The blood was allowed to clot and the serum collected after centrifugation. The volume of the urine collections, including the volume of water used to rinse the metabolic cages was recorded. The ionic fluoride content of the serum and urine was determined at pH 5.0. The total fluorine content of the serum and urine was determined by the oxygen-bomb reverse extraction technique.
For clearance studies of APFO and inulin the rats were anesthetized and the femoral vein was cannulated for continuous infusion of 5% mannitol in isotonic saline and the femoral artery was cannulated for drawing blood samples. The urinary bladder was also cannulated to obtain serial collections of urine. Intravenous doses of APFO and inulin were given to each animal and, following a 45-min equilibration period, blood and urine samples were collected. When collections were completed, probenecid was administered and additional clearance tests were performed to test the effects of probencid on the organic acid transport system.

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In the cumulative excretion study, the rats were prepared as described for the clearance tests except that the arterial cannulation was not needed. The rats were dosed iv with a mixture of radiolabeled APFO and unlabeled APFO.

RESULTS

Detailed results:

24 hours after administration of the dose, female rats excreted 76 $\pm$ 2.7 percent of the dose of nonionic fluorine (as APFO) in urine and had a mean serum nonionic fluorine level of 0.35 ppm.

In males, 9.2 $\pm$ 3.5 percent of the dose of nonionic fluorine (as APFO) was excreted. A level of 44.0 $\pm$ 1.7 ppm nonionic fluorine was in the serum.

APFO was bound to a similar extent in the plasma of male and female rats (97.5% bound).

In females, APFO was markedly reduced by probenecid (from 5.8 to 0.11 ml/min/100 g), indicating that elimination occurs by an active secretory mechanism, which is inhibited by probenecid.

The APFO/inulin clearance ratio was 14.5 for female rats. The ratio was decreased to 0.46 in females after probenecid. The APFO/inulin clearance ratio was 0.22 for males, and decreased to 0.12 after probenecid.

CONCLUSIONS

The authors concluded that the high APFO/inulin clearance ratio in females provided evidence that APFO is excreted in part by an active secretion mechanism. The decrease in the APFO/inulin clearance ratio in females from 14.5 to 0.46 after the administration of probenecid supports this conclusion. The lower APFO than inulin clearances in both sexes after administration of probenecid indicated partial tubular reabsorption of APFO in both sexes. This secretory mechanism is lacking or inactive in males and accounts for the greater toxicity of APFO in male rats.

REFERENCE

Hanhijarvi, H., R. Ophaug, and L. Singer. 1982. The sex-related difference in perfluorooctanoate excretion in the rat. Proceedings of the Society for Experimental Biology and Medicine; 171:50-55.

PHARMACOKINETIC STUDY

Title: Elimination and Toxicity of Perfluorooctanoic Acid During Subchronic Administration in the Wistar Rat

TEST SUBSTANCE

Identity: Perfluorooctanoic acid (PFOA)

Remarks: Purity was not specified

METHOD

Method/guideline followed: No specific guideline was listed

Test type: 28-Day subchronic toxicity and elimination study

Species/strain/cell type or line: Wistar rat

Sex: Female and male

Age and body weight range of animals used: Newly-weaned

Number of animals/sex/dose: 6 animals/sex/dose

Route of administration: Oral gavage

Vehicle: 0.9% NaCl solution

Doses: 0, 3, 10, 30 mg/kg/day

Excretion routes, body fluids, and tissues monitored and/or sampled during study: During the study period, the animals were weighed once a week and 24 hour urine was collected on study days 7 and 28. The behavior and feed and water consumption were observed regularly. At the end of the study period, blood and tissue samples were collected from control and 30 mg/kg rats.

Statistical methods used: Student's t-test, Pearson product moment correlation and Fisher's exact test were the principal statistical procedures used for the evaluation of the findings.

Method remarks: PFOA treatment occurred over 28 consecutive days. The administered volume of the saline solutions was always 0.5 ml/100g animal weight. PFOA concentrations in blood and urine samples were analyzed by gas chromatography.

RESULTS

Detailed results from Day 7: On the seventh day of the study period, the mean urinary excretion of PFOA (mg/24 hrs/kg) was lower than the daily dose in all three groups of males. A statistically significant difference from the daily dose was found in all three groups of male rats ($P<0.05$ and $P<0.001$, t-test). In the female animals, none of the three groups excreted significantly less PFOA in the urine on day 7 than they were administered. The mean urinary PFOA concentration of the female rats in the lowest

dose group was significantly higher ($P<0.01$) than that of the male animals. In the group receiving the highest daily dose of PFOA, the urinary PFOA concentration was significantly higher ($P<0.05$) in the male animals; however, this can be explained by the significantly lower ($P<0.05$) mean urinary volume of the male rats.

Detailed results from Day 28: By test day 28, the males seemed to have also reached the steady state, with the exception of the 10 mg/kg group which showed a significantly lower ($P<0.05$) excretion of PFOA than the administered daily dose.
At each dose level, the mean PFOA concentrations in the plasma of the male rats were significantly higher than those of the female animals ($P<0.001$ for the two highest dose groups and $P<0.01$ for the lowest dose group).
A detailed statistical analysis of organ weights did not reveal any differences. The weights of the liver relative to body weights of male rats showed a significant positive dose response ($P<0.001$, Pearson). A possible explanation was the significant dose-related decrease in the bodyweights found in the males ($P<0.05$, Pearson); however, the difference between the control males and the lowest-dose group was more obvious than between any of the dosed groups ($P<0.01$, t-test).
In the high-dose group females, one or more mild inflammatory foci were observed in four livers, which was not significantly different from control females with only one small chronic focus (Fisher's exact test).

Metabolites measured: None

CONCLUSIONS

The mean urinary excretion suggests that the average renal clearance of PFOA in the female rats is about 10 times higher than in the male animals. The sex-related difference in plasma PFOA concentrations are best explained by active tubular secretion in the female kidney. The steady state was achieved by 7 days in the female animals only. By the 28^{th} day, both males and females excreted roughly the same amount of PFOA in 24 hours as their daily dose.

REFERENCE

Hanhijarvi, H., M. Ylinen, A. Kojo, and V. Kosma. 1987. Elimination and toxicity of perfluorooctanoic acid during subchronic administration in the Wistar rat. Pharmacology and Toxicology; 61:66-68.

PHARMACOKINETIC STUDY

Title: A Proposed Species Difference in the Renal Excretion of Perfluorooctanoic Acid in the Beagle Dog and Rat

TEST SUBSTANCE

Identity: Perfluorooctanoic Acid (PFOA)

Remarks: No information on supplier, lot number, or purity of substance tested was given.

METHOD

Method/guideline followed: NA

Test type: In vivo

Species/strain/cell type or line: Beagle dogs

Sex: male/female

Age and body weight range of animals used: Not stated

Number of animals/sex/dose:
Group 1: 6 (3 males and 3 females) given 30 mg/kg PFOA with continuous infusion of 5% mannitol solution at 1.7 ml/min. Probenecid was then administered at 30 mg/kg intravenously.
Group 2: 4 (2 males and 2 females) given 30 mg/kg PFOA only and kept in metabolic cages.

Route of administration: Intravenous

Vehicle: Not stated

Doses: 30 mg/kg – see above (under Number of animals/sex/dose)

Excretion routes, body fluids, and tissues monitored and/or sampled during study: Group 1: urine and blood samples
Group 2: blood samples

Statistical methods used: Paired Student's t-test used to determine statistical significance of the reduction of PFOA clearance in each dog before and after administration of probenecid.

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Method remarks:
Group 1: Dogs were anaesthetized with methoxyflurane. Catheters were fixed in both ureters after laparotomy and cystotomy. Urine was collected at 10 min intervals for 60 min. (Dosing and other methods described above.)
Group 2: Dogs were kept in metabolic cages after the injection. Half-lives were determined for each animal separately.
PFOA concentrations were analyzed with a GLC-method.

RESULTS

Detailed results: Probenecid effectively and statistically significantly reduced PFOA clearance in each dog ($P < 0.05$ for each dog).
PFOA plasma half-lives (males): 473 and 541 hours
PFOA plasma half-lives (females): 202 and 305 hours

Metabolites measured: None. It appears that only PFOA was measured.

CONCLUSIONS

Because of the statistical significance of the reduction of PFOA clearance by probenecid, males and females have an active secretion mechanism for PFOA. Plasma half-lives were longer in males than females.

The authors also compare the clearance rates with those in rats, noting that the glomular filtration rate of PFOA is similar in both species. However, the active tubular secretion rate of PFOA in the rat and dog are quite different.

REFERENCE

Hanhijarvi, H., M. Ylinen, T. Haaranen, and T. Nevalainen. 1988. A proposed species difference in the renal excretion of perfluorooctanoic acid in the beagle dog and rat In: Beynen, A.C. and H.A. Solleveld (Eds). *New Developments in Biosciences: Their Implications for Laboratory Animal Science*. Martinus Nijhoff Publishers. Dordrecht, Netherlands.

PHARMACOKINETIC STUDY

Title: Tissue Distribution, Metabolism, and Elimination of Perfluorooctanoic Acid in Male and Female Rats

TEST SUBSTANCE

Identity: [1-^{14}C]Perfluorooctanoic acid (PFOA, 99% pure, specific activity 5.44 mCi/mmol)

Remarks: Synthesized and purified according to methods described previously

METHOD

Method/guideline followed: NA

Test type: In vivo

Species/strain/cell type or line: Sprague-Dawley rats

Sex: males and females

Age and body weight range of animals used:
Age = six weeks
Weight (males) = 170-195 grams
Weight (females) = 130-155 grams

Number of animals/sex/dose: 4 of each sex for tissue distribution experiments;
32 males and 16 females for tissue elimination experiments

Route of administration: intraperitoneal

Vehicle: Propylene glycol/water (1:1 v/v)

Doses: 4 mg/kg

Excretion routes, body fluids, and tissues monitored and/or sampled during study: urine, plasma, liver, bile, feces (e.g., tissues able to metabolize endogenous fatty acids or were target tissues for PFOA toxicity)

Statistical methods used: Not stated

Method remarks: none

RESULTS

Males:

Tissue distribution – Percent dose on a per gram basis (from highest to lowest) in monitored organs was: liver, plasma, kidneys, heart, testes, fat (epididymal fat pad), gastrocnemius muscle. The liver and plasma were the primary tissues of distribution. [See Table 1 of the study for percentages by time after administration.] The high concentration in liver was examined further in males by an *in situ* nonrecirculating liver perfusion technique – '11% of the PFOA infused was extracted by the liver in a single pass'.

Elimination – Urine and feces were both major routes of elimination. Cumulative excretion of PFOA in urine and feces after 28 days was 36.4% and 35.1% respectively. The apparent half-life for whole-body elimination of PFOA was 15 days (360 hours).

Half-lives –

Organ	Hrs	Days
Liver	271.2	11.3
Plasma	216	9.0
Kidney	225.6	9.4
Heart	249.6	10.4
Gastrocnemius	220.8	9.2
Fat	192	8.0
Testis	216	9.0
Blood	211.2	8.8

Females:

Tissue distribution – Percent dose on a per gram basis (from highest to lowest) in monitored organs was: plasma, kidneys, liver, ovaries. The liver, plasma, and kidneys were the primary tissues of distribution. [See Table 2 of the study for percentages by time after administration.]

Elimination – Urine was the major route of elimination. Cumulative excretion of PFOA in urine after 1 day was 91%, whereas the amount in feces was negligible. The apparent half-life for whole-body elimination of PFOA was < 1 day.

Half-lives –

Organ	Hrs
Liver	3.8
Plasma	2.9
Kidney	3.2
Blood	3.8

Metabolites measured: Defluorination of PFOA was measured to determine whether it was metabolized. Specific metabolites were not discussed.

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CONCLUSIONS

A sex difference in urinary elimination of PFOA was observed, resulting in different whole-body elimination half-lives. However, there was no apparent difference in biliary excretion – both males and females eliminated less than 1% of the PFOA dose by this route. Second, the greater persistence of PFOA in male rats was not due to greater formation of PFOA-lipid conjugates. Third, the parent compound appears to be the only tissue storage form of PFOA in both sexes as well as the only compound excreted in urine and bile; therefore, a differential rate of metabolite formation by sex was not responsible for sex differences. (The authors also discuss the unpublished results of Vanden Heuvel et al. 1992, noting the possible link between testosterone and inhibition of PFOA excretion.)

REFERENCE

Vanden Heuvel, J.P., Kuslikis, B.I., Van Rafelghem, M.L. and Peterson, R.E. 1991. Tissue distribution, metabolism, and elimination of perfluorooctanoic acid in male and female rats. J. Biochem. Toxicol. 6(2):83-92.

PHARMACOKINETIC STUDY

Title: Renal Excretion of Perfluorooctanoic Acid in Male Rats: Inhibitory Effect of Testosterone

TEST SUBSTANCE

Identity: [^{14}C]-Perfluorooctanoic acid (PFOA, 99% pure, specific activity 5.44 mCi/mmol)

Remarks: Synthesized according to methods described previously

METHOD

Method/guideline followed: NA

Test type: In vivo

Species/strain/cell type or line: Sprague-Dawley rats

Sex: males and females

Age and body weight range of animals used:
Age = six weeks
Weight (males) = 170-195 grams
Weight (females) = 130-155 grams

Number of animals/sex/dose: 4/sex/dose

Route of administration: intraperitoneally (i.p.)

Vehicle: Propylene glycol/water (1:1 v/v)

Doses: 4 mg/kg (administered as [1-^{14}C]PFOA one week after vehicle, 17beta-estradiol, or testosterone implantation)

Excretion routes, body fluids, and tissues monitored and/or sampled during study:
Urine, plasma, feces, liver tissue, kidney tissue

Statistical methods used: Differences between treatment groups were determined by one-way analysis of variance (ANOVA). Duncan's t-test was performed to compare individual mean values when ANOVA showed differences. (Level of significance tested was $p<0.05$.)

Method remarks: To test differences in elimination of PFOA between males and females, rats were castrated, ovariectomized, or sham operated; estrogen or testosterone was administered.

Estrogen (as 17beta-estradiol) capsules were inserted subcutaneously into castrated males (through an incision posterior to the scapulae).

Testosterone capsules were inserted subcutaneously in castrated males or ovariectomized females (through an incision made posterior to the scapulae).

Groups of sham-operated males and females, castrated males, and ovariectomized females were implanted with tubing only and served as controls for the implantation procedure.

In castrated male rats, probenecid was administered (at 65 mg/kg i.p.) 30 min prior to administration of PFOA. 7 hours after PFOA administration, rats were anaesthetized with Nembutal (50 mg/kg i.p.).

RESULTS

Males:

Tissue PFOA Concentrations*
(% Dose ^{14}C-PFOA/g)**

	Liver	Kidney	Plasma
sham/vehicle	1.91 ± 0.12	0.91 ± 0.07	1.48 ± 0.29
castrated/vehicle	0.11 ± 0.03	0.08 ± 0.03	0.021 ± 0.006
castrated/17beta-estradiol	0.02 ± 0.001	0.02 ± 0.004	0.006 ± 0.002
castrated/testosterone	1.53 ± 0.05	0.40 ± 0.03	1.07 ± 0.05

*Significant differences in liver, kidney, and plasma concentrations of PFOA were observed among treatment groups (sham/vehicle, castrated/vehicle, castrated/17beta-estradiol, castrated/testosterone). In liver and kidneys, PFOA concentration was highest in the sham/vehicle group, and 2nd highest in the castrated/testosterone group. In plasma, PFOA was highest (and similar in concentration) in the sham/vehicle and castrated/testosterone groups. Plasma PFOA concentrations in castrated/vehicle and castrated/17beta-estradiol groups were very low.
** Source: Table 1, pg. 33

Urinary Elimination

sham/vehicle	16% of dose in 4 days
castrated/vehicle	> 1 time greater than sham/vehicle
castrated/17beta-estradiol	> 1 time greater than sham/vehicle
castrated/testosterone	similar to sham/vehicle

Fecal excretion: *This was less than urinary excretion. Regardless of treatment group, the cumulative percent of PFOA dose eliminated in feces over 4 days was similar in sham/vehicle, castrated/vehicle, castrated/17beta-estradiol groups. Excretion was higher in the castrated/testosterone group.*

Effect of administering probenecid: ***This had no effect on urinary PFOA elimination in sham/vehicle, but reduced PFOA elimination in castrated groups. There were no differences in plasma concentrations of PFOA among treatment groups after probenecid.***

Hyaline droplet formation (a test for PFOA binding to male-specific protein alpha2u-globulin): Histopathologic analysis did not reveal formation of hyaline droplets in the male kidney.

Females: *Tissue concentrations:* No significant differences in liver, kidney, and plasma concentrations of PFOA were observed among treatment groups (sham/vehicle, ovariectomized/vehicle, ovariectomized/testosterone). *Urinary elimination:* No significant differences; all rats eliminated about 70% of the PFOA dose in urine in 4 days. *Fecal excretion*: no significant differences; all excreted less than 0.5% of the dose.

Metabolites measured: None

CONCLUSIONS

These results suggest that testosterone pretreatment inhibits elimination of PFOA in the male. Also, probenecid reduced PFOA elimination in castrated male rats that had increased PFOA urinary excretion. Testosterone is a key determinant of the sex difference in PFOA elimination in rats.

Remarks: none

REFERENCE

Vanden Heuvel, J.P., Davis, J.W., Sommers, R., and Peterson, R.E. 1992. Renal excretion of perfluorooctanoic acid in male rats: Inhibitory effect of testosterone. J. Biochem. Toxicol. 7(1):31-36.

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PHARMACOKINETIC STUDY

Title: Covalent Binding of Perfluorinated Fatty Acids to Proteins in the Plasma, Liver, and Testes of Rats

TEST SUBSTANCE

Identity: [1-^{14}C]Perfluorooctanoic acid ([1-^{14}C]PFOA)

Remarks: 99% pure, specific activity = 51.7 mCi/mmol

METHOD

Method/guideline followed: Not specified

Test type: In vivo

Species/strain/cell type or line: Rat/Harlan Sprague-Dawley, obtained from Harlan Sprague-Dawley (Indianapolis, IN)

Sex: Male

Age and body weight range of animals used: Six-weeks, 170-195 grams

Number of animals/sex/dose: Not specified

Route of administration: Intraperitoneal (i.p.)

Vehicle: Propylene glycol/water (1:1, v/v; 1 ml/kg)

Doses: 9.4 umol/kg

Excretion routes, body fluids, and tissues monitored and/or sampled during study: Liver, plasma, testes

Statistical methods used: Differences from control were determined by one-way analysis of variance. The least significant differences test was used to compare individual means where analysis of variance indicated differences. The level of significance for all analyses was $P < 0.05$.

Method remarks:

Test animals and test substances

[1-^{14}C]Perfluorooctanoic acid was synthesized and purified as described by I.L. Reich et al (1987). Hemoglobin (2 x crystallized, dialyzed and lyophilized), fatty acid free bovine serum albumin, and all tissue culture media and supplements were obtained from Sigma Chemical Company (St. Louis, MO). Reagent grade solvents were obtained from Aldrich Chemical Company (Milwaukee, WI). Water purification was performed by passing distilled water through a four-bowl Milli-Q water purification system and a 0.25 m filter (Millipore Corp, Milford, MA). Rats were individually housed in suspended stainless-steel cages in a temperature-controlled room (approximately 21 C). A 12-hour light and 12-hour dark photoperiod was

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maintained. Food (Purina Rat Chow No. 5012, Ralston Purina Co, St. Louis, MO) and water were provided *ad libitum* throughout the study. An acclimation period of at least 1-week was allowed before initiation of the experiment.

Covalent binding of PFOA to proteins in vivo

[1-^{14}C]Perfluorooctanoic acid ([1-^{14}C]PFOA) was administered to rats in propylene glycol/water (1:1, v/v; 1 ml/kg) at a dose of 9.4 mol/kg, i.p. At designated times post-treatment, rats were anesthetized with Nembutal (50 mg/kg, i.p.); tissues were quickly removed, freeze clamped, and stored at -70 C. Blood was removed in a lightly heparinized needle and plasma was separated by centrifugation. Macromolecular binding was determined on tissue homogenates, which were brought up to 10% TCA and put on ice for 10 minutes. To remove any unbound perfluorinated acid, samples were washed with 3 mL of methanol/ether (3:1) 6 times and then 3 more times using 3 mL of ethyl acetate. Washing was continued, if necessary, until no additional PFOA-derived ^{14}C could be extracted from the protein. One milliliter of 1 N NaOH was added to the protein precipitate and placed in a shaking water bath overnight at 37 C to solubilize the protein precipitate. The sample was then neutralized with 1 M acetic acid and 1 mL was used for liquid scintillation counting,while another aliquot was used for protein analysis. The protein analysis was performed according to O.H. Lowry et al (1951). PFOA-derived radioactivity in all samples was quantitated using a Packard Liquid Scintillation Analyzer with quench correction performed with a Packard DPM 1-2-3 software program. Radioactivity in hydrolysates was determined in Hionic-Fluor scintillation cocktail. Binding was expressed as pmol PFOA equiv/mg protein.

Covalent binding of PFOA to hemoglobin and albumin in vitro

^{14}C-labeled PFOA was added to tubes containing albumin or hemoglobin in 1.0 mL of phosphate buffer to give a 2, 4, 8, or 100 M solution. If methionine or cysteine were present, they were added in 1 mL of phosphate buffer to bring the reaction mixture up to 2 mL. All experiments were done using a final volume of buffer of 2 mL. The time of incubation was 1 hour at 37 C. Protein was precipitated with 10% TCA. To remove any free unbound perfluorinated acid, samples were washed with 5 mL of acetone 5 times. Samples were vortexed for 1 minute and spun at 2500 revolutions/minute for 5 minutes after each acetone wash step. The acetone was transferred off. Protein samples were then washed with 5 mL of ether 2 additional times. Washing was continued if necessary until no additional PFOA (as determined by liquid scintillation counting) could be extracted from the protein. The protein powder was hydrolyzed with 1 mL of 1 N NaOH at 37 C in a shaking water bath overnight. Binding was expressed as pmol PFOA equiv/mg of protein.

Selection of target tissues for the analysis of PFOA-binding to proteins

The liver, plasma, and testis were selected as target tissues for the analysis of PFOA-binding to proteins because they are either major tissues of distribution for PFOA in rats (liver and plasma) or they are target organs for perfluorinated acid toxicity (liver and testes).

RESULTS

Detailed results:

Covalent binding of PFOA to target tissues in rats

The covalent binding data for PFOA for each tissue (2 hour, 1 day, and 4 days after treatment) were pooled because no time-dependent changes in the absolute and relative concentrations of covalently bound protein were observed. The tissue elimination half-life for PFOA (average tissue $t_{1/2}$ = 9 days) showed that there was little difference in tissue concentrations of PFOA in the

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range of 2 hours to 4 days. In PFOA-treated rats, the absolute concentration of covalently bound PFOA was significantly higher in the plasma than in the liver. Of the tissues examined, the testes had the highest relative concentration of PFOA-derived radioactivity covalently bound to protein. Approximately 0.4% of the testes PFOA concentration was covalently bound. To determine which proteins were modified following in vivo administration of PFOA, tissues were homogenized and subjected to SDS-PAGE/autoradiography with fluorography. Due to the small amount of covalently bound ^{14}C found within these tissues, no radioactive protein bands were detected when 50 µg protein was separated on SDS-PAGE and the X-ray film was exposed to the gel for up to 2 months at -70°C.

Covalent binding of PFOA to hemoglobin and albumin in vitro

The covalent binding of PFOA to hemoglobin was diminished by the addition of cysteine with no effect of methionine in parallel incubations.

Metabolites measured: [1-^{14}C]Perfluorooctanoic acid

CONCLUSIONS

Despite the metabolic inertness of the perfluorinated fatty acids, the results indicated that PFOA binds to proteins in the plasma, liver, and testes in a covalent manner. Although PFOA-derived radioactivity could be found covalently associated with protein following the organic extractions, the amount of radioactivity associated with any given protein was not enough to be seen following SDS-PAGE/autoradiography. The *in vitro* covalent binding of PFOA was reduced following the addition of cysteine to the incubation, but not the addition of methionine to the incubation. Thus, the ability of cysteine to inhibit PFOA covalent binding of proteins suggested that protein sulfhydryl groups may be involved; sulfhydryl groups may be the sites of covalent attachment of PFOA.

REFERENCE

Vanden Heuvel, J.P., Kuslikis, B.I., and Peterson, R.E. 1992. Covalent binding of perfluorinated fatty acids to proteins in the plasma, liver and testes of rats. Chem.-Biol. Interact. 82:317-328.

PHARMACOKINETIC STUDY

Title: Cholestyramine-Enhanced Fecal Elimination of Carbon-14 in Rats after Administration of Ammonium [^{14}C]Perfluorooctanoate or Potassium [^{14}C]Perfluorooctanesulfonate

TEST SUBSTANCE

Identity: Ammonium [^{14}C]perfluorooctanoate ([^{14}C]-APFO)

Remarks: [^{14}C]-APFO: specific activity = 0.51 μCI/mg, radiochemical purity >98%

METHOD

Method/guideline followed: NA

Test type: in vivo

Species/strain/cell type or line: rat, Charles River CD

Sex: male

Age and body weight range of animals used: 12 weeks, 300 – 342 grams

Number of animals/sex/dose: 5

Route of administration: intravenous

Vehicle: 0.9% NaCl

Doses: Single doses were administered as follows: animals treated with cholestyramine =13.3 mg/kg mean, animals not treated with cholestyramine = 13.5 mg/kg mean (control group)

Excretion routes, body fluids, and tissues monitored and/or sampled during study: Urine, feces, plasma, red blood cells, and liver were monitored and/or sampled during the study.

Statistical methods used: Data were expressed as means ± standard deviations. The student's t-test was used to evaluate significance of difference between two groups at a significance level of $p < 0.05$.

Method remarks: Rats were housed in individual stainless-steel metabolism cages and fasted with free access to water for 24 hours prior to administration of the test substance. Carbonyl-labeled [^{14}C]APFO was obtained from 3M. Cholestyramine was obtained from Mead Johnson. The dosing solutions were prepared by dissolving the test substance in 0.9% NaCl (the [^{14}C]APFO solution contained 2.1 mg/mL). Cholestyramine (dried and ground resin Z-620) was mixed, 4% by weight, with Purina Lab Chow. The radio-labeled test substance was administered as a single intravenous dose (lateral tail vein). Two mL of the dosing solution was administered to each of 10 rats. Five rats were given 4% cholestyramine in feed (ad libitum); the other rats were given normal Purina Lab Chow. In order to allow a comparison of the radiometric results on an absolute basis, the radio-labeled doses were not adjusted for individual body weights. The average doses as administered to each group were 13.3 mg/kg (cholestyramine-treated animals) and 13.5 mg/kg (control animals). Urine and feces samples were collected at intervals for individual rats 14 days after administration of the test substance. At this time, the rats were anesthetized

with diethyl ether and exsanguinated by drawing blood from the descending aorta. Plasma and red blood cells were prepared promptly by centrifugation. The liver was collected as a whole organ and stored frozen until analysis.

RESULTS

Detailed results: After 14 days of cholestyramine treatment, the mean percentage of dose eliminated via feces (43.2 ± 5.5) was 9.8-fold the mean percentage of dose eliminated via feces by control rat (4.4 ± 1.0). The difference was found to be significant. After adjustment for the amount of carbon-14 excreted in the urine (67% for controls and 41% for cholestyramine-treated), the amounts of carbon-14, which remained to be excreted were 16% for chloestyramine-treated rats and 28% for control rats. The mean liver carbon-14 content represented 4- and 8% of the dose for cholestyramine-treated and control rats, respectively. The mean carbon-14 concentrations of plasma and red blood cells with cholestyramine-treatment were significantly less than the mean concentration in controls. The mean data from analyses of liver, plasma, and red blood cells for carbon-14 content 14 days after [14C]APFO administration are shown in Table 1.

Table 1. Effect of Cholestyramine Treatment on Concentration of Carbon-14 in Rat Liver, Plasma, and Red Blood Cells After a Single Intravenous Dose of [^{14}C]APFO

Treatment Group	Carbon-14 Concentration (µg eq/g tissue or mL fluid)		
	Liver	Plasma	Red BloodCells
Cholestyramine-treated	12.1 ± 2.1*	5.1 ± 1.7*	1.8 ± 0.7*
Controls	22.3 ± 6.2	14.7 ± 6.8	4.2 ± 2.4

*Significantly different from control values ($p < 0.05$)

Metabolites measured: None

CONCLUSIONS

The data support the possible utility of cholestyramine as a promotor of the excretion of perfluorooctanoate in humans.

Reviewer's Remarks: The author's conclusions appear to be supported by the data.

REFERENCE

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

PHARMACOKINETIC STUDY

Title: Metabolic Handling of Perfluorooctanoic Acid in Rats

TEST SUBSTANCE

Identity: Perfluorooctanoic Acid (PFOA)

Remarks: Perfluorooctanoic acid (a mixture of linear and branched isomers) was supplied by Minnesota Mining and Manufacturing Company, St. Paul, MN 55101. Its purity was not indicated.

METHOD

Method/guideline followed: Not specified

Test type: Fluoride determinations and ultrafiltration studies were utilized—Rat *in vivo* and *in vitro*; Human serum-*in vitro*

Species/strain/cell type or line: Holtzman rat; Human serum

Sex: Rat-female; Human-not specified

Age and body weight range of animals used: Rats weighed approximately 250 grams, age of rats was not specified; Human serum-age and weight of source was not specified

Number of animals/sex/dose: Fluoride determination—not specified; Ultrafiltration studies—Rats serum-two aliquots; Human serum-three aliquots

Route of administration: Rats were dosed via stomach intubation

Vehicle: Rats were dosed with an aqueous solution of perfluorooctanoic acid

Doses: Rats were dosed with 2 mg nonionic fluorine as perfluorooctanoic acid; additionally, four rats were fed a low fluoride (<0.5 ppm) diet; Human serum-0, 75, or 1500 µg nonionic fluorine (as perfluorooctanoic acid)

Excretion routes, body fluids, and tissues monitored and/or sampled during study: Rat-serum, urine, and feces were analyzed for fluoride determinations and ultrafiltration studies; Human-serum was analyzed for ultrafiltration studies
Statistical methods used: Means were statistically compared by calculating Student's t-value. A P-value of <0.025 was chosen as indicating significance.

Method remarks:

Fluoride determinations—Rats were administered 2 mL of an aqueous solution containing 2 mg of nonionic fluorine, as perfluorooctanoic acid, by stomach intubation. The animals were placed in individual metabolic cages and fed rat chow (Purina) and tap water (1ppm fluoride) *ad libitum* for 4.5, 8, 24, or 52.5 hours. In addition, four rats were placed in metabolic cages and fed a low fluoride (<0.5 ppm) diet and distilled water for a period of 96 hours. This resulted in a substantial decrease in ionic fluoride content of the feces and facilitated the analysis for nonionic fluorine. A few crystals of thymol were added

to the urine containers to inhibit bacterial growth during the collection period. At the end of the experimental period, the urine and feces were collected and the anesthetized animals were sacrificed by cardiac puncture. The blood was allowed to clot and the serum was collected after centrifugation and stored frozen until analyzed. Urine, serum, and feces were also obtained from undosed animals to provide baseline data. The volumes of urine collections, which included water needed to rinse the metabolic cages, and the weight of the feces were recorded. A small known quantity of distilled water was added to the feces samples, which were then homogenized to a thick slurry. The serum and urine were analyzed for ionic fluoride at pH 5.0 with the fluoride ion-specific electrode. The ionic fluoride in the diffusate of the feces was determined at pH 5.0 with the fluoride electrode after isolation of the fluoride by diffusion from perchloric acid at 60 C (the fluoride in perfluorooctanoic acid is not acid labile under these conditions). The total fluorine content of serum, urine, and feces was determined with the oxygen-bomb reverse extraction technique. Fifteen mL of redistilled water was added to the oxygen bomb (prior to combustion of the samples) to act as a fluoride trap. Up to 0.3 mL of serum or urine was pipetted onto a 0.3-gram pellet of filter paper pulp and fired without drying in the oxygen bomb. Larger volumes (up to 3 mL) of serum low in fluoride were pipetted onto the pellet and lyophilized prior to combustion. The method was slightly modified for the analysis of the feces in that 15 mL of total ionic strength activity buffer (TISAB, Orion Research, Inc.) was added to the oxygen bomb prior to firing instead of 15 mL of water. Blank and recovery samples were carried through the entire procedure.

Ultrafiltration studies—The pH of two 90-mL aliquots of human serum was adjusted to 7.4 by equilibration with a mixture of 95% air and 5% carbon dioxide. Seventy-five g of nonionic fluorine (as perfluorooctanoic acid) was added to one aliquot and 1500 g was added to the second. After 2 hour, 10 mL of the spiked serum was removed for determination of the initial concentration of perfluorooctanoic acid and the remaining serum containing perfluorooctanoic acid was transferred to an Amicon TCFIO ultrafiltration unit fitted with a Diaflo XM50 membrane, which retains molecules >50,000 daltons. The sample chamber was flushed with 95% air, 5% carbon dioxide, pressurized with nitrogen, and 15 mL of ultrafiltrate was collected. An ultrafiltrate was also prepared from pooled serum obtained from four rats (dosed for the fluoride determination study) 4.5 hours after receiving the 2 mg of nonionic fluorine as perfluorooctanoic acid by stomach intubation and from 75 mL of buffered (pH 7.4) isotonic saline to which 900 g of nonionic fluorine had been added.

RESULTS

Detailed results:

Employing the oxygen bomb reverse extraction technique for determination of total fluorine, a recovery of 80 1.0 SEM% was obtained for perfluorooctanoic acid. The recovery of fluoride from perfluorooctanoic acid was increased to 94 1.5% when 15 mL of TISAB buffer, instead of water, was used as the fluoride trap inside the oxygen bomb. The quantity of nonionic fluorine was calculated by subtracting the ionic fluoride from the total fluorine determined after ashing in the oxygen bomb and dividing by either 80 or 94%. The blank for the procedure employing a 0.3-gram filter paper pulp pellet and 15 mL of redistilled water as a fluoride trap was 0.13 0.020 g. The modified procedure used to determine the total fluoride content of the feces had a blank of 0.40 0.043 g.

The results obtained in the ultrafiltration study of the binding of perfluorooctanoic acid in serum are given in Table 1. The control experiment in which perfluorooctanoic acid was added to an isotonic saline solution buffered at pH 7.4 provided evidence that the ultrafiltration membrane bound very little perfluorooctanoic acid. Virtually all of the nonionic fluorine (as perfluorooctanoic acid) added to the saline solution was ultrafilterable. The addition of perfluorooctanoic acid to human serum, in amounts

which elevated the nonionic fluorine to levels as high as 16 ppm, resulted in at least 99% of the added nonionic fluorine being bound to serum constituents and not being ultrafilterable. Serum harvested from rats 4.5 hour after the administration of a 2-mg dose of nonionic fluorine, as perfluorooctanoic acid, had a nonionic fluorine level in excess of 13 ppm and virtually all of this was bound to components in the serum and not ultrafilterable. Prior to intubation of perfluorooctanoic acid, the ionic and nonionic fluorine levels were 0.032 and 0.07 ppm, respectively. Within 4.5 hours after the administration of the perfluorooctanoic acid, the nonionic fluorine in the serum rose to 13.6 ppm. Despite the large increase (200-fold) in the nonionic fluorine level in the serum, the ionic fluoride level remained very low (0.03 ppm). The nonionic fluorine level in the serum decreased to 11.2 and 0.35 ppm at 8 hours and 24 hours, respectively. The level observed at 24 hours was still approximately seven times the baseline level. By 96 hours, the mean level of nonionic fluoride in the serum had decreased to 0.08 ppm, a value that was not statistically different from that of the undosed animals. Throughout the entire 96 hours, the ionic fluoride level of the serum remained very low.

Table 1. Fluoride Content of Serum, Serum Ultrafiltrates, and Buffered Isotonic Saline Before and After the Addition of Perfluorooctanoic Acid[a]

Specimen	Treatment	Specimen		Ultrafiltrate	
		Ionic	Total	Ionic	Total
Buffered Saline (pH 7.4)	Perfluorooctanoic acid added	-	12.1	-	11.1
Human Serum					
Aliquot 1	None	0.014	0.044	-	-
Aliquot 2	Perfluorooctanoic acid added	0.015	0.88	0.017	0.040
Aliquot 3	Perfluorooctanoic acid added	0.018	16.3	0.025	0.086
Rat Serum					
1	Control serum[b]	0.032	0.11	-	-
2	Experimental serum[c]	0.013	13.2	0.016	0.12

[a]Fluoride contents were reported as ppm and were the mean of two determinations.
[b]Pooled serum from normal rats.
[c]Pooled serum from rats 4.5 hours after administration of a 2-mg dose of nonionic fluorine as perfluorooctanoic acid.

The urine was analyzed for nonionic and ionic fluorine since the *in vivo* data showed a rapid removal of nonionic fluorine from the serum; the results are shown in Table 2. Although the urine from the undosed animals contained no detectable nonionic fluorine, within 4.5 hours after receiving the dose, an average of 749 g or 37% of the fluorine in the administered dose was recovered in the urine. The quantity of nonionic fluorine in the urine increased to 61% of the administered dose at 8 hours and by 24 hours 76% had been excreted in the urine. Between 24 and 96 hours of the experimental period, the quantity of nonionic fluorine found in the urine increased to 89%. The rate of excretion of nonionic fluorine thus increased from 0 g/hour in the undosed animals to 166 g/hour, 4.5 hours after receiving the dose of perfluorooctanoic acid. The rate of nonionic fluorine excretion rapidly decreased to 5 g/hour over the interval from 52.5 to 96 hours. The rate of excretion of ionic fluoride in the urine for the undosed animals, based on a 24-hour urine collection, was found to be 1.73 g F/hour. Although there was a tendency toward an increased ionic fluoride excretion in the dosed animals, ionic fluoride excretion rate was not statistically greater than that of the nondosed animals.

000105

Table 2. Urinary Excretion of Ionic and Nonionic Fluoride Following Administration of a 2-mg Dose of Nonionic (As Perfluorooctanoic Acid) to Rats

Time (hr)	Ionic[a] (g/hr)	Nonionic[a]	
		g/hr	Accumulative % of dose
Baseline	1.73 0.24[b]	0	0
4.5	1.73 0.22	166 39.8	37 9.0
8.0	2.36 0.34	134 33.6	61 5.8
24	2.36 0.54	19 3.2	76 2.7
52.5	1.83 0.32	5 1.2	81 2.7
96	-[c]	5 1.3	89 2.6

[a]Excretion rates for ionic and nonionic fluoride were calculated as g excreted/hr from the preceding time point. The baseline value was based on a 24-hour urine collection.
[b]Mean SEM
[c]The ionic fluoride excretion rate for the 96-hour period is not given since these animals were fed a low fluoride diet.

In an effort to account for the remaining nonionic fluorine, feces collected from dosed animals over 52.5- and 96-hour periods were analyzed for nonionic fluorine. The percentage of the administered dose of nonionic fluorine recovered in the urine and feces of animals after 52.5 and 96 hours is shown in Table 3. After 52.5 hours, a mean of 4.5% of the administered dose of nonionic fluorine was recovered in the feces. An additional 81.5% was present in the urine, which brought the total quantity of nonionic fluorine excreted in the feces and urine to 86%. By 96 hours, however, the quantity of nonionic fluorine excreted in the feces had increased to 14.3% and the entire dose of nonionic fluorine had been excreted in the urine and feces.

Table 3. Percentage of the Administered Dose of Nonionic Fluorine Recovered in the Urine and Feces after 52.5 and 96 hours

Time (hr)	Urine	Feces	Total
52.5	81.5 3.9	4.5 1.0	86.0 3.3
96	89.3 2.6	14.3 4.1	103.5 1.7

Metabolites measured: Ionic and nonionic fluorine

CONCLUSIONS

The nonionic fluorine level in the serum was increased 200-fold after administration of the dose, but returned to baseline levels by 52.5 hours. Although perfluorooctanoic acid is rapidly absorbed and bound to nonultrafilterable components in the serum, the entire dose of nonionic fluorine was recovered in the urine and feces after 96 hour. Neither the ionic fluoride level in the serum nor the rate of ionic fluoride excretion in the urine was significantly altered by the administration of perfluorooctanoic acid. Although perfluorooctanoic acid has not been identified in the urine, the available data suggest that it has been excreted intact or in, possibly, a conjugated form.

REFERENCE

00106

Ophaug, R.H. and L. Singer. 1980. Metabolic Handling of Perfluorooctanoic Acid in Rats. Proc Soc Exp Biol Med. 163:19-23.

000107

PHARMACOKINETIC STUDY

Title: Stimulation by oestradiol of the urinary excretion of perfluorooctanoic acid in the male rat

TEST SUBSTANCE

Identity: Perfluorooctanoic acid (PFOA); 98% pure.

Remarks: The test substance was dissolved in a mixture of propylene glycol-water (1:1) at a concentration of 25 mg/ml.

METHOD

Method/guideline followed: NA

Test type: *in vivo*

Species/strain/cell type or line: rat/Wistar

Sex: male and female

Age and body weight range of animals used: age not specified, male bw range 170-300g, female bw range 150-180g

Number of animals/sex/dose: Dosed with vehicle: 6 males and 6 females; Dosed with single intraperitoneal injection of PFOA: 6 females, 6 males, 10 castrated males, 10 castrated males treated with oestradiol, and 10 intact males treated with oestradiol

Route of administration: intraperitoneal injection

Vehicle: propylene glycol-water (1:1)

Doses: 50 mg/kg, single injection

Excretion routes, body fluids, and tissues monitored and/or sampled during study: urine, serum

Statistical methods used: one-way analysis of variance (differences between groups), Student's t-test for unpaired data (differences between means of two groups)

Method remarks: Rats were housed in metabolic cages in groups of 5. They were fed standard rat chow and tap water *ad libitum*. Twenty males were castrated at the age of 28 days and after 5 weeks they were used in the test. Half of the operated and 10 intact males were dosed with 500 µg/kg oestradiol valerate subcutaneously every second day for the 14 days before the test. Urine was collected in the cages during the 96 hours after a single dose of PFOA. Blood samples were collected by cardiac puncture. Serum samples were ultrafiltered to determine the protein binding of PFOA in serum. The PFOA contents of urine, serum, and ultrafiltrates were measured with gas chromatography. The free anionic and possible conjugated forms of PFOA in urine were separated and assayed for PFOA concentration.

OC0108

RESULTS

Detailed results: Castration and administration of oestradiol to the male rats had a significant stimulatory effect on the urinary excretion of PFOA. During the first 24 hours, female rats excreted 72 ± 5% (n=6) of the dose, whereas intact males excreted only 9 ± 4% (n=6). After the oestradiol treatment, both intact and castrated males were able to excrete PFOA in urine in similar amounts as females (61 ± 19% and 68 ± 14% at 24 h, respectively). The castrated males without oestradiol treatment excreted PFOA in urine faster than intact males (50 ± 13% at 24 h), but not as fast as females or oestradiol treated males during the whole test ($P < 0.01$). At the end of the test (96 h), the concentration of PFOA in the serum of intact males was considerably higher (17-40 times) than in the serum of other groups.

Females and intact males treated with PFOA excreted slightly more urine than control animals up to 72 h ($P < 0.05$) after treatment. PFOA may enhance the urine volume excreted.

Ultrafiltration and analysis of the urine samples indicated that PFOA is not metabolized and is secreted in its anionic form. Over 98% of PFOA was bound to proteins in the serum of females and intact males determined in the samples taken during 12 h after dosing.

Metabolites measured: none

CONCLUSIONS

No conclusions were given.

REFERENCE

Ylinen, M., Hanhijarvi, H., Jaakonaho, J., and P. Peura. 1989. Stimulation by oestradiol of the urinary excretion perfluorooctanoic acid in the male rat. Pharmacology & Toxicology 65: 274-277.

OC0109.

PHARMACOKINETIC STUDY

Title: Disposition of perfluorooctanoic acid in the rat after single and subchronic administration

TEST SUBSTANCE

Identity: Perfluorooctanoic acid (PFOA) from Aldrich-Chemie; purity was not indicated.

Remarks: The test substance was dissolved in:
a) 0.9% NaCl solution for intragavage administration and
b) propylene glycol-water mixture (1:1) for intraperitoneal administration.

METHOD

Method/guideline followed: NA

Test type: *in vivo*

Species/strain/cell type or line: rat/Wistar

Sex: male and female

Age and body weight range of animals used: a) newly weaned/bw not specified
b) 10 weeks/bw not specified

Number of animals/sex/dose: a) 18
b) 20

Route of administration: a) intragavage
b) intraperitoneal

Vehicle: a) 0.9% NaCl solution
b) propylene glycol-water (1:1)

Doses: a) 3, 10, and 30 mg/kg/day, daily doses for 28 consecutive days
b) 50 mg/kg, single injection, volume = 0.25 mL/100g

Excretion routes, body fluids, and tissues monitored and/or sampled during study: serum, brain, liver, kidney, lung, spleen, ovary, testis, and adipose tissue

Statistical methods used: The biological half-life of PFOA in the serum and other tissues was estimated from the equation (linear regression) of the linear relationship between time and concentration of PFOA in a semilogarithmic plot.

Method remarks: The animals were housed at 21 °C, with a dark period from 9 p.m. to 7 a.m. They were given tap water and regular rat chow *ad libitum*. The serum was collected by cardiac puncture. After decapitation the brain was sampled and the other tissues were sampled at necropsy. The concentrations of PFOA in the serum, tissues, and lipid extracts were determined using capillary gas chromatography.

OC0110

RESULTS

Detailed results: a) At all three dose levels in the gavage study, the levels of PFOA in the serum and other tissues analyzed were higher in males than females ($p < 0.05$). The distribution of PFOA was mainly to the serum, but liver, kidney, and lung tissues also had high concentrations. A significant ($p < 0.05$) positive correlation was observed between the dose and the concentration of PFOA found in the liver ($r^2 = 0.996$), kidney ($r^2 = 0.933$), spleen ($r^2 = 0.995$), and lung ($r^2 = 0.959$) of females. No similar significant correlation was found in males. However, the spleen, testis, and brain concentrations of PFOA correlated positively with the concentration in the serum ($r^2 = 0.969$, 0.971, and 0.976, respectively).

b) After intraperitoneal injection, the concentrations of PFOA in the serum and other tissues assayed were higher in males than in females during the whole test. PFOA was mainly distributed to the serum, but liver, kidney, spleen, and brain tissues also had high concentrations. Twelve hours after administration of PFOA, about 10% of the dose was found in the serum of females, compared to about 40% in males. After two weeks, about 3.5% of the dose was still left in the serum of males. In females, the PFOA levels in the serum, liver, and kidney decreased in a discontinuous fashion, indicating distinct phases. The half-lives of the concentration of PFOA in the serum were 24 h and 105 h in females and males, respectively. The half-life of PFOA in the liver of females during the first week was estimated to be 60 h, while in males, it was found to be 210 h. In males, a more linear relationship between time and PFOA concentration was observed. PFOA was not found in the assay of the lipid fraction of the liver. Similar levels of PFOA were found in the spleen as in the liver (females = 73 h and males = 170 h). In the kidney, half-lives of 145 h and 130 h were found for females and males, respectively.
Metabolites measured: none

CONCLUSIONS

No conclusions were given.

REFERENCE

Ylinen, M., Kojo, A., Hanhijdrvi, H., and P. Peura. 1990. Disposition of perfluorooctanoic acid in the rat after single and subchronic administration. Bulletin of Environmental Contamination & Toxicology 44: 46-53.

OC0111

EPIDEMIOLOGIC DATA

Title: Mortality Among Employees of a Perfluorooctanoic Acid Production Plant

TEST SUBSTANCE

Identity: Perfluoroctanoic acid (PFOA)

Remarks: The cohort was exposed to PFOA during production of the chemical. Many other chemicals (e.g., benzene, asbestos) were also used or produced at the plant. The workers were exposed to these chemicals as well.

METHOD

Study design: Retrospective cohort mortality study.

Manufacturing/Processing/Use: There was no information in the study on methods used to manufacture and process PFOA. PFOA is used as a surfactant, and it is used in a large number of industrial applications and consumer products including plasticizers, lubricants, wetting agents, and emulsifiers.

Hypothesis tested: To determine whether mortality from any cause at the 3M Cottage Grove (Minnesota) manufacturing facility was associated with occupational exposure to PFOA.

Study period: The study population worked at the plant from Jan 1, 1947 to Dec 31, 1983.

Setting: Minnesota 3M plant that produced PFOA and other chemicals.

Total population: 3537 employees participated in the study (2788 males and 749 females). 1339 of this total worked in the Chemical Division. 398 (348 males and 50 females) of these employees were deceased, of which 148 males and 11 females worked in the Chemical Division.

Subject selection criteria: All workers employed for at least 6 months at the plant between Jan 1, 1947 to Dec 31, 1983.

Comparison population: In SMR analyses: U.S. general population death rates; Minnesota population death rates. Women were compared only with the U.S. population because cause- and calendar period-specific Minnesota rates for women were not available. In proportional hazards analyses: non-Chemical division workers at the plant.

Participation rate: Six workers employed during this time were excluded because they had incomplete records, resulting in a 99.8% participation rate (3537/3543). Of this population, vital status (alive or dead) was obtained for 100% of the individuals, and death certificates were obtained for 99.5% of those who died.

Subject description: Individuals employed during the study period were identified through company personnel records. Mean age at employment was 27.3 years for males and 27.6 years for females. Mean age at death was 56.4 for males and 55.4 for females.

Health effects studied: Mortality

Data collection methods: Vital status was obtained from the Social Security Administration for 1947-1982, and from the National Death Index for 1979-1989. Work histories and other employee data were obtained from company personnel records. Death certificates were obtained from the state health departments for those employees presumed to be deceased.

Exposure period: The potential exposure period was from Jan. 1, 1947 to Dec. 31, 1983.

Description/delineation of exposure groups/categories: PFOA production was restricted to the Chemical Division of the plant. Employees who worked in the Chemical Division for at least 1 month were considered exposed to PFOA. Employees who never worked in the Chemical Division or worked there for less than 1 month were considered not exposed to PFOA.

Measured or estimated exposure: Cumulative exposure to PFOA was estimated using the surrogate measure of months of Chemical Division employment.

Exposure levels: Not measured. Employees were considered either exposed or not exposed.

Statistical methods: Stratified Standardized Mortality Ratios (SMRs), adjusted for age, sex, and race, were calculated and compared to U.S. and Minnesota white death rates for men. For women, only state rates were available. The SMRs for males were stratified for 3 latency periods (10, 15, and 20 years) and 3 periods of duration of employment (5, 10, and 20 years). Cause-specific mortality rates were compared between exposed on unexposed workers using stratified SMRs.

Relative risk and 95% confidence intervals were estimated for deaths from all causes, cancer, cardiovascular diseases, and other selected causes using proportional hazard models (SAS). Age at first employment, year of first employment, and duration of employment were included as covariates in the model. The appropriateness of the proportional hazard assumptions was tested using stratified models with graphical analysis of log versus follow-up time relationships and models that tested the significance of a product term between exposure and log follow-up time.

Other methodological information:
A nosologist coded the death certificates for underlying cause of death for the workers according to ICD 8.

Data collected included year of first employment, age at first employment, duration of employment, and duration of employment in the Chemical Division. Length of employment at the plant, length of employment in the Chemical Division, calendar year of first employment, and age at first employment were analyzed to determine whether they had an effect on numbers of deaths from all causes and from specific causes.

RESULTS

Describe results: For all female employees, the SMRs for all causes and for all cancers were less than 1. The only elevated (although not significant) SMR was for lymphopoietic cancer, and was based on only 3 deaths. When exposure status was considered, SMRs for all causes of death and for all cancers were significantly lower than expected, based on the U.S. rates, for both the Chemical Division workers and the other employees of the plant.

In male workers, the SMRs were close to 1 for most of the causes of death when compared to both the U.S. and the Minnesota death rates. When latency and duration of employment were considered, there were no elevated SMRs. When employee deaths in the Chemical Division were compared to Minnesota death rates, the SMR for prostate cancer for workers in the Chemical Division was 2.03 (95% CI .55 - 4.59).

The SMR for prostate cancer for workers in the Chemical Division was 2.03 (95% CI .55 - 4.59). This was based on 4 deaths (1.97 expected). There was also a statistically significant association with length of employment in the Chemical Division and prostate cancer mortality. Based on the results of proportional hazard models, the relative risk for a 1-year increase in employment in the Chemical Division was 1.13 (95% CI 1.01 to 1.27). It rose to 3.3 (95% CI 1.02 -10.6) for workers employed in the Chemical Division for 10 years when compared to the other employees in the plant. The SMR for workers not employed in the Chemical Division was less than expected for prostate cancer (.58).

Study strengths: Vital status was determined for 100% of the cohort.

Study weaknesses: There was a potential for misclassification of exposure because many of the non-Chemical Division employees may have been exposed to PFOA. In addition, exposures were not measured; therefore categories of exposure were very broad (ever vs. never exposed). This exposure misclassification would bias the effect estimates toward the null. Workers were also exposed to other chemicals in the workplace. The authors also note that there are differences in the distribution of age at risk among the Chemical Division and non-Chemical Division workers and that this could confound the results of the study. There were also small numbers of deaths in many of the categories for males and especially for females in all categories. The cohort needs to be followed for many years to come in order to develop an accurate picture of the mortality experience of the employees of this plant.

Research sponsors: National Institute for Occupational Safety and Health Grant and the 3M Corporation

Consistency of results: Currently there are no other mortality studies on PFOA workers.

CONCLUSIONS

Although an association between employment in the Chemical Division and prostate cancer was observed, the results must be interpreted carefully. Continued follow up of this study or other studies with direct exposure measurements might help to confirm the association.

REFERENCE

Gilliland, F.D. and Mandel, J.S. 1993. Mortality among employees of a perfluorooctanoic acid production plant. JOM. 35(9): 950-954.

OTHER

This study is the second update of the mortality study. Another update is expected to cover the years through 1997. It has not yet been submitted to EPA.

EPIDEMIOLOGIC DATA

Title: Mortality Study of Workers Employed at the 3M Cottage Grove Facility

TEST SUBSTANCE

Identity: Perfluoroctanoic acid (PFOA) and other fluorochemicals

Remarks: This study is an update of the study published by Gilliland and Mandel, J Occup Med 1993, 950-954.

METHOD

Study design: Retrospective cohort mortality study.

Manufacturing/Processing/Use: The 3M Cottage Grove, MN plant has produced perfluorinated compounds since 1947. A primary product from this plant is ammonium perfluorooctanoate (APFO), a potent synthetic surfactant used in industrial applications. APFO rapidly dissociates in biologic media to perfluorooctanoate (PFOA).

Hypothesis tested: To determine whether occupational exposure to PFOA and other fluorochemicals is related to the mortality experience of employees of the 3M facility in Cottage Grove, Minnesota.

Study period: The study population worked at the plant for at least 1 year since Jan. 1, 1947. The cohort was followed through Dec. 31, 1997. Currently employed workers were assigned Dec 31, 1997 as their last date of employment.

Setting: 3M plant in Cottage Grove, Minnesota that produced PFOA and other chemicals.

Total population: 6678 workers were identified. Of these workers, 3992 worked at the plant for at least one year. Eighty percent of the cohort was male.

Subject selection criteria: All workers employed at the Cottage Grove plant for at least 1 year. The cohort was followed through Dec. 31, 1997. Currently employed workers were assigned Dec 31, 1997 as their last date of employment.

Comparison population: In SMR analyses, Minnesota population death rates for whites were used. Mortality reference rates from 7 regional counties were also used to rule out large variations based on regional mortality reporting differences.

Participation rate: Death certificates were obtained for 97% (n = 590) of the cohort who were deceased.

Subject description: 80% (n = 3183) of the employees in the cohort were male. The mean age at follow-up was 56.6 years, and the mean number of years worked at the plant was 12.1. The number of person-years at follow-up was 108198. There were 607 deaths identified in the cohort.

Health effects studied: Mortality

Data collection methods: A review of employee work history records of any employee with at least 1 year employment were abstracted to record the workers' name, SSN, 3M identification number, date of birth, and dates of work history. This cohort was linked to records from the original cohort to update the employment information and verify other data. The National Death Index was searched for all of the workers. Discrepancies with the original cohort were resolved and deaths before 1979 were verified in the Social Security Death Index. A licensed nosologist coded the death certificates to ICD 8.

Exposure period: The potential exposure period was from Jan. 1, 1947 to Dec. 31, 1997.

Description/delineation of exposure groups/categories: Workers were placed into 3 exposure groups based on job history information. Those groups were: definite PFOA exposure (jobs where cell generation, drying, shipping and packaging of PFOA occurred throughout the history of the plant); probable PFOA exposure (other chemical division jobs where exposure to PFOA was possible but with lower or transient exposures); and not exposed to fluorochemicals (primarily non-chemical division jobs).

Measured or estimated exposure: estimated based on job history information.

Exposure levels: Not measured. Employees were considered exposed (492 workers), probably exposed (1685), or not exposed (1815).

Statistical methods: Standardized Mortality Ratios (SMRs) and 95% confidence intervals were derived using the PC Life Table Analysis System software developed by NIOSH. This program computes age, gender, and race-specific SMRs using standard life table methods. The expected number of deaths are estimated by multiplying the age, gender, race, and calendar period tabulated person-years of follow up to the corresponding cause-specific mortality reference rates. Mortality rates for white Minnesotans were used as reference data.

Other methodological information:

RESULTS

Describe results: 607 deaths were identified in the cohort. 46 of these deaths were in the PFOA exposure group, 267 in the probable exposure group, and 294 in the not exposed group. The authors also stratified by a minimum of one-year exposure to PFOA in both the definitely-exposed and probably exposed groups. 182 workers (17 deaths) were definitely exposed for at least 1 year and 1673 workers (219 deaths) had probable exposure for at least one year.

When all employees were compared to the state mortality rates, SMRs were less than 1 or only slightly higher for all of the causes of death analyzed. None of the SMRs were statistically significant at p = .05. The highest SMR reported was for bladder cancer (SMR = 1.31, 95% CI = 0.42 – 3.05). Five deaths were observed (3.83 expected).

A few SMRs were elevated for employees in the definite PFOA exposure group: 2 deaths from cancer of the large intestine (SMR = 1.67), 1 from pancreatic cancer (SMR = 1.34), and 1 from prostate cancer (SMR = 1.30). In addition, employees in the definite PFOA exposure group were 2.5 times more likely to die from cerebrovascular disease (5 deaths observed, 1.94 expected; 95% CI = 0.84 – 6.03).

In the probable exposure group, 3 SMRs were notable: cancer of the testis and other male genital organs (SMR = 2.75, 95% CI = 0.07 – 15.3); pancreatic cancer (SMR = 1.24, 95% CI = 0.45 – 2.70); and

malignant melanoma of the skin (SMR = 1.42, 95% CI = 0.17 – 5.11). Only 1, 6, and 2 cases were observed, respectively. The SMR for prostate cancer in this group was 0.86 (n = 5).

There were no notable excesses in SMRs in the non-exposed group, except for cancer of the bladder and other urinary organs. Four cases were observed and only 1.89 were expected (95% CI = 0.58 – 5.40).

The excess in prostate cancer deaths that was observed in the first study was not as strong in this updated cohort. Only 1 death was reported in the definite exposure group while 5 were observed in the probable exposure group. It is difficult to interpret these results since the exposure categories were modified since the last study. However, all of the employees in either group were assumed to have some exposure to PFOA. The new delineation of exposures further defined the chemical plant employees of the first study and placed them into 2 groups, while the film plant employees still remained in the non-exposed group. The number of years that these employees worked at the plant and/or were exposed to PFOA was not reported for prostate cancer.

The excess mortality in cerebrovascular disease noted in employees in the definite exposure group was further analyzed based on number of years of employment at the plant. Three of the 5 deaths occurred in workers who were employed in jobs with definite PFOA exposure for more than 5 years but < 10 years (SMR = 15.03, 95% CI = 3.02 – 43.91). The other 2 occurred in employees with less than 1 year of definite exposure. The SMR was 6.9 (95% CI = 1.39 – 20.24) for employees with greater than 5 years of definite PFOA exposure. When these deaths were further analyzed by cumulative exposure (time-weighted according to exposure category), workers with 27 years of exposure in probable PFOA exposed jobs or those with 9 years of definite PFOA exposure were 3.3 times more likely to die of cerebrovascular disease than the general population. A dose-response relationship was not observed with years of exposure.

The slight excess in bladder cancer in the cohort as a whole should be noted. PFOA exposures were reported in employee serum at the Decatur, Alabama plant, and bladder cancer mortality was 4 times higher in workers with high exposure jobs than the general population (SMR = 4.81, 95% CI = 0.99 – 14.06). Three deaths were reported, and all of them occurred in the high exposure group. PFOA has been used at this plant as an elastomer in fluoropolymer production or it is produced as a by-product. It is also now being manufactured at Decatur. Manufacture has been occurring since 1999.

Study strengths/weaknesses: It is difficult to compare the results of the first and second mortality studies at the Cottage Grove plant since the exposure categories were modified. Although the authors claim that the newer exposure categories are more accurate, there is still a great chance that exposure misclassification occurred. Without measured exposures, it is difficult to judge the reliability of the exposure categories that were defined. It should be noted that in this study the chemical plant employees were sub-divided into PFOA-exposed groups and the film plant employees remained in the "non-exposed" group. Nevertheless, all of the deaths were considered to probably have had exposure to PFOA in the workplace. Seventeen death certificates were not located and therefore were not included in the study. This is important because it could greatly change the analyses for the causes of death that had a small number of cases. There are other chemicals present in the plant. In order to confirm the results regarding cerebrovascular disease (was an artifact of death certificate coding), regional mortality rates were used for the reference population. The results did not change.

Research sponsors: University of Minnesota

Consistency of results: The excess in prostate cancer deaths that was observed in the first study was not as strong in this updated cohort. Only 1 death was reported in the definite exposure group while 5 were

observed in the probable exposure group. It is difficult to interpret these results since the exposure categories were modified since the last study. However, all of the employees in either group were assumed to have some exposure to PFOA. The new delineation of exposures further defined the chemical plant employees of the first study and placed them into 2 groups, while the film plant employees still remained in the non-exposed group. The number of years that these employees worked at the plant and/or were exposed to PFOA was not reported for prostate cancer.

CONCLUSIONS

Follow up of worker mortality at Cottage Grove needs to continue. Although there were more than 200 additional deaths included in this analysis, it is still a small number and the cohort is still relatively young. Given the results of studies on flurorchemicals in both animals and humans, further analysis is warranted. Of particular interest are bladder cancer, prostate cancer, cerebrovascular disease, cancer and disorders of the liver, and pancreatic cancer.

REFERENCE

Alexander, B.H. April 26, 2001. Mortality study of workers employed at the 3M Cottage Grove facility. Final Report. Division of Environmental and Occupational Health, School of Public Health, University of Minnesota.

OTHER

This study differs from the 1993 mortality study in that this one requires 1 year of exposure for inclusion in the cohort (as opposed to 6 months). In addition, the exposure categories were more specific. Additional cohort members were included (n = 169) that should have been included in the last study. It is not clear why these employees were not included in the 1993 study.

EPIDEMIOLOGIC STUDY

Title: An Epidemiologic Investigation of Reproductive Hormones in Men with Occupational Exposure to Perfluorooctanoic Acid

TEST SUBSTANCE

Identity: Perfluorooctanoic Acid (PFOA)

Remarks: This paper further examines the observation initially reported by Gilliland in his doctoral thesis that total serum organic fluorine may be associated with reproductive hormone changes in PFOA production workers.

METHOD

Study design: Two cross-sectional studies, utilizing general medical surveillance and analysis of eleven hormones in serum of male employees at a PFOA production facility.

Manufacturing/Processing/Use: PFOA was produced at the plant by an electrochemical process, which involved a four-stage process: isolating and converting the chemical to a salt slurry, converting the slurry to a salt cake, drying the cake, and packaging. The greatest likelihood for exposure to PFOA occurred in the drying area. PFOA, a potent synthetic surfactant, is used in industrial applications.

Hypothesis tested: Since PFOA has been shown to result in dose-related increases in hepatic, pancreatic acinar, and Leydig cell adenomas in laboratory animals and increased serum estradiol levels, PFOA may show a similar effect in humans with regard to reproductive hormones, particularly increased estradiol or decreased testosterone serum levels.

Setting: PFOA production plant (presumed to be 3M plant in Cottage Grove, MN)

Total # of subjects in study: 1993--111 production workers, 1995--80 production workers. Sixty-eight production workers were common to both cohort years.

Comparison group/population: Employees were divided into four serum PFOA level categories, 0-<1 ppm, 1-<10 ppm, 10-<30 ppm, and ≥30 ppm, in order to determine if an effect existed at the highest serum levels.

Participation rate: Not specified.

Subject description: Characteristics of subjects were not described.

Health effects studied: Potential changes in: serum cortisol, dehydroepiandrosterone sulfate (DHEAS), estradiol, follicle-stimulating hormone (FSH), 17α-hydroxyprogesterone (17-HP), free testosterone, total testosterone, luteinizing hormone (LH), prolactin, thyroid-stimulating hormone (TSH), and sex hormone-binding globulin (SHBG)

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Data collection methods: Medical surveillance consisted of a medical questionnaire, measurement of height, weight, and pulmonary function; standard biochemical and urinalysis tests; PFOA determination; and several hormone assays.

Details on data collection: The upper limit of detection of PFOA in 1993 was 80 ppm, whereas there was no upper limit of detection in 1995. A thermospray mass spectrophotometry assay was used to determine serum PFOA levels in 1993 and 1995. Eleven hormones were assayed. Cortisol was assayed using a fluorescence polarization immunoassay. Radioimmunoassays (RIA) were used for DHEAS, estradiol, 17-HP and total testosterone. Free testosterone was determined using equilibrium dialysis. LH, FSH, and prolactin were assayed using a microparticle enzyme immunoassay. TSH was determined using a chemiluminescence immunometric assay. SHBG was assessed via a radioimmunoassay after chromatographic sample purification. Bound testosterone was calculated as total testosterone less free testosterone. The same assays were used for both 1993 and 1995 analyses.

Exposure period: Not specified.

Description/delineation of exposure groups/categories: For the stratified analyses, employees were divided into four serum PFOA level categories: 0-<1 ppm, 1-<10 ppm, 10-<30 ppm, and ≥30 ppm in order to determine if an effect existed at the highest serum levels. Seventy-five percent of the employees with serum PFOA levels at 10 ppm or greater participated in both years.

Measured or estimated exposure: N/A

Statistical methods: Simple and stratified analyses, analysis of variance (ANOVA), Pearson correlation coefficients, and ordinary multivariable regression were used to evaluate associations between PFOA and each hormone, with adjustment for potential confounding variables. For stratified analyses, employees were divided into four serum PFOA level categories: 0-<1 ppm, 1-<10 ppm, 10-<30 ppm, and ≥30 ppm in order to determine if an effect existed at the highest serum levels. For multivariate evaluation, PFOA, age, body mass index (BMI), alcohol use, and cigarette use were examined as both categorical and continuous variables. Regression models were fitted with PFOA entered as a continuous variable using linear, square, and square root transformations. The possible nonlinear association of estradiol, free testosterone, and bound testosterone was evaluated. Nonlinear dose-response relationships were examined by model fit and by comparing parameter estimates, using indicator and continuous variables. Stepwise selection procedures were also used. Study results were analyzed using SAS.

Other methodological information: No additional comments.

RESULTS

Describe results: The range of serum PFOA was 0 – 80 ppm in 1993 and 0 – 115 ppm in 1995. Serum PFOA measurements were highly correlated among the 68 employees who participated in the study during both years.

PFOA was not highly correlated with any of the hormones or with the following covariates: age, alcohol consumption, BMI, or cigarettes. Most of the employees had PFOA serum levels less than 10 ppm. In 1993, only 12 employees had serum levels > 10 ppm, and 15 in 1995. However, these levels ranged from approximately 10 ppm to over 114 ppm. There were only 4 employees in the ≥30 ppm PFOA group in 1993 and only 5 in 1995. Therefore, it is likely that there was not enough power to detect differences in either of the highest categories. The mean age of the employees in the highest exposure category was the

lowest in both 1993 and 1995 (33.3 years and 38.2 years, respectively). Although not significantly different from the other categories, BMI was slightly higher in the highest PFOA category.

Estradiol was highly correlated with BMI ($r = .41$, $p < .001$ in 1993, and $r = .30$, $p < .01$ in 1995). In 1995, all 5 employees with PFOA levels > 30 ppm had BMIs > 28, although this effect was not observed in 1993. Estradiol levels in the 30 ppm group in both years were 10% higher than the other PFOA groups; however, the difference was not statistically significant. The authors postulate that the study may not have been sensitive enough to detect an association between PFOA and estradiol because measured serum PFOA levels were likely below the observable effect levels suggested in animal studies (55 ppm PFOA in the CD rat). Only 3 employees in this study had PFOA serum levels this high. They also suggest that the higher estradiol levels in the highest exposure category could suggest a threshold relationship between PFOA and estradiol.

Free testosterone was highly correlated with age in both 1993 and 1995. The authors did not report a negative association between PFOA serum levels and testosterone. There were no statistically significant trends noted for PFOA and either bound or free testosterone. However, 17-HP, a precursor of testosterone, was highest in the 30 ppm PFOA group in both 1993 and 1995. In 1995, PFOA was significantly associated with 17-HP in regression models adjusted for possible confounders. However, the authors state that this association was based on the results of one employee (data were not provided in the report). There were no significant associations between PFOA and cortisol, DHEAS, FSH, LH, and SHBG.

Study strengths and weaknesses: There are several design issues that should be noted when evaluating the results of this study. First, although there were 2 study years (1993 and 1995), the populations were not independent. Sixty-eight employees participated in both years. Second, there were 31 fewer employees who participated in the study in 1995, thus reducing the power of the study. There were also very few employees in either year with serum PFOA levels greater than 10 ppm. Third, the cross-sectional design of the study does not allow for analysis of temporality of an association. Since the half-life of PFOA is several years, the authors suggest that it is possible that there may be some biological accommodation to the effects of PFOA. Fourth, only one sample was taken for each hormone for each of the study years. In order to get more accurate measurements for some of the hormones, pooled blood taken in a short time period should have been used for each participant. Finally, there may have been some measurement error of some of the confounding variables.

Research sponsors: 3M Company

Consistency of results: An earlier study reported an association between total serum organic fluorine in workers and increased estradiol and decreased testosterone levels. However, it is difficult to compare the results of this study to the previous study because serum PFOA in workers was measured in this study.

CONCLUSIONS

In two cross-sectional studies in 1993 and 1995, significant hormonal changes among the male production employees were not apparent in relation to their measured serum PFOA levels as had been previously observed in laboratory animals. However, it should be noted that PFOA serum levels in workers were much lower than those levels reported to cause effects in laboratory animals.

REFERENCE

Olsen, G.W. et al. 1998. An Epidemiologic Investigation of Reproductive Hormones in Men with Occupational Exposure to Perfluorooctanoic Acid. JOEM. 40(7):614-622.

000122

EPIDEMIOLOGIC STUDY

Title: An epidemiologic investigation of plasma cholecystokinin and hepatic function in perfluorooctanoic acid production workers

TEST SUBSTANCE

Identity: Perfluorooctanoic acid (PFOA)

Remarks:

METHOD

Study design: 3 cross-sectional analyses based on workers' medical surveillance data collected in 1993, 1995, and 1997

Manufacturing/Processing/Use: The substance is a synthetic surfactant that is produced via a 4-stage electrochemical process involving converting the chemical to a salt slurry and then to a salt cake.

Hypothesis tested: To determine whether a positive association exists between plasma cholecystokinin (CCK) levels and serum PFOA levels in fluorochemical production workers, and to determine whether PFOA may modulate hepatic responses to obesity and alcohol.

Study period: Medical exams were performed in 1993, 1995, and 1997

Setting: PFOA production plant, Cottage Grove, MN

Total population: Not specified

Subject selection criteria: None specified, although participation in medical surveillance at the plant was voluntary.

Total # of subjects in study: The numbers of participants in 1993, 1995 and 1997 were 111, 80, and 74, respectively. There were 68 participants in common for 1993 and 1995, 20 in common for 1993 and 1997, and 17 in common for all three years.

Comparison population: N/A

Participation rate: approx. 70%

Subject description: Workers who engaged in the production of PFOA. No other information was provided.

Health effects studied: The effects of serum PFOA levels on hepatic responses to obesity and alcohol, and CCK levels.

Data collection methods: Plant workers voluntarily participated in biennial medical surveillance exams which consisted of a questionnaire, pulmonary function, height, and weight measurements, biochemical and urinalysis testing, PFOA determination, and some male reproductive hormone assays.

Details on data collection: Hormone data were only collected in 1993 and 1995. These results are provided in another robust summary (Olsen et al., 1998).

Serum biochemical tests included: alkaline phosphatase, gamma glutamyl transferase (GGT), serum glutamyl oxaloacetic transaminase (SGOT), serum glutamyl pyruvic transaminase (SGPT), total bilirubin, direct bilirubin, cholesterol, low-density lipoproteins (LDL), high-density lipoproteins (HDL), triglycerides, blood urea nitrogen (BUN), creatinine, and glucose. Hematology tests included: hematocrit, hemoglobin, red blood cells, platelets, and white blood cells.

Plasma CCK-33 was measured by direct radioimmunoassay. Serum PFOA was determined by thermospray (1993 and 1995) and electrospray (1997) high performance liquid chromatography mass spectrometry methods.

Exposure period: Not specified.

Description/delineation of exposure groups/categories: For the stratified analyses, employees were divided into four serum PFOA level categories: 0-<1 ppm, 1-<10 ppm, 10-<30 ppm, and 30 ppm in order to determine if an effect existed at the highest serum levels.

Measured or estimated exposure: N/A

Exposure levels: N/A

Statistical methods: Simple and stratified analyses, Pearson correlation coefficients, ANOVA, and ordinary multivariate regression with adjustment for potential confounding variables.

Other methodological information:

RESULTS

Describe results: The mean PFOA serum level in employees participating in the 1997 study period was 6.4 ppm (range 0.1 - 81.3 ppm). The mean CCK value was 28.5 pg/ml (range 8.8 - 86.7 pg/ml). The highest CCK values were reported in the 2 exposure categories less than 10 ppm. The means were 50% higher in these 2 categories than in the categories greater than 10 ppm ($p = .06$). When adjusted for potential confounders, multivariable regression models indicated a weak negative association between CCK and PFOA; however, these data were not included in the report. There were no statistically significant differences in serum chemistry values for participants with high CCK or PFOA levels compared to participants with low CCK or PFOA levels.

Serum PFOA levels were not consistently associated with any variables, such as alcohol or cigarette use, or with any hematological or chemistry parameters, and none of the values for such parameters were statistically significant at any of the PFOA levels. There was no evidence that PFOA levels modify hepatic responses to obesity and/or alcohol.

Study strengths and weaknesses: It should be noted that CCK was only measured in 1997. The authors indicate that the cross-sectional study format does not permit a direct analysis of an observed association with respect to time. Additionally, the number of participants in 1995 and 1997 was significantly lower than the number of participants in 1993.

Research sponsors: 3M Company

Consistency of results: The results of this study are inconsistent with earlier findings that PFOA modulates hepatic responses to alcohol and obesity and that there is a positive correlation between PFOA and CCK levels.

The following explanations may indicate why this study failed to find a positive association between PFOA and CCK values:

- It is possible that the hepatocarcinogenic effects of peroxisome proliferators in rodents do not act the same biochemically in humans.
- The serum PFOA levels observed in workers may have been too low to detect an effect. Effects in animals were observed at higher doses than most of the serum levels found in workers.
- CCK receptors may be different between rats and humans. Therefore, the monkey may be a more appropriate animal model to study the pancreatic effects of PFOA in humans.
- The involvement of CCK in the initiation or promotion of pancreatic cancer is controversial.
- The rat may not be an appropriate model in the study of pancreatic cancer in humans, since acinar cell malignancies induced by carcinogens in rats, are rare in humans.

CONCLUSIONS

The results do not suggest that there is an association between PFOA levels and increases in plasma CCK levels, nor do they suggest the presence of hepatic toxicity at the PFOA levels observed. It is unlikely that PFOA levels observed in this study modulate hepatic responses to obesity and alcohol use.

REFERENCE

Olsen, G.W., et al. 1998. 3M Final Report: An epidemiologic investigation of plasma cholecystokinin, hepatic function and serum perfluorooctanoic acid levels in production workers. St. Paul, 3M Company. Sept 4.

EPIDEMIOLOGIC STUDY

Title: Serum Perfluorooctanoic Acid and Hepatic Enzymes, Lipoproteins, and Cholesterol: a Study of Occupationally Exposed Men

TEST SUBSTANCE

Identity: Total serum fluorine was used as a surrogate for PFOA exposure. Serum PFOA was not measured due to the cost of analyzing the samples.

Remarks: Originally, employees were considered "unexposed" based on their job descriptions; however, when their serum levels were analyzed, these "unexposed" workers had PFOA levels higher than the general population. Therefore, total serum fluorine was used to classify workers into exposure groups.

METHOD

Study design: Cross-sectional study

Manufacturing/Processing/Use: This plant produces PFOA, as well as several other specialty chemicals

Hypothesis tested: To determine if workers exposed to PFOA experienced changes in hepatic enzymes and lipid metabolism. These effects have been observed in rodents exposed to PFOA.

Setting: PFOA production plant– 3M Chemolite plant in Cottage Grove, MN

Total # of subjects in study: 115

Subject selection criteria: Participants were recruited from all employees at the PFOA production plant who were employed during the period of 1985-1989.

Comparison group/population: All subjects potentially were exposed to PFOA. Therefore, differences in mean values of the biochemical endpoints were compared.

Participation rate: Presumed to be > 80%

Subject description: Mean age = 39.2; mean body mass index = 26.9; 76% drank less than 1 oz. alcohol per day and 17% drank 1-3 oz. alcohol/day; 74 percent smoked.

Health effects studied: Possible effects of total serum fluorine on levels of hepatic enzymes, lipoproteins, and cholesterol, including: serum glutamyl oxaloacetic transaminase (SGOT), serum glutamyl pyruvic transaminase (SGPT), gamma glutamyl transferase (GGT), cholesterol, low-density lipoproteins (LDL), and high-density lipoproteins (HDL).

Data collection methods: Participants completed a medical history questionnaire, were measured for height and weight, and donated blood samples by venipuncture.

Exposure period: Unknown.

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Description/delineation of exposure groups/categories: Workers were placed into exposure categories based on job descriptions; however, those workers presumed to have little or no PFOA exposure had total serum fluorine levels 20-50 times higher than those levels reported in the general population. Therefore, workers were classified into groups by total serum fluorine levels. Employees were placed into one of 5 groups of total serum fluorine levels: <1 ppm, 1-3 ppm, >3 - 10 ppm, >10 - 15 ppm, and > 15 ppm.

Measured or estimated exposure: Exposures were estimated. Total serum fluorine was used as a surrogate measure for PFOA exposure.

Statistical methods: Stratified analysis, ANOVA, Pearson correlation coefficients, and linear multivariate regression were used to evaluate associations between PFOA and biochemical endpoints. For stratified analyses, ANOVA procedures were used to assess differences in mean values. Age, body mass index (BMI), alcohol use, and tobacco use were included in the regression models as potential confounders. Number of cigarettes smoked per day was used in the model as a continuous variable if model fit was improved compared with the model using categorical variables.

RESULTS

Describe results: The range of the serum fluorine values was 0 to 26 ppm (mean 3.3 ppm). Approximately half of the workers fell into the > 1 - 3 ppm category, while 23 had serum levels < 1 ppm and 11 had levels > 10 ppm.

There were no significant differences between categories when analyzed using univariate analyses for cholesterol, LDL, and HDL. PFOA was, however, associated with HDL levels in moderate drinkers. In the multivariate analysis, there was not a significant association between total serum fluorine and cholesterol or LDL after adjusting for alcohol consumption, age, BMI, and cigarette smoking. There were no statistically significant differences among the categories of total serum fluorine for SGOT, SGPT, and GGT. Increases in SGOT and SGPT occurred with increasing total serum fluorine levels in obese workers (BMI = 35 kg/m^2). No workers reported hepatic disease diagnoses or signs, or symptoms consistent with hepatic disorders. No clinical cases of liver dysfunction associated with PFOA exposure were found in these workers.

Study strengths and weaknesses: PFOA was not measured directly and there is no exposure information provided on the employees (eg. length of employment/exposure). The authors state that no adverse clinical outcomes related to PFOA exposure have been observed in their employees; however, it is not clear that there has been follow-up of former employees. In addition, the standard deviations reported for the liver enzymes were very high for many of the exposure categories, indicating instability in the results. Many participants in the study were employed in production of compounds other than PFOA.

Research sponsors: A NIOSH grant and the 3M Medical Department

Consistency of results: There are no other known studies of this kind on PFOA.

CONCLUSIONS

PFOA was not associated with marked hepatic changes in humans as had been observed in rodents. However, PFOA may modulate the effect of alcohol use and obesity on hepatic lipid and xenobiotic metabolism.

000127

REFERENCE

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

000128

EPIDEMIOLOGIC DATA

Title: An Epidemiologic Analysis of Episodes of Care of 3M Decatur Chemical and Film Plant Employees, 1993-1998

TEST SUBSTANCE

Identity: POSF-based chemicals used at the Decatur plant

Remarks: Episodes of care analyses are not often used in occupational epidemiologic studies.

METHOD

Study design: Episode of care comparison

Manufacturing/Processing/Use: The 3M Decatur, Alabama plant began production in 1961. It is made up of the film plant and the chemical plant. The 3 major product groups in the chemical plant are protective chemicals, performance chemicals, and fluoroelastomers. Perfluorooctanesulfonyl fluoride (POSF) is the major sulfonate fluorochemical manufactured at Decatur and is used as the precursor to the production of a variety of perfluorinated amides, alcohols, acrylates, and other fluorochemical polymers.

Hypothesis tested: To use episodes of care methodology as a screen for morbidity outcomes associated with long-term, high exposure to POSF-based production at the 3M facility in Decatur, Alabama.

Study period: Episodes of care experience of 652 chemical employees and 659 film plant employees were analyzed for workers at the plant who were employed for at least 1 year between January 1, 1993 and December 31, 1998.

Setting: 3M plant in Decatur, Alabama.

Total population: 1311 workers were eligible for the cohort (at least 1 year of employment at the plant). The total worker population was not reported.

Subject selection criteria: All workers employed at the Decatur plant for at least 1 year between Jan. 1, 1993 and Dec. 31, 1998. Episodes of care were limited to their Decatur time of employment for employees hired, terminated, or died during the study period. However, records of employees on Medicare, long-term disability or who chose HMO coverage were not in the database and would not be included in the episodes of care for that employee.

Comparison population: Chemical and film plant employees were analyzed separately and then compared to each other. Employee comparison groups were defined according to their potential workplace exposure to POSF fluorochemical production. Group A: all chemical plant employees and all film plant employees eligible for the cohort. Group B: all chemical plant employees who worked solely in the chemical plant and all film plant employees who worked exclusively in the film plant. Group C: all chemical plant employees with high fluorochemical exposures compared to their job counterparts in the film plant. Group D: all plant workers with high fluorochemical exposure for at least 10 years prior to the study onset compared to their job counterparts in the film plant.

Participation rate: 97% of Decatur employees were eligible for participation in the study.

Subject description: 82% of the employees in the cohort were male (530 in the chemical plant and 558 in the film plant). The mean age was 45.1 in the chemical plant and 48.6 in the film plant. Sixty percent of the chemical plant employees had worked only in the chemical plant and a similar percentage of film plant workers had worked exclusively in the film plant. Seventy-six percent of the chemical plant workers had high exposure jobs.

Health effects studied: Morbidity. Based on animal data and epidemiologic studies on PFOA and PFOS, certain episodes of care were considered *a priori*. They included: liver and bladder cancer, endocrine disorders involving the thyroid gland and lipid metabolism, gastrointestinal disorders of the liver and biliary tract, and reproductive disorders.

Data collection methods: The Clinical Care Groups episode of care software developed by Ingenix, Inc. was used to provide a comprehensive grouping of all visits (inpatient and outpatient), procedures, ancillary services, and prescription drugs used in the diagnosis, treatment and management of more than 400 diseases or conditions. The software code constructs an episode of care around the index-eligible record by searching backward and forward in time for the health claims records that are related to the disease or condition on the index record. The index record consists of either procedure codes indicative of a face-to-face encounter or a pharmacy record for a delineating drug.

Exposure period: The episodes of care that were included in the study were those experienced between Jan. 1, 1993 to Dec. 31, 1998.

Description/delineation of exposure groups/categories: Workers were placed into groups according to potential workplace exposures: workers who were employed solely in the chemical or film plants, those who had high exposure jobs, and those who worked at least 10 years in jobs with high potential for fluorochemical exposure.

Measured or estimated exposure: estimated based on job history information.

Exposure levels: Not measured. Employees were placed into exposure categories based on job description.

Statistical methods: A risk ratio episode of care (RREpC) provided the estimate of risk between the observed to expected episodes of care for chemical plant employees compared to the observed to expected episodes of care among film plant employees. The expected number of episodes of care for both the film and chemical plant employees was calculated from health claims data of the 3M manufacturing population in the U.S. Because the chemical and film plant cohorts had slightly different age and gender structures, an adjusted ratio was calculated and compared to the unadjusted risk ratio. In most cases, the risk ratios were comparable. Therefore, 95% confidence intervals were only calculated for the unadjusted risk ratios.

Other methodological information: It should be noted that from an epidemiologic perspective, an episode of care could represent any and all incident cases, prevalent cases, and/or misclassified cases (both false positive and false negative). In addition, types and counts of episodes of care may differ by the software used, and it is possible that 2 different diagnoses may be assigned to the same episode. Certain services, such as lab procedures and prescriptions may not be reported for the episode. Also, the endpoint of an episode may vary among software programs. The clinical flexibility of the algorithm may differ depending on the software program.

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RESULTS

Describe results: The only increased risk of episodes for the conditions of a priori interest were for neoplasms of the male reproductive system and for the overall category of cancers and benign growths (which included cancer of the male reproductive system). There was an increased risk of episodes for the overall cancer category for all 4 comparison groups. The risk ratio was greatest in the group of employees with the highest and longest exposures to fluorochemicals (RREpC = 1.6, 95% CI = 1.2 – 2.1). Increased risk of episodes in long-time, high-exposure employees also was reported for male reproductive cancers (RREpC = 9.7, 95% CI = 1.1 - 458). It should be noted that the confidence interval is very wide for male reproductive cancers and the sub-category of prostate cancer. Five episodes of care were observed for reproductive cancers in chemical plant employees (1.8 expected), of which 4 were prostate cancers. One episode of prostate cancer was observed in film plant employees (3.4 expected). This finding is important because an excess in prostate cancer mortality was observed in the Cottage Grove plant mortality study. However, the update of the study did not confirm this finding.

There was an increased risk of episodes for neoplasms of the gastrointestinal tract in the high exposure group (RREpC = 1.8, 95% CI = 1.2- 3.0) and the long-term employment, high exposure group (RREpC = 2.9, 95% CI = 1.7 – 5.2). Most of the episodes were attributable to benign colonic polyps. Similar numbers of episodes were reported in film and chemical plant employees.

In the entire cohort, only 1 episode of care was reported for liver cancer (0.6 expected) and 1 for bladder cancer (1.5 expected). Both occurred in film plant employees. Only 2 cases of cirrhosis of the liver were observed (0.9 expected), both in the chemical plant. There was a greater risk of lower urinary tract infections in chemical plant employees, but they were mostly due to recurring episodes of care by the same employees. It is difficult to draw any conclusions about these observations, given the small number of episodes reported.

Chemical plant employees in the high exposure, long-term employment group were 2 ½ times more likely to seek care for disorders of the biliary tract than their counterparts in the film plant (RREpC = 2.6, 95% CI = 1.2 - 5.5). Eighteen episodes of care were observed in chemical plant employees and 14 in film plant workers. The sub-categories that influenced this observation were episodes of cholelithiasis with acute cholecystitis and cholelithiasis with chronic or unspecified cholecystitis. Most of the observed cases occurred in chemical plant employees.

Risk ratios of episodes of care for endocrine disorders, which included sub-categories of thyroid disease, diabetes, hyperlipidemia, and other endocrine or nutritional disorders, were not elevated in the comparison groups. Conditions which were not identified a priori but which excluded the null hypothesis in the 95% confidence interval for the high exposure, long-term employment group included: disorders of the pancreas, cystitis, and lower urinary tract infections.

Study strengths and weaknesses: See "other methodological information" section for limitations of episodes of care software. The results of this study should only be used for hypothesis generation. Although the episode of care design allowed for a direct comparison of workers with similar demographics but different exposures, there are many limitations to this design. Episodes of care are reported, not disease incidence; therefore, this parameter cannot be interpreted in any other manner. The data are difficult to interpret because a large RREpC may not necessarily indicate high risk of incidence of disease. In addition, many of the risk ratios for episodes of care had very wide confidence intervals. The analysis was limited to 6 years. Also, the utilization of health care services may reflect local medical practice patterns. Individuals may be counted more than once in the database because they can be categorized under larger or smaller disease classifications. Episodes of care may include the same

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individual several times. Not all employees were included in the database, such as those on long-term disability.

Research sponsors: 3M Company

Consistency of results: No other morbidity studies have been conducted on fluorochemicals.

CONCLUSIONS

This study should only be used for hypothesis generation regarding workers employed at the Decatur plant who are employed in jobs with high exposure to POSF-based fluorochemicals.

REFERENCE

Olsen, GW, Burlew, MM, Hocking, BB, Skratt, JC, Burris, JM, Mandel, JH. An epidemiologic analysis of episodes of care of 3M Decatur chemical and film plant employees, 1993-1998. Final Report. May 18, 2001.

GENETIC TOXICITY STUDIES

Title: An Assay of Cell Transformation and Cytotoxicity in C3H 10T ½ Clonal Cell Line for the Test Chemical T-2942 CoC

TEST SUBSTANCE

Identity: T-2942 CoC

Remarks: White powder dissolved in DMSO; composition and purity not indicated

METHOD

Method/Guideline followed: No guideline number specified

Test type: Cytotoxicity and cell transformation

Test system: Mouse embryo fibroblast

GLP: No

Year study performed: 1980

Species/Strain/cell-type/cell line: Cells of the C3H 10T-1/2 clone 8

Metabolic activation: None

Concentrations tested: 0.1, 1.0, 10, 50, 100, 200 g/mL

Statistical methods used: None

Remarks: The test material was dissolved in DMSO and <20µl of the solution was added to the cultures growing in Eagle Basal Medium (BME). Benzo(a)pyrene and di-epoxybutane were used as positive controls and DMSO as a solvent control. Prior to the performance of the transformation assay, dose range data were obtained in the form of cytotoxicity measurements as expressed by plating (cloning) efficiency. The approximate in vitro LD50 cytotoxicity dose was chosen as the median dose for the study of the transformation potential of the test chemical. The transformation assay was performed in two phases: assessment of cell transformation in the colony mode (phase 1) and determination of foci transformation potential (phase 2). Phase 1 was performed in six replicates. The test chemical was removed 24-hours after application and the cultures were re-fed every 3 days for 14 to 17 days. Plates were washed, fixed, and stained at 14 days to score for transformation. Phase 2 was also performed in 6 replicates per dose (1.0, 10, and 100 µg/mL) with butadiene epoxide as a positive control. In phase 2, the cultures were processed at 38 days after removal of the test substance.

RESULTS

Overall transformation results: Negative

Cytotoxic concentration: approximate LD_{50} = 50 µg/mL

Statistical results: None

CONCLUSIONS

There was no evidence of transformation observed at any of the dose levels tested in either the colony or foci assay methods.

REFERENCE

Garry, V.F., and R.L. Nelson. 1981. An Assay of Cell Transformation and Cytotoxicity in C3H 10 ½ Clonal Cell Line for the Test Chemical T-2942 CoC. 3M Company, St. Paul, MN.

Title: Mutagenicity Test on T-6564 in an *In Vivo* Mouse Micronucleus Assay

TEST SUBSTANCE

Identity: T-6564

Remarks: clear, colorless liquid; composition and purity not indicated

METHOD

Method/Guideline followed: Protocol No. 455, Edition 17, modified for 3M Corporation

Test type: *In vivo* mouse micronucleus assay

GLP: Yes

Year study performed: 1996

Species/Strain: Mouse/ Crl:CD-1®(ICR)BR

Sex: Male and female

No. animals/sex/dose: Five (micronucleus assay), Three (range-finding studies)

Vehicle (if used): Deionized water

Route of administration: Oral gavage

Doses:
Dose Selection Study I: 1000, 1510, 2010, 2540, 3010 mg/kg
Dose Selection Study II: 1010, 1520, 2020, 2530 mg/kg
Micronucleus Assay: 498, 995, 1990 mg/kg

Frequency of treatment: Once

Statistical methods used: Analysis of variance; Dunnet's t test

Remarks: The initial body weight of the animals was 22.4 – 28.3 g for females and 31.0 - 37.7 g for males; their age was 8 weeks. Dosing was achieved using a volume of 10 mL/kg. Based on the results of the dose selection study, the maximum tolerated dose was estimated as 2000 mg/kg. A vehicle control, using deionized water, and a positive control, using cyclophosphamide at 80 mg/kg, were implemented. Animals were sacrificed at 24, 48, and 72 hours after dosing and bone marrow was extracted, spread on slides and stained with May-Grunwald solution and Giemsa prior to analysis. The slides were coded for analysis, and scored for micronuclei and the polychromatic erythrocyte (PCE) to normochromatic erythrocyte (NCE) cell ratio. One thousand PCEs per animal were scored.

RESULTS

Effect on mitotic index or PCE/NCE ratio by dose level and sex: Negative

Genotoxic effects (unconfirmed, dose-response, equivocal): Negative

Statistical results: Negative

Remarks: All animals in the micronucleus test group appeared normal immediately after dosing; however, one male from a secondary dose group was found dead 22 hours after dosing. All other animals appeared normal. The positive control induced significant increases in micronucleated PCEs as compared to the vehicle control.
In the dose selection studies, there were mortalities at the 1510, 1520, and 300 mg/kg dose levels. Clinical signs of toxicity in the dose selection studies were hunched posture, hypoactivity, and rough hair coat.

CONCLUSIONS

The test article did not induce a significant increase in micronuclei in bone marrow polychromatic erythrocytes and is considered negative in the mouse micronucleus assay.

REFERENCE

Murli, H. 1996. Mutagenicity test on T-6564 in an *in vivo* mouse micronucleus assay. Study number 17750-0-455. 3M Corporation, St. Paul, MN.

000136

Title: Mutagenicity Test on T-6564 Measuring Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells: with a Confirmatory Assay with Multiple Harvests

TEST SUBSTANCE

Identity: T-6564, also referred to as L-13167 and FC-1015-X.

Remarks: The specific gravity of the test substance was 1.22 g/mL. Substance was a clear colorless liquid.

METHOD

Method/Guideline followed: Protocol No. 437C, Edition 4 was modified for 3M Corporation

Test type: In vitro cytogenetics

Test system: Chinese hamster ovary cells in culture

GLP: Y

Year study performed: 1996

Species/Strain/cell-type/cell line: Chinese hamster ovary cells/permanent cell line supplied by Dr. S. Wolff, University of California, San Francisco. Cell line had an average cycle time of 12 to 14 hours. Modal chromosome number was 21.

Type of metabolic activation used: Aroclor 1254 induced rat liver S9 homogenate, 15.0 µL/ml, plus NADP *at* 1.5 mg/ml and isocitric acid at 2.7 mg/ml.

Concentrations tested:
Rangefinding –
0.169, 0.508, 1.69, 5.08, 16.9, 50.8, 169, 508, 1690, 5080 µg/ml
(both with and without activation)
Initial study –
Without activation: 62.5, 125, 250, 500, 1000, 1500, 2000 µg/ml
With activation: 250, 500, 1000, 2000, 3000, and 4000 µg/ml
Confirmatory study –
Without activation/20.1 hours: 100, 200, 400, 600, 800, 1000, 1200 µg/ml
Without activation/44.2 hours: 50, 100, 200, 400, 600, 800, 1000, and 1200 µg/ml
With activation/20.1 + 44.2 hrs: 500, 1000, 1500, 2000, 2250, 2500, 2750, and 3000 µg/ml

Test conditions:

Number of cells: In the main studies (both with and without activation), cells were cultured for about 24 hours before treatment by seeding about 1.2×10^6 cells for the 17.8 hour assay and 0.8×10^6 cells for the 44.2 hour assay. The culture medium used was McCoy's 5a culture medium supplemented with nutrients and antibiotics.

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Negative Controls: In the nonactivation assays, negative controls were cultures with cells and culture medium only. Solvent controls contained only the solvent for the test article, sterile deionized water at 10.0 µl/ml. In the activation studies, negative and solvent controls were the same as those in the nonactivation studies but also included the S9 activation mix.

Positive Controls: Mitomycin C was used for nonactivation studies. Cyclophosphamide was used in the activation studies.

Only cells with 21 ± 1 centromeres were analyzed. One hundred cells from each replicate culture of treatment groups and negative and solvent control groups were analyzed. At least 25 cells from the positive control cultures were analyzed.

In the main study (after range-finding study was completed), both initial and confirmatory studies were done.

Statistical methods used: Fisher's Exact Test (Sokal and Rohlf 1981) was used to compare the percent of cells with aberrations in each treatment group with the control group. Linear trend tests of increasing numbers of cells with aberrations with increasing dose were also done. Tests were considered to be significant at a p value of < 0.01.

RESULTS

Overall results:

(1)Without activation –

Initial trial – negative (no significant increases in cells with chromosomal aberrations observed at concentrations tested.)

20.1-hour confirmatory study – negative (no significant increases in cells with chromosomal aberrations observed at concentrations tested.)

44.2-hour confirmatory study – negative (no significant increases in cells with chromosomal aberrations or polyploidy were observed at concentrations tested.)

(2) With activation –

Initial trial – negative (no significant increases in cells with chromosomal aberrations observed at concentrations tested.)

20.1-hour confirmatory study – positive (a significant increase in cells with chromosomal aberrations was observed at 2500 µg/ml.)

44.2-hour confirmatory study – positive (a significant increase in cells with chromosomal aberrations was observed at 2750 µg/ml. Also, a significant increase in polyploidy was observed in cultures dosed with 2250, 2500, and 2750 µg/ml.)

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Genotoxic effects: Positive results for chromosomal aberrations at two concentrations (2500 and 2750 μg/ml) in the presence of activation. Positive for polyploidy and endoreduplication in the presence of activation.

Cytotoxic concentrations: In the range-finding study, complete cytotoxicity was observed in cultures dosed with 5080 μg/ml (both with and without activation). The lowest doses at which cytotoxic effects were seen in the main study were:

Without activation
- Initial – 500 μg/ml
- 20.1-hr confirmatory – 600 μg/ml
- 44.2-hr confirmatory – 400 μg/ml

With activation
- Initial – 2000 μg/ml
- 20.1-hr confirmatory – 2000 μg/ml
- 44.2-hr confirmatory – 2250 μg/ml

Statistical results: Two concentrations in the main study (2500 and 2750 μg/ml) showed significantly greater numbers of chromosomal aberrations.

Remarks: Statistical significance was judged using a rule that was stricter than usually used to judge statistical significance (i.e., $p < 0.01$ was used rather than $p < 0.05$.) No information was available to determine whether chromosomal aberrations were significant at $p < 0.05$.

In the initial trial without activation, mitotic index reductions were 25%, 30%, 87%, and 96% at doses of 125, 250, 1000, and 1500 μg/ml, respectively when compared with solvent controls. Chromosomal aberrations were analyzed from cultures dosed with 125, 250, 500, and 1000 μg/ml. In the initial trial with activation, a mitotic index reduction of 2% was seen at 250 μg/ml when compared with solvent controls. Chromosomal aberrations were analyzed from cultures dosed with 250, 500, 1000, and 2000 μg/ml.

In the 20.1-hour confirmatory trial without activation, mitotic index reductions were 73%, 84%, and 95% at doses of 800, 1000, and 1200 μg/ml, respectively when compared with solvent controls. Chromosomal aberrations were analyzed from cultures dosed with 200, 400, 600, and 800 μg/ml.

In the 44.2-hour confirmatory trial without activation, mitotic index reductions were 12%, 61%, 63%, and 92% at doses of 50, 400, 600, and 800 μg/ml when compared with solvent controls. Chromosomal aberrations were analyzed from cultures dosed with 100, 200, 400, and 600 μg/ml.

In the 20.1-hour confirmatory trial with activation, mitotic index reductions were 13%, 38%, 15%, 11%, and 30% at doses of 1000, 1500, 2000, 2250, and 2500 μg/ml, respectively when compared with solvent controls. Chromosomal aberrations were analyzed from cultures dosed with 1500, 2000, 2250, and 2500 μg/ml.

In the 44.2-hour confirmatory trial with activation, mitotic index reductions were 14%, 17%, 56%, and 53% at doses of 1000, 2250, 2500, and 2750 μg/ml when compared with solvent controls. Chromosomal aberrations were analyzed from cultures dosed with 2000, 2250, 2500, and 2750 μg/ml.

The authors describe the following deviations from the protocol – endoreduplication and polyploidy were analyzed separately because some dose levels showed increased endoreduplication and some showed increased polyploidy.

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CONCLUSIONS

The authors state that T-6564 was considered negative for inducing chromosomal aberrations in Chinese hamster ovary cells expect at a single dose level (with activation) that induced significant toxicity.

Remarks: Significant toxicity was observed in the 20.1 and 44.2 h confirmatory trials with activation at the highest concentrations tested. These two concentrations were also positive for chromosomal aberrations. The significance of a positive response in the presence of excessive toxicity is questionable, however.

REFERENCE

Murli, H. 1996. Mutagenicity Test on T-6564 Measuring Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells: with a Confirmatory Assay with Multiple Harvests. Final Report. Corning Hazleton Inc. CHV Study No.: 17750-0-437CO. September 16.

000141

Title: Mutagenicity Test with T-6564 in the *Salmonella – Escherichia Coli*/Mammalian-microsome Reverse Mutation Assay with a Confirmatory Assay

TEST SUBSTANCE

Identity: T-6564, also referred to as L-13167 and FC1015-X

Remarks: The substance was a clear, colorless liquid. No other information was provided on the test substance.

METHOD

Method/Guideline followed: CHV Protocol 409R, Edition 4. Experimental methods and materials were based on Ames et al. (1975) and Green and Muriel (1976).

Test type: *Salmonella – Escherichia Coli*/Mammalian-microsome Reverse Mutation Assay with a Confirmatory Assay

Test system: *Salmonella typhimurium* and *Escherichia coli* strains

GLP: Y

Year study performed: 1996

Species/Strain/cell-type/cell line:

Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 from Dr. Bruce Ames, Department of Biochemistry, University of California, Berkeley.

Escherichia coli strain WP2*uvr*A from National Collection of Industrial Bacteria, Torrey Research Station, Scotland.

Metabolic activation: Aroclor 1254-induced rat liver S9 homogenate, plus NADP, H_20, NaH_2PO_4/Na_2HPO_4, glucose-6-phosphate, $KCl/MgCl_2$.

Concentrations tested:
Rangefinding –
10 doses from 6.67 to 5000 µg/plate
Main study –
100, 333, 1000, 3330, 5000 µg /plate (with and without activation)

Test conditions:

Number of cells: Density of tester strain cultures were $\geq 0.5 \times 10^9$ bacteria per ml.

Negative (vehicle) controls: These were plated for all tester strains both in the presence and absence of S9 mix. Positive controls: These were used both in the presence and absence of the S9 mix; different chemicals were used depending on the strain being tested.

During the range-finding study, the growth inhibitory effect (cytotoxicity) of the test article to the test system was determined using *S. typhimurium* strain TA100. Cytotoxicity was determined to be a decrease in the number of revertant colonies per plate or thinning of the background bacterial lawn. No cytotoxicity was observed in the range-finding study.

During the main study, both initial and confirmatory studies were conducted.

The authors' criteria for a positive test result was to produce at least a 2- or 3-fold increase (the amount depends on the strain tested) in mean revertants per plate compared with the vehicle control. The increase had to be accompanied by a dose response for increasing concentrations of the test article.

Statistical methods used: None indicated

Remarks:

RESULTS

Overall results: In the initial assay and confirmatory assay, all data were acceptable and there were no positive increases in numbers of revertants per plate both with and without activation. In the confirmatory assay, however, a non-dose responsive 5-fold increase in number of revertants was observed in *S. typhimurium* strain TA1537 in the absence of S9 mix. This strain was retested and all data were acceptable and no positive increases in numbers of revertants per plate were observed.

Genotoxic effects: A 5-fold increase in revertants occurred in one strain (see Overall Results), but was not confirmed upon retesting.

Cytotoxic concentration: No cytotoxicity observed in the range-finding test, and none was noted in the main study.

Statistical results: NA

Remarks:

Since the positive response seen with S. typhimurium TA1537 occurred at only one dose at the beginning of the dose response curve and was not repeatable, the test agent, T-6564 is considered negative in this assay.

CONCLUSIONS

The authors conclude that under the conditions of the study, T-6564 did not cause a positive increase in the number of revertants per plate of any tester strains, either in the absence or presence of the S9 mix.

REFERENCE

Lawlor, T.E. 1996. Mutagenicity Test with T-6564 in the *Salmonella – Escherichia Coli*/Mammalian-microsome Reverse Mutation Assay with a Confirmatory Assay. Corning Hazleton Inc. Final Report. CHV Study No: 17750-0-409R. September 13.

Title: Mutagenicity Test on T-6342, Measuring Chromosomal Aberrations in Human Whole Blood Lymphocytes With a Confirmatory Assay With Multiple Harvests

TEST SUBSTANCE

Identity: T-6342; clear, colorless liquid. No other information was given on solubility or purity.

Remarks:

METHOD

Method/Guideline followed: Protocol No.: 449CO, Edition No.: 2, Modified for 3M Corporation

Test type: *In vitro* mutagenicity assay

Test system: Human whole blood lymphocytes

GLP: Yes

Year study performed: 1996

Species/Strain/cell-type/cell line: Human/whole blood lymphocytes

Metabolic activation: With and *without* metabolic activation from the S9 supernatant fraction of homogenates from livers of rats pretreated with Aroclor 1254 for the nonspecific induction of metabolizing enzymes.

Concentrations tested:
Range-finding assay—0.167, 0.500, 1.67, 5.00, 16.7, 50.0, 167, 500, 1670, 5000 g/mL;
Initial trial (without activation)—127, 253, 505, 1010, 1510, 2010, 2510 g/mL;
Initial trial (with activation)— 253, 505, 1010, 1510, 2010, 2510, 3010, 4010 g/mL;
Confirmatory trial (replicate cultures, without activation, 22.1 hour assay)—125, 250, 500, 900, 1200, 1600, 2000 g/mL;
Confirmatory trial (replicate cultures, without activation, 46.0 hour assay)—62.5, 125, 250, 500, 900, 1200, 1600, 2000 g/mL;
Confirmatory trial (replicate cultures, with activation, 22.1 hour assay and 46.0 hour assay)—250, 500, 1000, 1500, 2000, 2500, 3000 g/mL

Statistical methods used: Statistical analysis employed a Cochran-Armitage test for linear trend and Fisher's Exact Test (Thakur et al., 1985) to compare the percentage of cells with aberrations (and, if applicable, the percentage of cells with more than one aberration), polyploidy, and endoreduplication in treated cells with results from vehicle controls. Test article significance was established where $p<0.01$. All factors as stated previously were taken into account and the final evaluation of the test article was based upon scientific judgement.

Remarks:

Experimental Design

Human venous blood from a single, normal, healthy male donor was drawn into sterile, heparinized Vacutainers. Cultures were initiated with 0.3 mL of blood/5 mL culture (dose range-finding assay) or 0.6 mL of blood/10.0 mL culture (chromosomal aberrations assays) in 15 mL centrifuge tubes. The cells were incubated at approximately 37 °C on a slope, with loose caps, in an atmosphere of about 5% CO_2 in air. The culture medium used was RPMI 1640 (JRH Biosciences) supplemented with 15% fetal bovine serum (FBS; Biochemed, Lot# E5331, dose range-finding assay; Lot No.: T06024, chromosomal aberrations assay), 1% phytohemagglutinin (PHA-M; Gibco), penicillin (100 units/mL; Quality Biologicals) and streptomycin (100 ug/mL; Quality Biologicals), and 2mM L-glutamine (Quality Biologicals). Deionized water (Prepared at CHV, Lot# 20) was the solvent of choice for this assay. The test article was dissolved in deionized water at a concentration of 500 mg/mL. The test article solutions and the vehicle control, deionized water, were dosed with a dosing volume of 1% (10 uL/mL) for this assay.

Negative and Solvent Controls

In the nonactivation assays, negative controls were cultures that contained only cells and culture medium. Solvent controls were cultures that contained deionized water at the highest concentration used in test cultures (1% or 10.0 L/mL). In the activation assays, the negative and solvent controls were the same as described in the nonactivation assays, but with the S9 activation mix included.

Positive Control Agents

The positive control agents, which were used in the assays, were mitomycin C (MMC; CAS# 50-07-7, Sigma, Lot# 40H2508) for the nonactivation series and cyclophosphamide (CP; CAS# 6055-19-2, Sigma, Lot# 43H0269) in the metabolic activation series. In the chromosomal aberrations assays, three concentrations of MMC (0.08 and 0.10 g/mL, initial and confirmatory trials; 0.1, 0.2, and 0.3 g/mL, third trial) and CP (20, 30, and 50 g/mL) were used to induce chromosomal aberrations. One of the dose levels was analyzed in each of the aberration assays. Both MMC and CP were dissolved in water.

Range-finding Assays

In these assays, cultures were initiated with 0.3 mL of blood/5 mL culture and were incubated for 2 days prior to treatment.

Assay Without Metabolic Activation

The lymphocytes were incubated with the test article for 19.5 hours at 37 C. The test article was washed from the cells with phosphate-buffered saline and fresh complete medium containing Colcemid (final concentration = 0.1 g/mL) was added. The cultures were harvested 2.0 hours later.

Assay With Metabolic Activation

In this assay, the lymphocytes were incubated with the test article for 3 hours at 37 C in the presence of a rat liver S9 reaction mixture (S9=15 l/ml, NADP = 1.5 mg/mL, and isocitric acid = 2.7 mg/mL). The S9 fraction (Molecular Toxicology, Inc., Lot# 0667) was derived from the liver of male Sprague-Dawley rats, which had been previously treated with Aroclor 1254. In order to avoid possible inactivation of short lived and highly reactive intermediates produced by the S9 enzymes through binding to serum proteins, the medium did not have FBS during the exposure period. After the exposure period, the cells were washed twice with buffered saline.

Complete RPMI culture medium was added to the cultures, which were incubated for 18.5 hours. Colcemid (final concentration = 0.1 g/mL~~), which~~ was added for the last 2.0 hours to collect metaphase cells.

Assay Evaluation

Mitotic index was analyzed from the surviving dose levels by analyzing the number of metaphases present in 1000 consecutive cells.

Chromosomal Aberration Assay—With and Without Metabolic Activation

In the chromosomal aberration assays, replicate cultures were used at each dose level and for negative controls, positive controls, and solvent controls. The aberration assays were conducted with a 22.0-hour harvest time in the initial trial and with 22.1- and 46.0-hour harvest times in the confirmatory trials. Chromosomal aberrations were analyzed from the cultures treated at 4 dose levels and from one of the positive control doses.

Aberration Assay Without Metabolic Activation

Cultures were initiated 2 days prior to treatment with 0.6 mL of whole blood/10.0 mL culture in 15 mL centrifuge tubes. Two days after culture initiation, cells were treated with the test article at predetermined concentrations for approximately 19.3 and 43.3 hours. The cultures were washed with buffered saline. Complete RPMI 1640 medium, containing 0.1 g/mL Colcemid , was placed back onto the cells. Two hours later, the cells were harvested and air-dried slides were made. The slides were stained in 5% Giemsa solution for the analysis of chromosomal aberrations. Table 1 contains a summary of the treatment schedule of the aberrations assay without metabolic activation.

Aberration Assay With Metabolic Activation

Cultures were initiated 2 days prior to treatment with 0.6 mL of whole blood/10.0 mL culture in 15 mL centrifuge tubes. Two days after culture initiation, the cultures were incubated at 37 C for 3 hours in the presence of the test article and the S9 reaction mixture. After the 3-hour exposure period, the cells were washed twice with buffered saline and the cells were refed with complete RPMI 1640 medium. One-tenth g/mL Colcemid was added to the cultures during the last 2.0 hours of incubation. The metaphase cells were harvested and prepared for cytogenetic analysis.

Harvest Procedure

The cell suspension was centrifuged, the supernatant was discarded, and the cells were treated with hypotonic KCl (0.075 M) for 10 minutes. After centrifugation and removal of the KCl, the cells were washed 3 times with freshly prepared fixative (absolute methanol:glacial acetic acid, 3:1, v:v). Air-dried slides were prepared from the harvested cells.

Slide Preparation and Staining

Slides were prepared by dropping the harvested cultures on clean slides. The slides were stained with 5% Giemsa solution for the analysis of mitotic index and chromosomal aberrations. All slides were air-dried and cover slipped.

Aberration Analysis and Assay Evaluation

Cells were selected for good morphology and only cells with the number of centromeres equal to the modal number 46 were analyzed. One hundred cells, if possible, from each replicate culture at 4 dose levels of the test article, the negative control, the solvent control, and positive control

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cultures were analyzed for the different types of chromosomal aberrations (Evans, 1962). At least 25 cells were analyzed for chromosomal aberrations from those cultures with >25% cells with chromosomal aberrations. Cells with aberrations were recorded. Mitotic index was assessed by analyzing the number of mitotic cells/1000 cells; the ratio was expressed as a percentage of mitotic cells. Chromatid and isochromatid gaps, if observed, were noted in the raw data and were tabulated. They were not, however, considered in the evaluation of the ability of the test article to induce chromosomal aberrations. Percent polyploidy and endoreduplication were analyzed and the results were tabulated.

RESULTS

Overall results: positive, negative, ambiguous: Negative

Genotoxic effects (unconfirmed, dose-response, equivocal – with/without activation): Negative

Cytotoxic concentration:

Range-finding Assay Without Metabolic Activation

Hemolysis was observed prior to wash in the cultures dosed with 5000 g/mL. Reductions of 35%, 6%, 38%, 15%, 18%, 57%, and 100% were observed in the mitotic indices of the cultures dosed with 0.500, 1.67, 5.00, 16.7, 500, 1670, and 5000 g/mL.

Range-finding Assay With Metabolic Activation

Hemolysis was observed prior to wash in the cultures dosed with 5000 g/mL. Reductions of 8%, 5%, 4%, 1%, and 100% were observed in mitotic indices of the cultures dosed with 1.67, 50.0, 500, 1670, and 5000 g/mL, as compared with the solvent control culture.

Chromosomal Aberration Assay Without Metabolic Activation

Initial Trial—Hemolysis was observed prior to wash in the cultures dosed with 2510 g/mL. Reductions of 38%, 18%, 18%, 50%, 55%, and 95% in the mitotic indices, as compared with the solvent control cultures, were observed in the cultures treated with 127, 253, 505, 1010, 1510, and 2010 g/mL, respectively.

Confirmatory Trial—In the 22.1-hour confirmatory trial, hemolysis was observed prior to wash of the cultures dosed with 1600 and 2000 g/mL, and a slight evidence of hemolysis was evident at harvest of the cultures dosed with 1600 g/mL. Reductions of 2%, 14%, 64%, 74%, 93%, and 93% in the mitotic indices, as compared with the solvent control cultures, were observed in the cultures dosed with 125, 500, 900, 1200, 1600, and 2000 g/mL, respectively. In the 46.0-hour confirmatory trial, hemolysis was observed prior to wash of the cultures dosed with 1600 and 2000 g/mL. Reductions of 59%, 93%, 98%, and 98% in the mitotic indices, as compared with the solvent control cultures, were observed in the cultures dosed with 900, 1200, 1600, and 2000 g/mL, respectively.

Chromosomal Aberration Assay With Metabolic Activation

Initial Trial—Hemolysis was observed prior to wash in the cultures dosed with 2010, 2510, 3010, and 4010 g/mL. No cells were visible prior to the addition of Colcemid to the cultures dosed with 4010 g/mL. Hemolysis was observed prior to the addition of Colcemid to the cultures dosed with 3010 g/mL. Reductions of 15%, 20%, 15%, 43%, 77%, and 95% in the mitotic

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indices, as compared with the solvent control cultures, were observed in the cultures treated with 253, 505, 1010, 1510, 2010, and 2510 μg/mL, respectively.

Confirmatory Trial—In the 22.1-hour confirmatory trial, hemolysis was observed prior to wash and prior to harvest of the cultures dosed with 2000, 2500, and 3000 μg/mL. Reductions of 15%, 5%, 69%, 82%, 97%, and 100% in the mitotic indices, as compared with the solvent control cultures, were observed in the cultures treated with 500, 1000, 1500, 2000, 2500, and 3000 μg/mL, respectively. In the 46.0-hour confirmatory trial, hemolysis was observed prior to wash and prior to harvest of the cultures dosed with 2000, 2500, and 3000 μg/mL. Reductions of 15%, 80%, 100%, and 100% in the mitotic indices, as compared with the solvent control cultures, were observed in the cultures treated with 1500, 2000, 2500, and 3000 μg/mL, respectively.

Statistical results: The test substance did not significantly increase chromosomal aberrations with or without metabolic activation.

Remarks:

Chromosomal Aberration Assay Without Metabolic Activation

Initial Trial—No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed at the concentrations analyzed (253, 505, 1010, 1510 μg/mL).

Confirmatory Trial—In the 22.1-hour confirmatory trial, no significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed at the concentrations analyzed (125, 250, 500, and 900 μg/mL). In the 46.0-hour confirmatory trial, no significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed at the concentrations analyzed (125, 250, 500, and 900 μg/mL). The sensitivity of the cell culture for induction of chromosomal aberrations was shown by the increased frequency of aberrations in the cells exposed to MMC, the positive control agent.

Chromosomal Aberrations Assay With Metabolic Activation

Initial Trial—No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed at the concentrations analyzed (505, 1010, 1510, and 2010 μg/mL).

Confirmatory Trial—In the 22.1-hour confirmatory trial, no significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed at the concentrations analyzed (250, 500, 1000, and 1500 μg/mL). In the 46.0-hour confirmatory trial, due to toxicity, only 52 metaphases were available for analysis in one of the cultures dosed with 2000 μg/mL. No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed at the concentrations analyzed (500, 1000, 1500, 2000 μg/mL), except for a weak increase in endoreduplication at 2000 μg/mL. The successful activation of the metabolic system was illustrated by the increased incidence of cells with chromosomal aberrations in the cultures treated with cyclophosphamide, the positive control agent.

CONCLUSIONS

The test article, T-6342, was considered negative for inducing chromosomal aberrations in cultured whole blood human lymphocytes cells with and without metabolic activation. These results were verified in independently conducted confirmatory trials.

REFERENCE

Murli, Hemalatha. 1996. Mutagenicity Test on T-6342, Measuring Chromosomal Aberrations in Whole Blood Lymphocytes With a Confirmatory Assay With Multiple Harvests. Corning-Hazelton, Inc. (CHV). Vienna, VA. CHV Study No.: 17073-0-449CO.

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Title: Mutagenicity Test on T-6342, Measuring Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells With a Confirmatory Assay With Multiple Harvests

TEST SUBSTANCE

Identity: T-6342 C; clear, colorless liquid.

Remarks: T-6342 was soluble in sterile, deionized water >4.980 g/L (the test substance at a concentration of 4980 ug/mL in culture medium remained completely soluble at a pH of 7.5).

METHOD

Method/Guideline followed: Protocol No.: 437CO, Edition No.: 4, Modified for 3M Corporation

Test type: *In vitro* mutagenicity assay

Test system: Chinese hamster ovary (CHO) cells

GLP: Yes

Year study performed: 1996

Species/Strain/cell-type/cell line: Chinese hamster ovary cells (CHO-WBL) from a permanent cell line, originally obtained from the laboratory of Dr. S. Wolff, University of California, San Francisco. The cells used in this study had been recloned to maintain karyotypic stability. This cell line had an average cycle time of 12-14 hours with a modal chromosome number of 21. The CHO cells were grown in McCoy's 5a culture medium, which was supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin and streptomycin, at approximately 37 C, in an atmosphere of about 5% CO_2 in air.

Metabolic activation: With and without rat liver S-9

Concentrations tested:
Range-finding assay—0.166, 0.498, 1.66, 4.98, 16.6, 49.8, 166, 498, 1660, 4980 g/mL;
Initial trial (without activation)—125, 250, 500, 750, 1000, 1500, 2000 g/mL;
Initial trial (with activation)—250, 500, 1250, 2500, 3750, 5000 g/mL;
Confirmatory trial (without activation)—125, 249, 498, 746, 995, 1490, 1990 g/mL;
Confirmatory trial (with activation)—249, 498, 1250, 2490, 3730, 4970 g/mL;
Repeat trial (without activation)—250, 500, 1250, 2490, 3740, 4980 g/mL

Statistical methods used: The Fisher's Exact Test with an adjustment for multiple comparisons (Sokal and Rohlf, 1981) was employed to compare the percentage of cells with aberrations in each treatment group with the results from the solvent controls. A linear trend test of increasing number of cells with aberrations with increasing dose (Armitage, 1971) was also performed. Test article significance was established where $p<0.01$.

Remarks:
Experimental Design
In the chromosomal aberrations assays, replicate cultures were used at each dose level and for negative and solvent controls. Single cultures were used for each of two doses of the positive

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control. The aberrations assays were conducted with a 20.0-hour harvest time in the initial trial and with 20.0- and 44.1-hour harvest times in the confirmatory trials. Chromosomal aberrations were analyzed from the cultures treated at 4 dose levels and from only one of the positive control doses.

Negative and Solvent Controls

In the nonactivation assays, negative controls were cultures that contained only cells and culture medium. Solvent controls were cultures containing the solvent for the test article at the highest concentration used in test cultures. In the activation assays, the negative and solvent controls were the same as described in the nonactivation assays, but with the S9 activation mix included.

Positive Control Agents

The positive control agents, which were used in the assays, were mitomycin C (MMC; CAS# 50-07-7, Sigma, Lot# 25H0619) for the nonactivation series and cyclophosphamide (CP; CAS# 6055-19-2, Sigma, Lot#67F0155) in the metabolic activation series. In the chromosomal aberrations assays, two concentrations of MMC (0.08 and 0.10 g/mL, initial and confirmatory trials; 0.50 and 1.0 g/mL, third trial) and CP (5.0 and 10.0 g/mL) were used to induce chromosomal aberrations in the CHO cells. One of the dose levels was analyzed in each of the aberration assays. Both MMC and CP were dissolved in water.

Range-finding Assays

In these assays, the cells were cultured for approximately ( ) 24 hours prior to treatment by seeding 0.3 x 10^6 cells/25 cm^2 flask into 5 mL of complete McCoy's 5a culture medium. Table 1 contains a summary of the treatment schedule of the range-finding assay. All dosing was achieved with a 1% (10 L/mL) dosing of each stock solution and the solvent control culture was dosed with 10 L/mL of sterile deionized water

Assay Without Metabolic Activation

The cultures were incubated with the test article for 17.8 hours at 37 C. The test article was washed from the cells with phosphate-buffered saline and fresh complete medium containing Colcemid (final concentration = 0.1 g/mL) was added. The cultures were then trypsinized and harvested 2.0 hours later.

Assay With Metabolic Activation

In this assay, the CHO cells were exposed to the test article for 3 hours at 37 C in the presence of a rat liver S9 reaction mixture (S9=15 l/ml, NADP = 1.5 mg/mL, and isocitric acid = 2.7 mg/mL). The S9 fraction (Molecular Toxicology, Inc., Lot# 0583) was derived from the liver of male Sprague-Dawley rats, which had been previously treated with Aroclor 1254. In order to avoid possible inactivation of short lived and highly reactive intermediates produced by the S9 enzymes through binding to serum proteins, the medium did not have FBS during the exposure period. After the exposure period, the cells were washed twice with buffered saline. Complete McCoy's 5a medium was added to the cultures, which were incubated for 16.8 hours. ~~with~~ Colcemid (final concentration = 0.1 g/mL)~~-which~~ was added for the last 2.0 hours to collect metaphase cells. The cultures were trypsinized, harvested, fixed, and slides were prepared and stained as described for the nonactivation range-finding assay.

Assay Evaluation

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Mitotic index was analyzed from the highest five surviving dose levels by analyzing the number of metaphases present in 1000 consecutive cells.

Aberration Assay Without Metabolic Activation

Cultures were initiated by seeding approximately 1.2 x 10^6 cells (20.0 hour assay) and 0.8 x 10^6 cells (44.0 hour assay) per 75 cm^2 flask into 10 mL of complete McCoy's 5a medium. One day after culture initiation, for the initial and confirmatory trials, the cells were incubated at 37 C with the test article at predetermined doses for about 17.7 (20.0 hour assay) and 41.8 (44.1 hour assay) hours. All dosing was achieved with a 1% (10 L/mL) dosing of each stock solution and the solvent control culture was dosed with 10 L/mL of sterile deionized water. The cultures were washed with buffered saline. Complete McCoy's 5a medium, containing 0.1 g/mL Colcemid , was placed back onto the cells. Approximately 2 hours later, the cells were harvested and air-dried slides were made. The slides were stained in 5% Giemsa solution for the analysis of chromosomal aberrations. For the third trial, one day after culture initiation, the cultures were incubated at 37 C for 3 hours in the presence of the test article in McCoy's 5a medium without FBS. After the 3-hour exposure period, the cells were washed twice with buffered saline and the cells were refed with complete McCoy's 5a medium. One-tenth g/mL Colcemid was added during the last 2.0 hours of incubation. The metaphase cells were harvested and prepared for cytogenetic analysis.

Aberration Assays With Metabolic Activation

Cultures were initiated by seeding approximately 1.2 x 10^6 cells (20.0 hour assay) and 0.8 x 10^6 cells (44.1 hour assay) per 75 cm^2 flask into 10 mL of complete McCoy's 5a medium. One day after culture initiation, the cultures that were treated under the conditions of metabolic activation were incubated at 37 C for 3 hours in the presence of the test article and the S9 reaction mixture in McCoy's 5a medium without FBS. All dosing was achieved with a 1% (10 L/mL) dosing of each stock solution and the solvent control culture was dosed with 10 L/mL of sterile deionized water. After the 3-hour exposure period, the cells were washed twice with buffered saline and the cells were refed with complete McCoy's 5a medium. One-tenth g/mL Colcemid was added to the culture medium during the last 2.0 hours of incubation. The metaphase cells were harvested and prepared for cytogenetic analysis.

Harvest Procedure

Prior to the harvest of the cultures, visual observations of toxicity were made. These observations included an assessment of the percent confluence of the cell monolayer within the culture flasks. The cultures were also evaluated for the presence of mitotic or dead cells floating in the medium. The cultures from the dose range-finding assay were trypsinized first to collect mitotic and interphase cells and were treated with 0.075 M KCl hypotonic solution. The cultures were fixed with an absolute methanol:glacial acetic acid (3:1, v:v) fixative and washed several times before air-dried slides were prepared.

Slide Preparation and Staining

Slides were prepared by dropping the harvested cultures on clean slides. The slides were stained with 5% Giemsa solution for the analysis of mitotic index and chromosomal aberrations. All slides were air-dried and cover slipped using Depex mounting medium.

Analysis of Aberrations and Assay Evaluation

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Cells were selected for good morphology and only cells with the number of centromeres equal to the modal number 21 1 (range = 20-22) were analyzed. One hundred cells, if possible, from each replicate culture at 4 dose levels of the test article and from the negative and solvent control cultures were analyzed for the different types of chromosomal aberrations (Evans, 1962). At least 25 cells were analyzed for chromosomal aberrations from one of the positive control cultures. Cells with aberrations were recorded. Mitotic index was assessed by analyzing the number of mitotic cells/1000 cells; the ratio was expressed as a percentage of mitotic cells. Chromatid and isochromatid gaps, if observed, were noted in the raw data and were tabulated. They were not, however, considered in the evaluation of the ability of the test article to induce chromosomal aberrations. Percent polyploidy was analyzed from the 44.0-hour assay.

RESULTS

Overall results: positive, negative, ambiguous: Positive

Genotoxic effects (unconfirmed, dose-response, equivocal – with/without activation): The tested substance was positive for clastogenic activity for highly toxic treatments of short duration (3 hours) both in the presence and absence of an S9 metabolic activation system. The tested substance was also positive for polyploidy for the highly toxic treatments.

Cytotoxic concentration:
This section only describes the cytoxicity observed at concentrations at which statistically significant increases in chromosomal aberrations occurred.

Chromosomal Aberrations Assay With Metabolic Activation

Initial Trial—The treatment with 3750 ug/mL was only slightly toxic, but the 5000 ug/mL treatment was highly toxic, causing about 95% reduction in monolayer confluency for one culture and about 75% reduction for the replicate culture. Floating dead cells and debris were abundant, and only 94 metaphases were analyzable from the culture with greater toxicity (5000 ug/mL).

Confirmatory Trial—
For the 20 hour harvest, the confluency of the cultures treated with 4970 ug/mL was reduced by about 70% and the mitotic index was reduced by 43%. For the cultures treated with 3730 ug/mL (20 hour harvest), there was approximately a 30% reduction in confluency and an 18% reduction in mitotic index.

Aberration Assays Without Metabolic Activation; 3-Hour Treatment

20 hour harvest—A 30% reduction in confluency occurred in the cultures treated with 2490 ug/mL. The next higher dose, 3740 ug/mL, was so toxic that only a small number of metaphases could be examined.

44 hour harvest—A 55% reduction in monolayer confluency occurred in the cultures treated with 3740 ug/mL and the 2490 ug/mL treatment resulted in a 30% reduction. The mitotic index was significantly reduced (57%) for the high dose (3740 ug/mL).

Statistical results:

Chromosomal Aberration Assays With Metabolic Activation

Initial Trial—Significant increases in cells with chromosomal aberrations were observed in the cultures dosed with 3750 and 5000 g/mL.

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Confirmatory trial—For the 20 hour harvest, a significant increase in cells with chromosomal aberrations (simple and complex) was observed for the cultures dosed with 4970 g/mL. Although the 4970 g/mL treatment was too toxic to yield sufficient metaphases for analysis, it was, nevertheless, noted that the percent polyploid cells increased significantly.

Chromosomal Aberration Assays Without Metabolic Activation; 3-Hour Treatment

A significant increase in the number of cells with chromosomal aberrations was observed in the cultures dosed with 3740 g/mL (7.5%). A significant increase in percent polyploidy was also observed in the cultures dosed with 3740 g/mL. The mitotic index was significantly reduced (57%) for the 3740 g/mL dose.

Remarks:

Range-finding Assay Without Metabolic Activation

In the culture treated with 4980 g/mL, no visible mitotic cells were observed; the cell monolayer was <5% confluent and the culture consisted only of debris and dead cells, floating or attached. A severe reduction in the number of visible mitotic cells was observed in the culture dosed with 1660 g/mL. The cell monolayer looked unhealthy and the degree of confluency was reduced about 15%; floating dead cells and debris were noted. Floating debris was also observed in the culture dosed with 498 g/mL, but otherwise the culture was similar to the negative control. Mitotic indices were analyzed from the cultures dosed with 16.6, 49.8, 166, 498, and 1660 g/mL and compared to the solvent control. At 1660 g/mL, the mitotic index was essentially zero. However, little or no reduction in mitotic index was observed at the next lower dose of 498 g/mL.

Range-finding Assay With Metabolic Activation

An unhealthy cell monolayer was observed in the culture dosed with 4980 g/mL. The cell monolayer confluence was reduced by approximately 85% and a severe reduction in the number of visible mitotic cells occurred (about 63%). At the next lower dose of 1660 g/mL, the monolayer confluence was the same as the solvent control and the mitotic index was only somewhat reduced (about 27%).

Chromosomal Aberration Assays Without Metabolic Activation

Initial Trial—No significant increases in cells with chromosomal aberrations were observed for the cultures analyzed (750, 1000, 1500, and 2000 g/mL). Unhealthy cell monolayers, floating dead cells and debris, severe loss of visible mitotic cells, and about 25% reduction in cell monolayer confluence were observed in the cultures dosed with 1500 and 2000 g/mL. Slightly unhealthy cell monolayers, floating dead cells and debris, a reduction in the number of visible mitotic cells, and about 15% reduction in cell monolayer confluence were observed in the cultures dosed with 1000 g/mL. At the lowest analyzed dose of 750 g/mL, the cell monolayers appeared to be almost normal, with only a slight reduction in the number of visible mitotic cells and about a 15% reduction in the degree of confluence. Severe reductions in mitotic index, relative to the solvent control cultures, occurred at all dose levels (95%, 86%, 67%, and 57% at 2000, 1500, 1000, and 750 g/mL, respectively).

Confirmatory Trial—For the 20-hour harvest, no significant increases in cells with chromosomal aberrations were observed for any of the cultures analyzed (746, 995, 1490, and 1990 g/mL). A 95% reduction in mitotic index was observed at 1990 g/mL; whereas, a 25% reduction in

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mitotic index was observed at 746 g/mL. The cell monolayer confluence was reduced by about 25% at 1990 g/mL and only 83 metaphases could be scored from one of the replicate cultures. To compensate, 117 metaphases were scored from the replicate culture, in order to score a total of 200 metaphases for this dose. For the 44.1-hour harvest, chromosomal aberrations were analyzed from the cultures dosed with 249, 498, 746, and 995 g/mL. No significant increases in cells with chromosomal aberrations were observed for any of the cultures analyzed. Percent polyploidy was scored at this harvest time and no significant increases were noted. The two highest applied doses of 1990 and 1490 g/mL were lethal. Treatment with 995 g/mL was severely toxic, resulting in about 40% reductions in monolayer confluence and 79% reduction in mitotic index. At 746 g/mL, however, the toxicity was much less (about 15% reduction in confluency and 18% reduction in mitotic index, compared to the solvent control cultures).

Chromosomal Aberration Assays With Metabolic Activation

Initial Trial—Chromosomal aberrations were analyzed from the cultures dosed with 1250, 2500, 3750, and 5000 g/mL in the presence of the rat liver S9 metabolic activation system. Significant increases in cells with chromosomal aberrations were observed in the cultures dosed with 3750 and 5000 g/mL. The treatment with 3750 g/mL was slightly toxic, but the 5000 g/mL treatment was highly toxic, causing about 95% reduction in monolayer confluency for one culture and about 75% reduction for the replicate culture. Floating dead cells and debris were abundant and only 94 metaphases were analyzable from the culture with greater toxicity. However, the mitotic index was reduced by only 13%, compared to the solvent control cultures.

Confirmatory Trial—For the 20 hour harvest, a significant increase in cells with chromosomal aberrations (simple and complex) was observed for the cultures dosed with 4970 g/mL. This toxic treatment reduced the confluency by about 70% and caused a 43% reduction in the mitotic index. The treatment with 3730 g/mL caused about 30% reduction in the confluency and 18% reduction in the mitotic index. There was no clastogenic activity observed at 3730 g/mL. For the 44.1-hour harvest, no significant increases in cells with chromosomal aberrations were observed for any of the cultures analyzed (498, 1250, 2490, 3730 g/mL). Although the 4970 g/mL treatment was too toxic to yield sufficient metaphases for analysis, it was, nevertheless, noted that the percent polyploid cells increased significantly. At 3730 g/mL, there was little or ~~not~~ *no* toxicity evident. The successful activation by the metabolic system was illustrated by the large increase in percent cells with chromosomal aberrations (at 20 hours harvest) in the cultures exposed to cyclophosphamide.

Chromosomal Aberration Assays Without Metabolic Activation; 3-Hour Treatment

For the 20-hour harvest, no significant increases in cells with chromosomal aberrations were observed in the cultures analyzed (250, 500, 1250, 2490 g/mL). At 2490 g/mL, a 30% reduction in confluency was observed. However, the next highest dose, 3740 g/mL, was so toxic that only a small number of metaphases could be examined. For the 44-hour harvest, chromosomal aberrations were analyzed from the cultures dosed with 500, 1250, 2490, and 3740 g/mL. A significant increase in the number of cells with chromosomal aberrations was observed in the cultures dosed with 3740 g/mL (7.5%). A significant increase in percent polyploidy was also observed in the cultures dosed with 3740 g/mL. Relative to the solvent controls, the 3740 g/mL treatment resulted in about 55% reduction in monolayer confluency and the 2490 g/mL treatment resulted in about 30% reduction. The mitotic index was also significantly reduced (57%) for the high dose.

CONCLUSIONS

The test article, T-6342, was evaluated as being clastogenic to cultured CHO cells for highly toxic treatments of short duration (eg., 3 hours) both in the presence and absence of an S9 metabolic activation system. Polyploidy was also induced for the highly toxic treatments.

REFERENCE

Murli, Hemalatha. 1996. Mutagenicity Test on T-6342, Measuring Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells: with a Confirmatory Assay with Multiple Harvests. Corning-Hazelton, Inc. (CHV). Vienna, VA. CHV Study No.: 17073-0-437CO.

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Title: Mutagenicity Test on T-6342, Measuring Chromosomal Aberrations in Human Whole Blood Lymphocytes With a Confirmatory Assay With Multiple Harvests

TEST SUBSTANCE

Identity: T-6342; clear, colorless liquid,

Remarks: No other information was given about purity or solubility

METHOD

Method/Guideline followed: Protocol No.: 449CO, Edition No.: 2, Modified for 3M Corporation

Test type: *In vitro* mutagenicity assay

Test system: Human whole blood lymphocytes

GLP: Yes

Year study performed: 1996

Species/Strain/cell-type/cell line: Human/whole blood lymphocytes

Metabolic activation: With and without metabolic activation from S9 supernatant fraction of livers of rats pretreated with Aroclor 1254 for the non-specific induction of metabolizing enzymes.

Concentrations tested:
Range-finding assay—0.167, 0.500, 1.67, 5.00, 16.7, 50.0, 167, 500, 1670, 5000 g/mL;
Initial trial (without activation)—127, 253, 505, 1010, 1510, 2010, 2510 g/mL;
Initial trial (with activation)— 253, 505, 1010, 1510, 2010, 2510, 3010, 4010 g/mL;
Confirmatory trial (replicate cultures, without activation, 22.1 hour assay)—125, 250, 500, 900, 1200, 1600, 2000 g/mL;
Confirmatory trial (replicate cultures, without activation, 46.0 hour assay)—62.5, 125, 250, 500, 900, 1200, 1600, 2000 g/mL;
Confirmatory trial (replicate cultures, with activation, 22.1 hour assay and 46.0 hour assay)—250, 500, 1000, 1500, 2000, 2500, 3000 g/mL

Statistical methods used: Statistical analysis employed a Cochran-Armitage test for linear trend and Fisher's Exact Test (Thakur et al., 1985) to compare the percentage of cells with aberrations (and, if applicable, the percentage of cells with more than one aberration), polyploidy, and endoreduplication in treated cells with results from vehicle controls. Test article significance was established where $p<0.01$. All factors as stated previously were taken into account and the final evaluation of the test article was based upon scientific judgement.

Remarks:
Experimental Design
Human venous blood from a single, normal, healthy male donor was drawn into sterile, heparinized Vacutainers. Cultures were initiated with 0.3 mL of blood/5 mL culture (dose range-finding assay) or 0.6 mL of blood/10.0 mL culture (chromosomal aberrations assays) in 15 mL

centrifuge tubes. The cells were incubated at approximately 37 C on a slope, with loose caps, in an atmosphere of about 5% CO_2 in air. The culture medium used was RPMI 1640 (JRH Biosciences) supplemented with 15% fetal bovine serum (FBS; Biochemed, Lot# E5331, dose range-finding assay; Lot No.: T06024, chromosomal aberrations assay), 1% phytohemagglutinin (PHA-M; Gibco), penicillin (100 units/mL; Quality Biologicals) and streptomycin (100 ug/mL; Quality Biologicals), and 2mM L-glutamine (Quality Biologicals). Deionized water (Prepared at CHV, Lot# 20) was the solvent of choice for this assay. The test article was dissolved in deionized water at a concentration of 500 mg/mL. The test article solutions and the vehicle control, deionized water, were dosed with a dosing volume of 1% (10 uL/mL) for this assay.

Negative and Solvent Controls

In the nonactivation assays, negative controls were cultures that contained only cells and culture medium. Solvent controls were cultures that contained deionized water at the highest concentration used in test cultures (1% or 10.0 L/mL). In the activation assays, the negative and solvent controls were the same as described in the nonactivation assays, but with the S9 activation mix included.

Positive Control Agents

The positive control agents, which were used in the assays, were mitomycin C (MMC; CAS# 50-07-7, Sigma, Lot# 40H2508) for the nonactivation series and cyclophosphamide (CP; CAS# 6055-19-2, Sigma, Lot# 43H0269) in the metabolic activation series. In the chromosomal aberrations assays, three concentrations of MMC (0.08 and 0.10 g/mL, initial and confirmatory trials; 0.1, 0.2, and 0.3 g/mL, third trial) and CP (20, 30, and 50 g/mL) were used to induce chromosomal aberrations. One of the dose levels was analyzed in each of the aberration assays. Both MMC and CP were dissolved in water.

Range-finding Assays

In these assays, cultures were initiated with 0.3 mL of blood/5 mL culture and were incubated for 2 days prior to treatment.

Assay Without Metabolic Activation:

The lymphocytes were incubated with the test article for 19.5 hours at 37 C. The test article was washed from the cells with phosphate-buffered saline and fresh complete medium containing Colcemid (final concentration = 0.1 g/mL) was added. The cultures were ~~and~~ harvested 2.0 hours later.

Assay With Metabolic Activation

In this assay, the lymphocytes were incubated with the test article for 3 hours at 37 C in the presence of a rat liver S9 reaction mixture (S9=15 l/ml, NADP = 1.5 mg/mL, and isocitric acid = 2.7 mg/mL). The S9 fraction (Molecular Toxicology, Inc., Lot# 0667) was derived from the liver of male Sprague-Dawley rats, which had been previously treated with Aroclor 1254. In order to avoid possible inactivation of short lived and highly reactive intermediates produced by the S9 enzymes through binding to serum proteins, the medium did not have FBS during the exposure period. After the exposure period, the cells were washed twice with buffered saline. Complete RPMI culture medium was added to the cultures, which were incubated for 18.5 hours. Colcemid (final concentration = 0.1 g/mL~~), which~~ was added for the last 2.0 hours to collect metaphase cells.

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Assay Evaluation

Mitotic index was analyzed from the surviving dose levels by analyzing the number of metaphases present in 1000 consecutive cells.

Chromosome Aberration Assay—With and Without Metabolic Activation

In the chromosomal aberration assays, replicate cultures were used at each dose level and for negative controls, positive controls, and solvent controls. The aberration assays were conducted with a 22.0-hour harvest time in the initial trial and with 22.1- and 46.0-hour harvest times in the confirmatory trials. Chromosomal aberrations were analyzed from the cultures treated at 4 dose levels and from one of the positive control doses.

Chromosome Aberration Assay Without Metabolic Activation

Cultures were initiated 2 days prior to treatment with 0.6 mL of whole blood/10.0 mL culture in 15 mL centrifuge tubes. Two days after culture initiation, cells were treated with the test article at predetermined concentrations for approximately 19.3 and 43.3 hours. The cultures were washed with buffered saline. Complete RPMI 1640 medium, containing 0.1 g/mL Colcemid, was placed back onto the cells. Two hours later, the cells were harvested and air-dried slides were made. The slides were stained in 5% Giemsa solution for the analysis of chromosomal aberrations. Table 1 contains a summary of the treatment schedule of the aberrations assay without metabolic activation.

Chromosome Aberration Assay With Metabolic Activation

Cultures were initiated 2 days prior to treatment with 0.6 mL of whole blood/10.0 mL culture in 15 mL centrifuge tubes. Two days after culture initiation, the cultures were incubated at 37 C for 3 hours in the presence of the test article and the S9 reaction mixture. After the 3-hour exposure period, the cells were washed twice with buffered saline and the cells were refed with complete RPMI 1640 medium. One-tenth g/mL Colcemid was added to the cultures during the last 2.0 hours of incubation. The metaphase cells were harvested and prepared for cytogenetic analysis. Table 1 contains a summary of the treatment schedule of the aberrations assay with metabolic activation.

Harvest Procedure

The cell suspension was centrifuged, the supernatant was discarded, and the cells were treated with hypotonic KCl (0.075 M) for 10 minutes. After centrifugation and removal of the KCl, the cells were washed 3 times with freshly prepared fixative (absolute methanol:glacial acetic acid, 3:1, v:v). Air-dried slides were prepared from the harvested cells.

Slide Preparation and Staining

Slides were prepared by dropping the harvested cultures on clean slides. The slides were stained with 5% Giemsa solution for the analysis of mitotic index and chromosomal aberrations. All slides were air-dried and cover slipped.

Aberrations Analysis and Assay Evaluation

Cells were selected for good morphology and only cells with the number of centromeres equal to the modal number 46 were analyzed. One hundred cells, if possible, from each replicate culture at 4 dose levels of the test article, the negative control, the solvent control, and positive control cultures were analyzed for the different types of chromosomal aberrations (Evans, 1962). At least 25 cells were analyzed for chromosomal aberrations from those cultures with >25% cells with chromosomal aberrations. Cells with aberrations were recorded. Mitotic index was assessed by analyzing the number of mitotic cells/1000 cells; the ratio was expressed as a percentage of mitotic cells. Chromatid and isochromatid gaps, if observed, were noted in the raw data and were tabulated. They were not, however,

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considered in the evaluation of the ability of the test article to induce chromosomal aberrations. Percent polyploidy and endoreduplication were analyzed and the results were tabulated.

RESULTS

Overall results: positive, negative, ambiguous: Negative

Genotoxic effects (unconfirmed, dose-response, equivocal – with/without activation): Negative.

Cytotoxic concentration:

Range-finding Assay Without Metabolic Activation

Hemolysis was observed prior to wash in the cultures dosed with 5000 g/mL. Reductions of 35%, 6%, 38%, 15%, 18%, 57%, and 100% were observed in the mitotic indices of the cultures dosed with 0.500, 1.67, 5.00, 16.7, 500, 1670, and 5000 g/mL.

Range-finding Assay With Metabolic Activation

Hemolysis was observed prior to wash in the cultures dosed with 5000 g/mL. Reductions of 8%, 5%, 4%, 1%, and 100% were observed in mitotic indices of the cultures dosed with 1.67, 50.0, 500, 1670, and 5000 g/mL, as compared with the solvent control culture.

Chromosomal Aberration Assays Without Metabolic Activation

Initial Trial—Hemolysis was observed prior to wash in the cultures dosed with 2510 g/mL. Reductions of 38%, 18%, 18%, 50%, 55%, and 95% in the mitotic indices, as compared with the solvent control cultures, were observed in the cultures treated with 127, 253, 505, 1010, 1510, and 2010 g/mL, respectively.

Confirmatory Trial—In the 22.1-hour confirmatory trial, hemolysis was observed prior to wash of the cultures dosed with 1600 and 2000 g/mL, and a slight evidence of hemolysis was evident at harvest of the cultures dosed with 1600 g/mL. Reductions of 2%, 14%, 64%, 74%, 93%, and 93% in the mitotic indices, as compared with the solvent control cultures, were observed in the cultures dosed with 125, 500, 900, 1200, 1600, and 2000 g/mL, respectively. In the 46.0-hour confirmatory trial, hemolysis was observed prior to wash of the cultures dosed with 1600 and 2000 g/mL. Reductions of 59%, 93%, 98%, and 98% in the mitotic indices, as compared with the solvent control cultures, were observed in the cultures dosed with 900, 1200, 1600, and 2000 g/mL, respectively.

Chromosomal Aberration Assays With Metabolic Activation

Initial Trial—Hemolysis was observed prior to wash in the cultures dosed with 2010, 2510, 3010, and 4010 g/mL. No cells were visible prior to the addition of Colcemid to the cultures dosed with 4010 g/mL. Hemolysis was observed prior to the addition of Colcemid to the cultures dosed with 3010 g/mL. Reductions of 15%, 20%, 15%, 43%, 77%, and 95% in the mitotic indices, as compared with the solvent control cultures, were observed in the cultures treated with 253, 505, 1010, 1510, 2010, and 2510 g/mL, respectively.

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Confirmatory Trial—In the 22.1-hour confirmatory trial, hemolysis was observed prior to wash and prior to harvest of the cultures dosed with 2000, 2500, and 3000 g/mL. Reductions of 15%, 5%, 69%, 82%, 97%, and 100% in the mitotic indices, as compared with the solvent control cultures, were observed in the cultures treated with 500, 1000, 1500, 2000, 2500, and 3000 g/mL, respectively. In the 46.0-hour confirmatory trial, hemolysis was observed prior to wash and prior to harvest of the cultures dosed with 2000, 2500, and 3000 g/mL. Reductions of 15%, 80%, 100%, and 100% in the mitotic indices, as compared with the solvent control cultures, were observed in the cultures treated with 1500, 2000, 2500, and 3000 g/mL, respectively.

Statistical results: The test substance did not significantly increase chromosomal aberrations with or without metabolic activation.

Remarks:

Chromosomal Aberration Assays Without Metabolic Activation

Initial Trial—No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed at the concentrations analyzed (253, 505, 1010, 1510 g/mL).

Confirmatory Trial—In the 22.1-hour confirmatory trial, no significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed at the concentrations analyzed (125, 250, 500, and 900 g/mL). In the 46.0-hour confirmatory trial, no significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed at the concentrations analyzed (125, 250, 500, and 900 g/mL). The sensitivity of the cell culture for induction of chromosomal aberrations was shown by the increased frequency of aberrations in the cells exposed to MMC, the positive control agent.

Chromosomal Aberration Assays With Metabolic Activation

Initial Trial—No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed at the concentrations analyzed (505, 1010, 1510, and 2010 g/mL).

Confirmatory Trial—In the 22.1-hour confirmatory trial, no significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed at the concentrations analyzed (250, 500, 1000, and 1500 g/mL). In the 46.0-hour confirmatory trial, due to toxicity, only 52 metaphases were available for analysis in one of the cultures dosed with 2000 g/mL. No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed at the concentrations analyzed (500, 1000, 1500, 2000 g/mL), except for a weak increase in endoreduplication at 2000 g/mL. The successful activation of the metabolic system was illustrated by the increased incidence of cells with chromosomal aberrations in the cultures treated with cyclophosphamide, the positive control agent.

CONCLUSIONS

The test article, T-6342, was considered negative for inducing chromosomal aberrations in cultured whole blood human lymphocytes cells with and without metabolic activation. These results were verified in independently conducted confirmatory trials.

REFERENCE

Murli, Hemalatha. 1996. Mutagenicity Test on T-6342, Measuring Chromosomal Aberrations in Whole Blood Lymphocytes With a Confirmatory Assay With Multiple Harvests. Corning-Hazelton, Inc. (CHV). Vienna, VA. CHV Study No.: 17073-0-449CO.

GENETIC TOXICITY (IN VIVO)

Title: Mutagenicity test on T-6342 in an *in vivo* mouse micronucleus assay

TEST SUBSTANCE

Identity: T-6342

Remarks: It is a clear, colorless liquid. The purity and chemical identity of the test substance were not indicated.

METHOD

Method/Guideline followed: This study was conducted using modifications of the procedures suggested by Heddle et al. (1983). Protocol No. 455, Edition 17 (no reference given), modified for 3M Corporation.

Test type: Mouse micronucleus assay

GLP: Yes

Year study performed: 1995

Species/Strain: Mouse/Crl:CD-1®(ICR)BR

Sex: Male and female

No. animals/sex/dose: 15 for treatment groups, 5 for control groups

Vehicle (if used): Deionized water

Route of administration: Oral gavage

Doses: 1250, 2500, and 5000 mg/kg; Doses selected on the basis of a previous study.

Frequency of treatment: Single dose

Statistical methods used: The analysis of the data was performed using ANOVA (Winer, 1971) on either untransformed (when variances were homogeneous) or rank transformed (when variances were heterogeneous) proportions of cells with micronuclei per animal. If the ANOVA was significant ($P<0.05$), a Dunnett's t-test (Dunnett, 1955; 1964) was used to determine which dose groups, if any, were significantly different from the negative control. Analyses were performed separately for each harvest time and sex combination.

Remarks: The animals were dosed with the appropriate amount of the test substance and euthanized approximately 24, 48, or 72 hours after dosing for extraction of the bone marrow. Ten animals (5 males and 5 females) were randomly assigned to each dose/harvest time group. Ten animals (5 males and 5 females) were assigned to the control groups. The negative control animals were dosed with the vehicle, deionized water. The positive control animals were dosed with 80 mg/kg cyclophosphamide (CP). Both control groups were euthanized after 24 hours. After the bone marrow was collected, it was spread onto

slides, dried, and stained with May-Grunwald solution and Giemsa. The slides were scored for micronuclei and the polychromatic erythrocyte (PCE) to normochromatic erythrocyte (NCE) cell ratio. One thousand PCEs per animal were scored. The frequency of micronucleated cells was expressed as percent micronucleated cells based on the total PCEs present in the scored optic field. The normal frequency of micronuclei in this strain is about 0.0-0.4%.

RESULTS

Effect on mitotic index or PCE/NCE ratio by dose level and sex:

Treatment	Harvest time	% Micronucleated PCEs		Ratio PCE:NCE	
		Males	Females	Males	Females
Vehicle control:	24h	0.14 . 0.02	0.02 . 0.02	0.64 . 0.07	0.63 . 0.05
Positive control (CP):	24h	5.44 . 0.37*	2.50 . 0.33*	0.51 . 0.06	0.69 . 0.11
1250 mg/kg:	24h	0.28 . 0.05	0.00 . 0.00	0.69 . 0.09	0.75 . 0.17
	48h	0.00 . 0.00	0.02 . 0.02	0.68 . 0.08	0.74 . 0.06
	72h	0.06 . 0.02	0.02 . 0.02	0.36 . 0.08	0.59 . 0.06
2500 mg/kg:	24h	0.12 . 0.04	0.04 . 0.02	0.69 . 0.07	0.64 . 0.05
	48h	0.02 . 0.02	0.02 . 0.02	0.79 . 0.07	0.65 . 0.01
	72h	0.20 . 0.07	0.06 . 0.02	0.47 . 0.07	0.64 . 0.07
5000 mg/kg:	24h	0.06 . 0.04	0.10 . 0.04	0.80 . 0.08	0.79 . 0.09
	48h	0.15 . 0.09	0.06 . 0.02	0.56 . 0.10	0.63 . 0.06
	72h	0.17 . 0.03	0.03 . 0.03	0.17 . 0.04*	0.24 . 0.05*

*Significantly different from the corresponding vehicle control ($p < 0.05$).

Genotoxic effects (unconfirmed, dose-response, equivocal): Negative

Statistical results: No significant increases in micronucleated polychromatic erythrocytes were observed.

Remarks: Due to toxicity, the PCE/NCE ratios of the males and females from the 5000 mg/kg dose group at the 72 hour harvest group were significantly lower than the vehicle control animals.

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CONCLUSIONS

The test material, T-6342, did not induce a significant increase in micronuclei in bone marrow polychromatic erythrocytes under the conditions of this assay and is considered negative in the mouse micronucleus assay.

REFERENCE

Murli, H. 1995. Mutagenicity test on T-6342 in an *in vivo* mouse micronucleus assay. Corning Hazleton Inc. Vienna, Virginia. 3M Corporation. St. Paul, MN.

GENETIC TOXICITY (IN VITRO)

Title: Evaluation of the Ability of T-7524 to Induce Chromosome Aberrations in Cultured Peripheral Human Lymphocytes

TEST SUBSTANCE

Identity: T-7524

Remarks: The specific gravity of the test substance was 1.23. Substance was a brown paste of 98% purity; the remaining 2% was 1-methyl-20pyrrolidinone.

METHOD

Method/Guideline followed: OECD Guideline 473; EEC Directive 67/548/EEC; OPPTS Guideline 870.5375; Japanese Ministry of Health and Welfare and Japanese Ministry of International Trade and Industry.

Test type: In vitro cytogenetics

Test system: Cultured human lymphocytes

GLP: Y

Year study performed: 2000

Species/Strain/cell-type/cell line: cells obtained from healthy adult male volunteers

Type of metabolic activation used: rat liver microsomal enzymes routinely prepared from adult male Wistar rats, obtained from Charles River, Sulzfeld, Germany. Animals were housed at NOTOX in a special room under standard laboratory conditions, as described in the Standard Operating Procedures (SOP's). Rats were injected intraperitoneally with Aroclor 1254 induced in corn oil. Livers were washed and minced and supernatant was used for activation.

Concentrations tested:

Without S9 -

First Experiment: 1000, 3330 and 5000 g/ml

Second Experiment: 333, 560, 1000, 1300, 1800, 2400, and 3330 g/ml.
333, 420, 560, 750, 1000, 1300, and 1800 g/ml.

With S9 (1.8% v/v) -

Experiment 1: 1000, 3333 and 5000 g/

Experiment 2-1000, 3333, and 5000 g /ml

Test conditions:

Range-Finding Test: Lymphocyte cultures (0.4 ml blood of a healthy male donor plus 5 ml or 4.8 ml culture medium, with and without S9 respectively and 0.1 ml (9 mg/ml) phytohaemagglutinin) were cultured for 48 hours and thereafter exposed to selected doses of T-7524 for 3 h, 24 h, and 48 h in the absence of S9-mix or for 3 h in the presence of S9 mix. After 3 hrs, the cells exposed to T-7524 were rinsed once with 5 ml of HBSS and incubated in 5 ml culture medium for another 20-22 h (24 h fixation time). The cells treated for 24 hr and 48 hr in absence of S9-mix were not rinsed after treatment but were fixed immediately after 24 h and 48 h. Based on the results of dose range-finding test, an appropriate range of dose levels was chosen for the cyotgenetic assay.

Experiment 1: Lymphocytes were cultured for 48 hours and then exposed in duplicate to selected doses of T-7524 for 3 hours with and without S9-mix. After 3 hrs, the cells were rinsed with 5 ml HBSS and incubated in 5 ml culture medium for another 20-22 hr (24 h fixation time). Based on the mitotic index of the dose range finding test and the first cytogenetic assay, appropriate dose levels were selected for the second cytogenetic assay - the highest dose level tested inhibited the mitotic index by approximately 50% or more; the mitotic index of the lowest dose tested was approximately the same as the solvent control.

Experiment 2: Cells were cultured for 48 h and thereafter exposed in duplicate to selected doses of T-7524 for 24 h and 48 h in the absence of S9-mix or for 3 h in the presence of S9-mix. After 3 h treatment, cells in the presence of S9-mix were rinsed once with 5 ml HBSS and incubated in 5 ml culture medium for 44-46 h (48 h fixation time). The cells treated for 24 h and 48 h without S9-mix were not rinsed after treatment but were harvested immediately after 24 h and 48 h fixation time.

Chromosome preparation: During the last 3 h of culture, cell division was arrested by addition of the spindle inhibitor colchicine (0.5 ug/ml medium). Thereafter the cell cultures were centrifuged for 5 min at 1300 rpm and the supernatant was removed. Cells in the remaining cell pellet were swollen by 5 min treatment with hypotonic 0.56% (w/v) potassium chloride solution at 37C. After hypotonic treatment, the cells were fixed with 3 changes of methanol: acetic acid fixative (3:1 v/v).

Slide preparation: Fixed cells were dropped on cleaned slides that were immersed for 24 hr in a 1:1 mix of 96% (v/v) ethanol/ether and cleaned with a tissue. The slides were marked with the NOTOX study identification number and group number. Two slides were prepared/culture. Slides were set to dry and then stained for 10-30 min with 5% (v/v) Giemsa solution in tap water. Slides were then rinsed in tap-water and set to dry. The dry slides were cleared by dipping in xylene before they were embedded in MicroMount and mounted with coverslip.

Mitotic index scoring: The mitotic index of each culture was determined by counting the number of metaphases per 1000 cells. At least 3 analysable concentrations were used; the highest concentrations analyzed were those cultures where the mitotic index was inhibited approximately 50% or more compared to the solvent control. The mitotic index of the lowest dose level analyzed was about the same as the mitotic index of the solvent. Cultures treated with an intermediate dose were also examined for chromosome aberrations.

Counting chromosome aberrations: To prevent bias, slides were randomly coded prior to examination of chromosome aberrations and scored. An adhesive label with the NOTOX study ID number and code was stuck over the marked slide. At least 100 metaphase chromosome spreads per culture were examined by light microscopy for chromosome aberrations. If the number of aberrant cells (gaps excluded) were 25 or more in 50 metaphases, no more metaphases were examined. Only metaphases with 46 chromosomes were analyzed. Numbers of cells with aberrations and numbers of aberrations were calculated.

RESULTS

Genotoxic effects: T-7524 did not induce a statistically or biologically significant increase in numbers of cells with chromosome aberrations.

Cytotoxic concentrations:
Without S-9:

3 hr treatment; 24 hr fixation: no toxic dose; highest dose tested 5000 g/ml.
24 h treatment; 24 h fixation: 2400 and 3330 g/ml
48 h treatment; 48 h fixation: 1300 and 1800 g/ml

With S-9

3 hr treatment; 24 hr fixation: no toxic dose; highest dose tested 5000 g/ml.
3 hr treatment; 48 hr fixation: no toxic dose; highest dose tested 5000 g/ml.

CONCLUSIONS

When tested without S-9 and with 24 h continuous treatment, T-7524, induced a statistically significant increase in the number of chromosome aberrations at 1000 g/ml when gaps were included in the total number of aberrations. However, when gaps are excluded the number of aberrations are within the historical control range of the laboratory. This increase was not seen at 1000 g/ml with 48 hour continuous treatment. Because this increase occurred at the middle dose only, included gaps, and the chromosome aberrations seen, excluding gaps are within the historical control range of the performing laboratory and no increase occurred with 48 h continuous treatment, the increase is not considered to constitute a positive response.

Based on the above results, T-7524 is not clastogenic in human lymphocytes under the experimental conditions of the study.

Remarks: none

REFERENCE

NOTOX. 2000. Evaluation of the Ability of T-7524 to Induce Chromosome Aberrations in Cultured Peripheral Human Lymphocytes. NOTOX Project Number 292062. Hertogenbosch, The Netherlands.

ACUTE TOXICITY

Title: An Acute Inhalation Toxicity Study of T-2305 CoC in the Rat

TEST SUBSTANCE

Identity: T-2305CoC

Remarks: Fine white powder, received from 3M Company; composition and purity not indicated.

METHOD

Method/guideline followed: Guideline number not stated.

GLP: No

Year study performed: 1979

Species/Strain: Rat/Sprague-Dawley (211 to 264 grams)

Sex (Males/females/both): Both

No. of animals/sex/dose: 5/sex/group

Route of Administration: Inhalation

Remarks: The nominal test concentration was 18.6 mg/L, with a total of 17.90 grams of test material delivered in a total volume of 960 liters of dry air. The test material was sieved and packed into the cylinder of a Wright dust-feed mechanism. The flow rate of dry air was 16 liters/minute. The resulting dust-laden air was passed into the 32.3 liter glass exposure chamber housing the test animals for an exposure period of one hour. The animals were observed for abnormal signs at 15-minute intervals during the exposure, upon removal from the chamber, hourly for 4 hours after removal from test chamber, and daily thereafter for 14 days prior to sacrifice.

RESULTS

Number of deaths at each dose level (by sex): There were no deaths.

Remarks: During the exposure period, the study animals exhibited excessive lacrimation and salivation, decreased activity, labored breathing, gasping, closed eyes, mucoid nasal discharge, and irregular breathing. After removal from the exposure chamber, red nasal discharge (10/10), yellow staining of the anogenital fur (9/10), dry rales (6/10), red material around the eyes (5/10), excessive salivation (4/10), excessive lacrimation (1/10), and body tremors (1/10) were observed in the rats. Excessive salivation, lacrimation and body tremors had abated by the one-hour post-exposure observation and there was no red

material observed around the eyes at the two hour post exposure observation. Red nasal dischage (10/10) yellow staining of the anogenital fur (6/10) and dry rales (5/10) were still seen at the one hour post-exposure observation. During the 14-day observation period, excessive lacrimation (6/10) and salivation (3/10), mucoid nasal discharge (10/10), dry rales (8/10), dry red material around the nose (2/10), and moist rales (1/10) were observed in the rats. Weight gains appeared normal. Necropsy findings showed lung discoloration in 8/10 rats (a higher than normal incidence).

CONCLUSIONS

It was concluded from the study results that the test substance is not fatal to rats at a nominal exposure of 18.6 mg/L for one hour. Immediate effects of treatment were excessive lacrimation, salivation, decreased activity. Labored breathing, gasping, closing of the eyes, mucoid nasal discharge, irregular breathing, yellow staining of the ano-genital fur, and dry rales. The persistence of lacrimation, salivation, mucoid nasal discharge, dry rales, dry red material around the nose and moist rales along with lung discoloration seen at necropsy indicate that the material may have a prolonged or residual effect at 14 days.

REFERENCE

Bio/dynamics Inc. 1979. An Acute Inhalation Study of T-2305 CoC in the Rat. 3M Company, St. Paul, MN. Project No. 78-7184.

ACUTE TOXICITY

Title: Acute Oral Toxicity (LD_{50}) Study in Rats

TEST SUBSTANCE

Identity: Fluorad Fluorochemical FC-143,

Remarks: White powder, 3M Lot No. 340; composition and purity not indicated

METHOD

Method/guideline followed: Guideline number not stated

GLP (Y/N): No

Year study performed: 1978

Species/Strain: Rat/Charles River CD

Sex (Males/females/both): both

Number of animals/sex/dose: 5/sex/dose

Vehicle: 40% acetone/60% corn oil

Route of Administration: Oral gavage

Remarks: The test material was administered at the following dose levels: 100, 215, 464, 1000, and 2150 mg/kg in a volume of 10 mL/kg. The rats weighed 180-218 g at study initiation. Animals were observed for mortality and pharmcotoxic signs during the first four hours after dosing, at 24 hours and daily thereafter for a total of 14 days. Body weights were recorded immediately prior to dosing and at 7 and 14 days. Animals that died on study were subjected to gross necropsy as were all survivors at the end of the 14 day observation period.

RESULTS

The following clinical signs were observed: ptosis, piloerection, hypoactivity, decreased limb tone, ataxia, corneal opacity, hypothermic to touch, and death. Signs observed at necropsy of animals which were found dead or sacrificed moribund included: 1) congestion, pitting and red foci in the lungs; 2) distended stomachs; or stomachs filled with fluid including dark red or red fluid, stomachs containing dark red particulate material; stomachs with hyperemic or thickened mucosa or erosion of the glandular mucosa; 3) intestines filled with red fluid; and 4) pale discoloration of the liver;5) red staining around the nose and mouth. Animals that survived to day 14 had thickened stomach mucosa, hyperemic glandular stomach mucosa, hydrometra of the uterus and mottled coloration of the kidneys. Signs were observed at all

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treatment levels. Treatment did not appear to have an effect upon weight of the animals that survived to terminal sacrifice.

Number of deaths at each dose level (by sex):

100 mg/kg: 1/5 males, 0/5 females

215 mg/kg: 0/5 males, 0/5 females

464 mg/kg: 2/5 males, 3/5 females

1000 mg/kg: 3/5 males, 5/5 females

2150 mg/kg: 5/5 males, 5/5 females

Remarks:

CONCLUSIONS

LD_{50} (male rats) = 680 mg/kg, with 399 – 1157 mg/kg 95% confidence limit
LD_{50} (female rats) = 430 mg/kg, with 295 – 626 mg/kg 95% confidence limit
LD_{50} (combined sexes) = 540 mg/kg, with 389 – 749 mg/kg 95% confidence limit

REFERENCE

Fluorad Fluorochemical FC-143 Acute Oral Toxicity (LD_{50}) Study in Rats. 1978. Study No. 137-091, International Research and Development Corporation. Sponsored by 3M Company.

ACUTE TOXICITY

Title: Acute Oral Toxicity Study of T-6669 in Rats (OECD Guidelines)

TEST SUBSTANCE

Identity: T-6669, a fine white powder.

Remarks: None

METHOD

Method/guideline followed: OECD Guidelines

GLP (Y/N): Y

Year study performed: 1997

Species/Strain: albino rats of Crl:CD (SD)BR strain

Sex (Males/females/both): both

Number of animals/sex/dose: 5/sex/dose

Vehicle: distilled water

Route of Administration: gavage

Remarks: Doses of 250 and 500 mg/kg were tested. All dose levels were administered as volumes of 10 ml/kg body weight. The rats weighed 208-269 g and were 8-12 weeks old. Clinical observations were conducted at 1, 2.5, and 4 hours after test material administration and each day for 14 days. Mortality checks were conducted twice a day (morning and afternoon) for 13 days and on the morning of Day 14. Body weights were determined on Days 0, 7, and 14 or at death if survival exceeded 1 day. All animals were subject of an abbreviated gross necropsy.

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RESULTS

LD50: Males: > 500 mg/kg
Females: between 250 and 500 mg/kg

Number of deaths at each dose level (by sex):
250 mg/kg – none; 500 mg/kg – 2/5 males and 5/5 females.

Remarks: All animals exhibited body weight gain throughout the study. All animals treated at 250 mg/kg appeared normal during the study except for two females that exhibited red-stained faces and/or a wet urogenital area within 24 hours of test material administration. Clinical signs of toxicity observed in the animals treated with 500 mg/kg were: red-stained face, yellow-stained or wet urogenital area, hypoactivity, hunched posture, staggered gait, and excessive salivation. There were no test-material related lesions observed at necropsy, although at 250 mg/kg one female had large pelvises in both kidneys. At 500 mg/kg, one male had a cannibalised right flank, one female had multiple dark brown areas in the glandular mucosa of the stomach, and a second female had a clear fluid in the lumen of the bilateral horns of the uterus.

CONCLUSIONS

None were specified beyond the results.

REFERENCE

Glaza, S.M. 1997. Acute Oral Toxicity Study of T-6669 in Rats. Corning Hazleton Inc. CHW 61001760. January 10. Sponsored by 3M, St. Paul, Minnesota.

OTHER

ACUTE - SKIN IRRITATION

Title: Primary Skin Irritation Study – Rabbits

TEST SUBSTANCE

Identity: T-1395

Remarks: no other identifying information was given.

METHOD

Note pH of test material: Not specified

Method/Guideline followed: Similar to test in Section 1500.41 – Hazardous Substances and Articles, Administration and Enforcement Regulations, *Federal Register*, Vol. 38, No. 187, p. 27019+, 27 September 1973.

Test Type: in vivo

Species/strain/cell type: albino rabbits

Sex (males/females/both): Not specified

Number of animals/sex/dose: 6 total

Total dose: Doses of 0.5g were placed on an intact site and an abraded site. Sites were examined while dry and after application of water at the end of the experiment.

Vehicle: Not specified

Length of time test material is in contact with animal/cell: Wrappings were removed after 24 hours but there was no indication about whether the site was washed. Readings were also made at 72 hours.

Grading scale: Scores for erythema and eschar formation as well as edema formation were presented.

Remarks: Six albino rabbits had their hair clipped from their backs and flanks, and five tenths of one gram (0.5 g) of test material was placed on abraded-dry or intact-dry prepared test sites, then covered with gauze patches and an impervious material was wrapped around the site to keep the bandage in place. After 24 hours and 72 hours the coverings were removed and the degree of erythema and edema was recorded according to a standardized scale (the Draize method).

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RESULTS

In all cases it is reported the primary skin irritation scores were 0, indicating no reddening or swelling detected. No clinical signs were reported.

Primary irritation score: zero

Remarks: There was no indication of reliability and no QA/QC.

CONCLUSIONS

Based on the results, the substance was not classified as a primary irritant.

REFERENCE

Gabriel, Karl. Summary of: Primary Skin Irritation Study – Rabbits. Performed by: Biosearch. Submitted to 3M Company, 3M Center, St. Paul, MN.

OTHER

General remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

ACUTE - EYE IRRITATION

Title: Primary Eye Irritation Study - Rabbits

TEST SUBSTANCE

Identity: T-1395

Remarks: no other identifying information was given.

METHOD

Note pH of test material: Not specified

Method/Guideline followed: Similar to test in Section 1500.42 – Hazardous Substances and Articles, Administration and Enforcement Regulations, *Federal Register*, Vol. 38, No. 187, p. 27019+, 27 September 1973.

Test Type: in vivo

Species/strain/cell type or line: adult albino rabbits

Sex (males/females/both): Not specified

Number of animals/sex/dose: 6/single dose

Total dose: 0.1 gram per eye.

Length of time test material is in contact with animal/cell:
5 seconds in 3 animals; 30 seconds in 3 animals

Observation period: 1 hr, 24 hr, 48 hr, 72 hr, and 5 and 7 days

Remarks: The test material was washed from the eyes in 3 animals after 5 seconds with 200 cc of water and after 30 seconds in 3 animals with 200 cc of water. No other information on the methods was given.

RESULTS

Corrosive: Not specified

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Irritation score:

After 5 sec: All scores for Cornea and Iris were zeros (for all rabbits). In two rabbits, scores for conjunctivae were 4, and in 1 rabbit, the score was 2, throughout the observation period.

After 30 sec: All scores for Cornea and Iris were zeros (for all rabbits). In one rabbit, scores for Conjunctivae were 4 at 1-48 hrs, and increased to 6 at 72 hrs and at 5 and 7 days. In 1 rabbit, the score was 4 throughout the observation period and in 1 rabbit the score was 6 throughout the observation period.

Tool used to assess score: Scoring was done according to the Illustrated Guide for Grading Eye Irritation By Hazardous Substances.

Description of lesions: None

Remarks: None

CONCLUSIONS

The only conclusion provided in study was that the test substance was not an ocular irritant.

Remarks: This conclusion is suspect due to the positive scores observed during the study. However, without additional information on the scoring method, there is no way to provide an informed judgment about the results.

REFERENCE

Gabriel, Karl. Summary of: Primary Eye Irritation Study – Rabbits. Performed by: Biosearch. Submitted to 3M Company, 3M Center, St. Paul, MN.

OTHER

General remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

ACUTE - EYE IRRITATION

Title: Primary Eye Irritation-Rabbit

TEST SUBSTANCE

Identity: 3M identified the test substance as T-1395.

Remarks: The test substance was not further characterized.

METHOD

Note pH of test material: Not specified

Method/Guideline followed: The methods employed in the testing, evaluation, and grading of the test material are those described in Section 1500.42-Hazardous Substances and Articles, Administration and Enforcement Regulations, Federal Register, Vol. 38, No. 187, p. 27019, 27 Sept 1973.

Test Type: *in vivo*

Species/strain/cell type or line: Albino rabbit/Not specified

Sex (males/females/both): Not specified

Number of animals/sex/dose: 6/single dose

Total dose: 0.1 gram

Test conditions: The test substance was instilled into the right eyes of the test animals; the left eyes were not treated and served as controls. The test substance was not washed from the treated eyes.

Length of time test material is in contact with animal/cell: The exposed eye was not washed following administration of the test substance.

Observation period: 1, 24, 48, and 72 hours post-exposure; 5 and 7 days post-exposure

Scoring method used: Interpretation of the results was made in accordance with the grading system outlined in "Illustrated Guide for Grading Eye Irritation By Hazardous Substances".

Remarks: Healthy young adult animals were used in this study.

RESULTS

Corrosive: Not specified

Irritation score: No overall irritation score was given. Individually, 1 hour after application of the test substance, 6/6 animals exhibited iris irritation scores of 5 and 4/6 animals exhibited conjunctivae irritation

scores of 10; the remaining two animals exhibited conjunctivae irritation scores of 6 and 8. 6/6 animals continued to display irritation of the iris and conjunctivae (irritation scores: 5 and 2-6, respectively) up to 48 hours after administration of the test substance. Irritation scores and incidence of irritation had decreased by day 7 of the study, at which time 2/6 animals exhibited iris irritation scores of 5 and 1/6 animals exhibited a conjunctivae irritation score of 2. No corneal irritation was observed in any of the animals throughout the study.

Tool used to assess score: Interpretation of the results was made in accordance with the grading system outlined in "Illustrated Guide for Grading Eye Irritation By Hazardous Substances".

Description of lesions: Not specified

Remarks: None

CONCLUSIONS

Remarks: Based on the results of this study and the source used for interpreting the results, the authors considered the test substance to be a primary ocular irritant; I agree with this conclusion.

REFERENCE

3M Company. 1976. Primary Eye Irritation Study-Rabbits.

OTHER

Remarks: This summary was based on a summary report; therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

ACUTE - SKIN IRRITATION

Title: Primary Skin Irritation Test with T-3371 in Albino Rabbits

TEST SUBSTANCE

Identity: Riker Laboratories, Inc. identified the test substance as T-3371.

Remarks: The test substance was not further characterized.

METHOD

Note pH of test material: Not specified

Method/Guideline followed: ICAO

Test Type: in vivo

Species/strain/cell type: Rabbit/New Zealand White Albino

Sex (males/females/both): Female

Number of animals/sex/dose: 3/exposure period

Total dose: 0.5 gram

Vehicle: None

Length of time test material is in contact with animal/cell: 3 minutes, 1 hour, or 4 hours

Test conditions: One day prior to application of the test substance, the hair was shaved from the back and flanks of each rabbit. For each rabbit, two test sites were selected. The test sites were lateral to the midline of the back and approximately 10 centimeters apart. The test substance was applied to each of the test sites and occluded with 2-inch square gauze patches. The patches were secured with gauze wrap; then, the trunk of each animal was wrapped with impervious plastic sheeting for the duration of the exposure period. After the exposure period, all wrappings were removed and the test sites were examined for irreversible damage and scored for erythema and edema.

Grading scale: The authors presented separate irritation scores for erythema and edema, each based on a grading scale of 0 - 4. Test sites were also observed for the presence or absence of chemical burn, eschar, necrosis, and epithelial sloughing.

Remarks: Animals were housed in standard wire-mesh cages in temperature and humidity controlled rooms with food and water offered *ad libitum*.

RESULTS

Results: The test substance produced irreversible tissue damage following a 3-minute, 1- hour, and 4-hour contact period. Moderate erythema and edema, as well as chemical burn, eschar, and necrosis were produced following all three contact periods. An endpoint was not achieved due to the extreme irritation following each contact period.

Primary irritation score: Not reported

Remarks:

CONCLUSIONS

The chemical produced irreversible tissue damage to the skin of female albino rabbits; including moderate erythema and edema, chemical burn, eschar, and necrosis. Inadequate information was presented in the report to evaluate quality of the study and validity of the conclusion.

REFERENCE

Primary Skin Irritation Test with T-3371 in Albino Rabbits. 1983. Safety Evaluation Laboratory, Riker Laboratories, Inc. St. Paul, Minnesota. Experiment #0883EB0079

ACUTE TOXICITY

Title: Acute Oral Toxicity Study-Rats

TEST SUBSTANCE

Identity: 3M identified the test substance as T-1585.

Remarks: The test substance was not further characterized.

METHOD

Method/guideline followed: Not specified

GLP (Y/N): No

Year study performed: 1976

Species/Strain: Rat/Sherman-Wistar, 200-300 grams

Sex (Males/females/both): Male and female

Number of animals/sex/dose: 5/sex/single dose

Vehicle: 50% water

Route of Administration: Oral

Test conditions: Each animal was weighed and dosed by direct administration of the test substance into the stomach by means of a syringe and dosing needle. The dosage-level was 1,000 mg/kg and the test substance was dosed as a 50% w/v suspension in water. Following administration of the test substance, the animals were allowed food and water *ad libitum* for the 14-day observation period, during which time the rats were observed for signs of toxicity and mortalities.

Remarks: The test animals were deprived of food (not water) for 24 hours prior to dosing.

RESULTS

LD_{50}: <1,000 mg/kg

Number of deaths at each dose level (by sex): 1,000 mg/kg-3/5 males, 4/5 females

Remarks: 60% of males (3/5) and 80% of females (4/5) died following administration of the test substance; therefore, the LD50 was determined to be < 1,000 mg/kg. Signs of toxicity included an eroded

gastric mucosa and writhing; the number of animals, which exhibited these signs, was not specified. The three surviving animals exhibited no change in initial and final body weight.

CONCLUSIONS

Remarks: The authors concluded the LD_{50} to be < 1,000 mg/kg; I agree with this conclusion.

REFERENCE

Acute Oral Toxicity in Rats-T-1585. 1976. 3M Company.

OTHER

Remarks: This summary was based on a summary report submitted by 3M; therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

000184

ACUTE - EYE IRRITATION

Title: Primary Eye Irritation Study - Rabbits

TEST SUBSTANCE

Identity: T-1585

Remarks: No other specifications regarding the test substance were noted.

METHOD

Note pH of test material: Not specified

Method/Guideline followed: Section 1500.42 – Hazardous Substances and Articles, Administration and Enforcement Regulations, Federal Register, Vol. 38, No. 187, p.27019, September 27, 1973.

Test Type: *in vivo*

Species/strain/cell type or line: Rabbit/Albino

Sex (males/females/both): Not specified

Number of animals/sex/dose: 6/single dose

Total dose: 0.1 g

Length of time test material is in contact with animal/cell: 7 days

Observation period: 1h, 24 h, 48 h, 72 h, 5 d, and 7 d

Scoring method used: Used grading system outlined in "Illustrated Guide for Grading Eye Irritation by Hazardous Substances"

Remarks: The test substance (0.1 g) was administered to the right eye of each rabbit, while the untreated eye served as a control. The test material was not washed from the eyes. At each observation, the treated eyes were scored for corneal opacity and area, iris, and conjunctival redness, chemosis, and discharge.

RESULTS

Corrosive: Not specified

Irritation score: Corneal opacity and area = 4; Iris = 2; Conjunctival redness = 2; Conjunctival chemosis = 4; Conjunctival discharge = 3

Tool used to assess score: "Illustrated Guide for Grading Eye Irritation By Hazardous Substances."

Description of lesions: None

Remarks: The scores for all the rabbits were the same for each category and observation. The scores remained the same through day 7. Only a summary table of scores was given, with no description of the effects.

CONCLUSIONS

The test substance is a primary ocular irritant and requires cautionary labeling. Inadequate information is presented in the report to evaluate the quality of the study and the validity of the conclusions.

REFERENCE

Biosearch, Inc. 1976. Primary Eye Irritation Study – Rabbits. Philadelphia, PA. 3M Company. St. Paul, MN.

ACUTE TOXICITY

Title: Acute Dermal Toxicity Study of T-6342 in Rabbits

TEST SUBSTANCE

Identity: T-6342; a clear, colorless liquid.

Remarks: None

METHOD

Method/Guideline followed: OECD Guideline TP2071. Most likely this should be OECD Guideline 404 for Dermal Toxicity. OECD Guidelines do not begin with letters and do not go as high as 4 numbers.

GLP: Yes

Year study performed: 1995

Species/strain: Rabbits/Hra(NZW)SPF

Sex (males/females/both): Both

Number of animals/sex/dose: 5 males, 5 females/single dose

Total dose: 2000 mg/kg

Vehicle: None

Route of administration: Dermal

Remarks: The rabbits had the hair clipped from their backs before the appropriate amount of the test substance was applied to the intact skin. The area of application was covered with a gauze patch and an occlusive dressing. Collars were used to restrain the animals. After the 24 hour exposure period, the collars and dressings were removed. The test sites were washed with tap water and disposable paper towels. Clinical observations and mortality checks were made at approximately 1, 2.5, and 4 hours after test material administration and twice daily thereafter for 14 days. Body weights were determined before test material was applied, at Day 7 and on Day 14. The initial dermal irritation reading was made 30 minutes after removal of the test material and on Days 3, 7, 10, and 14. At the end of the study, the animals were euthanized and subjected to necropsy.

000187

RESULTS

Number of deaths at each dose level (by sex): none

Remarks: All animals appeared normal and exhibited body weight gains throughout the study, with the exception of one male that lost weight during the first week. Dermal irritation consisted of slight to moderate erythema, edema, and atonia, and slight desquamation, coriaceousness, and fissuring. No visible lesions were observed at necropsy. The dermal LD50 in rabbits was greater than 2000 mg/kg.

CONCLUSIONS

The dermal LD50 of T-6342 was greater than 2000 mg/kg in male and female rabbits. The test substance produced slight to moderate dermal irritation. No clinical signs during the study or visible lesions at necropsy were noted.

REFERENCE

Glaza, S. 1995. Acute dermal toxicity study of T-6342 in rabbits. Corning Hazelton, Inc. Madison, WI. Project ID: HWI 50800374. 3M Company. St. Paul, MN.

000188

SKIN SENSITIZATION DATA

Title: Assessment of Contact Hypersensitivity to T-7524 in the Albino Guinea Pig (Maximisation-Test)

TEST SUBSTANCE

Identity: T-7524

Remarks: Purity was 98%; the remaining 2% was 1-methyl-2-pyrrolidinone. Substance was a brown paste, with a specific gravity of 1.23.

METHOD

Method/guideline followed: EC – methods for the determination of toxicity, as last amended by Commission Directive 96/54/EC; OECD Guideline Number 406; Magnusson and Kligman 1970.

Study duration: about 90 days

GLP (Y/N): Y

Year study performed: 2001

Species/strain: Guinea pig; Dunkin Hartley strain

Sex: Females

Number of animals per dose group: 10/treatment group; 5/control group

Route of administration: Intradermal and epidermal administration

Range-finding study:

Concentrations: Starting and subsequent concentrations were taken from the following series: 1%, 2%, 5%, 10%, 20%, 50%, and 100%, and doses lower than 1% if needed. There was no indication about which doses were used for induction or challenge. Vehicle not indicated.

Vehicle: not indicated

Induction method: Intradermal injections of four concentrations were used. Dermal reactions were assessed at 24 and 48 hours.

Challenge method: epidermal application was done using 4 test substance concentrations. Dermal reactions were assessed 24 and 48 hours after exposure.

Main study:

Induction (concentration and method):

Day 1: Three injections were made at a clipped scapular region:
(1) 1:1 w/w mixture of Freund's Complete Adjuvant with water,
(2) test substance at 5% concentration, and
(3) a 1:1 w/w mixture of 10% test substance with Freund's Adjuvant.
The vehicle was not indicated.

Day 7: Ten percent sodium-dodecyl-sulfate (SDS) was applied epidermally to a clipped area between injection sites; this provoked a mild inflammatory reaction.

Day 8: Fifty percent test substance concentration was applied epidermally to the SDS-treated area and held in place with Micropore tape. The dressing was removed after 48 hrs.

Induction vehicle: Not indicated.

Challenge (concentration and method):

Day 22: A fifty percent concentration of the test substance was applied epidermally to the clipped flank. Vehicle was not indicated.

Challenge vehicle: Not indicated.

Post-observation period:

Sites were assessed 24 and 48 hours after induction and challenge phases.

Grading scale: The source of the scale was not indicated, but both irritation and challenge reactions were graded. Results were evaluated according to EC criteria for classification and labeling requirements for dangerous substances and preparations.

Statistical methods used: None

Remarks: A reliability check of the test (study date not indicated) was done using alpha-hexylcinnamicaldehyde as a positive control. Skin reactions were indicative of a sensitisation rate of 100 percent for this compound.

RESULTS

Toxic response/effects by dose level: No mortality occurred and no systemic toxicity was observed. Body weights and body weight gain were similar between controls and treated groups.

Induction and challenge scores: During induction, skin effects were enhanced by the 10% SDS treatment. During challenge, no skin reactions were evident after challenge in treated groups and controls. Light yellow staining was seen at the test substance-treated skin sites (24 and 48 hours post challenge). The staining did not hamper scoring of the skin reactions.

Sensitization: A sensitization rate of 0 percent was determined.

Remarks: none.

CONCLUSIONS

There was no evidence that T-7524 caused skin hypersensitivity in guinea pigs.

Remarks: none.

REFERENCE

NOTOX. 2001. Assessment of Contact Hypersensitivity to T-7524 in the Albino Guinea Pig (Maximisation-Test). NOTOX Project number 292027. Hertogenbosch, The Netherlands.

REPEAT DOSE DATA

Title: 5 Daily Dose Oral Toxicity Study with T-6669 in Rats

TEST SUBSTANCE

Identity: The test material was perfluorooctanoic acid, ammonium salt, T-6669 (FC-143), Lot No. 235., CASRN 3825-26-1.

Remarks: It is described as a white powder that is 93-97% ammonium perfluorooctanoate.

METHOD

Method/guideline followed: The study was conducted in accordance with a company protocol TP6785 dated December 20, 1996 included in the report.

Study duration: 20 days

GLP (Y/N): Y

Year study performed: 1997

Species/strain: Rat/Crl:CD®(SD)BR VAF/Plus®

Sex: Male

Number of animals per dose group: 10

Route of administration: Oral gavage

Doses tested and frequency: 0, 5.0, 14.0, or 42.0 mg/kg/day. Doses were administered once daily for 5 consecutive days.

Post-treatment observation period: 15 days

Statistical methods used: Methods are outlined in the report and include ANOVA (Winer, 1971) , Levene's Test (Levene, 1960), and Dunnett's multiple comparison t-test (Dunnett, 1964).

Remarks: In a deviation from the protocol, the animals weighed between 303 and 395 g at study initiation; the protocol stated weights between 210 and 250 g. The animals were provided food and water *ad libitum*. Reverse osmosis water was used as the vehicle and the dose volume was 5 mL/kg. The animals were observed twice a day for mortality and moribundity. Each animal was removed from its cage and examined for clinical signs before dosing and at approximately 1, 2.5, and 4 hours after each dose administration and daily thereafter. Bodyweights were measured daily. Blood samples were collected on Days –2 (predose, though the protocol states that the predose sample is collected on Day -1), 6, 9, 15, and 20. On Day 6, five animals per group were sacrificed, and the liver was removed from each animal and weighed. The right lateral lobe of the liver was collected from each animal, weighed, and

analyzed for palmitoyl CoA oxidase activity. The remaining liver tissue was also collected and weighed. On Day 20, the remaining animals were sacrificed and their livers were collected and weighed.

RESULTS

NOAEL (dose and effect): Greater than 42 mg/kg , the highest dose tested. A NOEL of < 5.0 mg/kg, the lowest dose tested based on higher hepatic palmitoyl CoA oxidase activity was determined by the study author.

LOAEL (dose and effect): Greater than 42 mg/kg , the highest dose tested. A LOEL of 5.0 mg/kg, based on higher hepatic palmitoyl CoA oxidase activity reported in study.

Toxic response/effects by dose level: All animals survived to scheduled sacrifice. No treatment-related clinical signs were observed in any groups.

5.0 mg/kg: higher hepatic palmitoyl CoA oxidase activity (24 IU/G compared to 5 IU/G for controls)

14.0 mg/kg: significantly reduced mean body weight gains during dosing period, higher hepatic palmitoyl CoA oxidase activity (39 IU/G compared to 5 IU/G for controls)

42.0 mg/kg: significantly reduced mean body weight gains during dosing period, higher hepatic palmitoyl CoA oxidase activity (39 IU/G compared to 5 IU/G for controls)

Statistical results: The lower mean body weight gains for the mid- and high-dose groups were statistically significant compared to controls ($p \leq 0.05$).

Remarks: Though the mid- and high-dose level groups exhibited lower body weight gains during the dosing period, they showed recovery usually within the first 3 days after dosing was completed. The overall mean body weight gains were not significantly different between any groups.

CONCLUSIONS

The author stated that based on significant increases in the levels of palmitoyl CoA oxidase activity, an indication of peroxisome proliferation, at all dose levels tested, the no effect level of T-6669 is less than 5.0 mg/kg when administered to male Crl:CD®(SD)BR VAF/Plus®rats for 5 consecutive days.

Remarks: The author designated the no effect level based on significant increases in the levels of palmitoyl CoA oxidase activity. However, enzyme induction may be transient and in the absence of other changes is not clearly indicative of a toxic effect, therefore no LOAEL was determined by this study.

REFERENCE

Henwood, S. 1997. 5 Daily Dose Oral Toxicity Study with T-6669 in Rats. Corning Hazleton, Inc., Madison, WI. Laboratory Project Identification: CHW 6329-197. 3M, St. Paul, MN.

REPEAT DOSE DATA

Title: 28-Day Oral Toxicity Study with FC-143 in Albino Mice; IBT NO. 8532-10655

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), T-1742CoC, Lot No. 269.

Remarks: The purity of the test substance was not indicated.

METHOD

Method/guideline followed: Not stated

Study duration: 28 days

GLP (Y/N): N

Year study performed: 1977

Species/strain: Charles River CD albino mice

Sex: male and female

Number of animals per dose group: 10 (5 males and 5 females)

Route of administration: Diet - Purina rat chow

Doses tested and frequency: 0, 30, 100, 300, 1000, 3000, 10000, 30000 ppm

Post-observation period: None

Statistical methods used: A test was used to compare mean body weights, liver weights, and liver to body weight ratios among groups, but the type of test was not noted.

Remarks: Daily observations for behavioral reactions or mortalities were made. Weekly body weight measurements were taken. Gross pathologic examination was conducted at final sacrifice on all surviving mice. It was stated that "a representative set of organs and other tissues was removed and preserved in neutral buffered formalin for future histopathologic examination." However, organs examined were not specified. Liver weights and liver to body weight ratios were calculated. Microscopic examination was conducted on livers from all sacrificed mice. Samples of livers and blood were sent to 3M for analysis, but the results, if the analyses were completed, are not included.

RESULTS

NOAEL (dose and effect): none; effects at all doses

LOAEL (dose and effect): 30 ppm (hepatocellular hypertrophy; hepatocellular degeneration and/or necrosis; cytoplasmic vacuoles; bile duct proliferation; increased liver weight; increased liver to body weight ratios; body weight loss).

Toxic response/effects by dose level:

30 ppm: [see LOAEL]
100 ppm: increased liver weight; cyanosis; hepatocellular degeneration and/or necrosis; bile duct proliferation; hepatocellular hypertrophy; cytoplasmic lipid vacuoles; body weight loss
300 ppm: muscular weakness; roughed fur; cyanosis; hepatocellular degeneration and/or necrosis; bile duct proliferation; body weight loss
1000 ppm: muscular weakness; roughed fur; cyanosis; hepatocellular degeneration and/or necrosis; hepatocellular hypertrophy; cytoplasmic vacuoles (before death by day 9)
3000 ppm: muscular weakness; roughed fur (before death during week 1)
10000 ppm: muscular weakness; roughed fur (before death during week 1)
30000 ppm: muscular weakness; roughed fur (before death during week 1)

Statistical results:

The following parameters were significantly different from controls (at a level of at least $p < 0.05$):
(A) Body weight loss:
30 ppm: females at week 4
100 ppm: males at weeks 1-4; females at weeks 2-4
300 ppm: males at weeks 1-4; females at weeks 1-3
1000 ppm: males and females at week 1 (animals died after week 1)

(B) Absolute liver weight increase:
30 ppm: males and females
100 ppm: males and females
Not reported at other doses because mice died before day 28.

(C) Relative liver weight increase:
30 ppm: females
100 ppm: males
Not reported at other doses because mice died before day 28.

Remarks: Body weight was suppressed in a dose-related manner; increased amounts of food were consumed although food waste was also seen.
1000 ppm and higher: all males and females died within the first 9 days of testing.
300 ppm: all mice except 1 male died within 26 days of testing.
30 and 100 ppm test groups: one animal died in each group. No other deaths occurred. Gross pathology showed enlargement and/or discoloration of 1 or more liver lobules in all animals sacrificed after 28 days.

000195

CONCLUSIONS

No conclusions were given by the study authors.

Remarks: All mice fed APFO lost weight. Reductions in body weight gain were followed by weight losses in mice fed 30, 100, or 300 ppm. A dose-related pattern was seen in the depressed body weights. Relative and absolute liver weights were increased in mice fed 30 ppm or more APFO. Gross pathological examination of kidneys or other organs besides livers is not discussed. Treatment-related changes were observed in the livers among all APFO treated animals including enlargement and/or discoloration of 1 or more liver lobes. Histopathologic examination of all APFO treated mice revealed diffuse cytoplasmic enlargement of hepatocytes throughout the liver (panlobular hypertrophy) accompanied by focal to multifocal cytoplasmic vacuoles. Degeneration and /or necrosis of hepatocytes and focal bile duct proliferation was also noted in mice within all groups.

REFERENCE

Christopher, B. and Marias, A.J. 1977. 28-Day Oral Toxicity Study with FC-143 in Albino Mice, Final Report, Industrial Bio-Test Laboratories, Inc. Study No. 8532-10655, 3M Reference No. T-1742CoC, Lot 269.

REPEAT DOSE DATA

Title: 28-Day Oral Toxicity Study with FC-143 in Albino 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).
T-1742CoC, Lot No. 269.

Remarks: Purity of the test substance was not indicated

METHOD

Method/guideline followed: Not stated

Study duration: 28 days

GLP (Y/N): N

Year study performed: 1977

Species/strain: Charles River CD albino rats

Sex: male and female

Number of animals per dose group: 10 (5 males and 5 females)

Route of administration: Diet - Purina rat chow

Doses tested and frequency: 0, 30, 100, 300, 1000, 3000, 10000, 30000 ppm

Post-observation period: None

Statistical methods used: A test was used to compare mean body weights, liver weights, and liver to body weight ratios among groups, but the type of test was not specified.

Remarks: Animals were observed daily for clinical signs of toxicity. Body weight and food consumption were determined weekly. Liver weights, body weights, and liver weight to body weight ratios were determined at terminal sacrifice. Gross pathological examination was conducted on all surviving rats. It is stated that the study included a complete examination of gross pathology and Aa complete set@ of tissues and organs were examined, but the specific list is not supplied. Livers were weighed to determine relative organ weight then stained for histopathologic examination. Samples of livers and blood were sent to 3M for analysis, but the results, if the analyses were completed, are not included.

RESULTS

NOAEL (dose and effect): none; effects observed at lowest test dose

LOAEL (dose and effect): 30 ppm (increased liver weight and hepatocyte hypertrophy)

Toxic response/effects by dose level: All animals given 10000 or 30000 ppm died within the first week of testing. There were no premature deaths or other clinical signs of toxicity in the other groups. Males at 300 ppm showed slightly reduced body weight gains. Body weight gains among animals fed 1000 ppm or more were significantly reduced in a dose-dependent manner. Reduced food intake was evident among rats fed either 1000 or 3000 ppm. Treatment-related morphologic changes in the liver were observed among all male and female test animals. The primary lesion consisted of focal to multifocal cytoplasmic enlargement (hypertrophy) of hepatocytes among animals in test groups 0, 30, and 100 ppm and multifocal to diffuse enlargement of hepatocytes was seen among animals in test groups 300, 1000, and 3000 ppm

Statistical results: Differences in body weight between the control and treatment groups were statistically significant at 1000 and 3000 ppm. Absolute liver weights were significantly increased for males at 300 ppm and in females at 1000 and 3000 ppm. Relative liver weight was significantly increased for males at 3000 ppm.

Remarks: Gross pathological exam did not reveal treatment-related effects in kidneys or other organs besides livers. The main effect was focal to multifocal cytoplasmic enlargement of hepatocytes among animals fed 300 ppm. In animals fed 1000 ppm or more the effect was multifocal to diffuse enlargement of hepatocytes. The severity/degree of tissue involvement of these lesions was more pronounced in male test animals. Absolute liver weights in males at 1000 and 3000 ppm are not significantly different than controls only because of body weight reduction in the higher dose animals.

CONCLUSIONS

The authors gave no conclusion.

Remarks: All animals in the 10,000 and 30,000 ppm groups died before the end of the first week. Body weight gains were reduced in the groups receiving 1000 or more ppm. Reduced food intake was observed in rats fed 1000 ppm or higher in a dose-related manner.

Relative liver weights were increased in males fed 30 ppm or more and females fed 300 ppm or more. Treatment-related changes were observed in the livers among all APFO treated animals. The severity of effects was more pronounced in male test animals

REFERENCE

Metrick, M. and Marias, A.J. 1977. 28-Day Oral Toxicity Study with FC-143 in Albino Rats, Final Report, Industrial Bio-Test Laboratories, Inc. Study No. 8532-10654, 3M Reference No. T-1742CoC, Lot 269, September 29, 1977.

REPEAT DOSE DATA

Title: Ninety-Day Subacute Rhesus Monkey Toxicity Study

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 used in this study was obtained from the sponsor, 3M. Company, St. Paul, MN. The lot number of FC-143 used in this study was 340. The test substance was a white powder.

METHOD

Method/guideline followed: Not stated

Study duration: 90-day

GLP (Y/N): No

Year study performed: 1978

Species/strain: Rhesus monkey

Sex: Male and female

Number of animals per dose group: Two per sex per group

Route of administration: Oral gavage

Doses tested and frequency: 0 (control group), 3, 10, 30, and 100 mg/kg/day, 7 days/week, for 90 days

Post-observation period: None

Statistical methods used: All statistical analyses compared the treatment groups with the control group, by sex. The tests were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie (1960) using Dunnett's multiple comparison tables to judge significance of differences.

Remarks: Ten male rhesus monkeys (2.60 – 3.90 kg) and 10 female rhesus monkeys (2.95 – 3.80 kg) were used in this study. The monkeys were housed individually in hanging wire mesh, "squeeze type" cages and maintained in a temperature, humidity, and light controlled environment. Purina Monkey Chow was fed twice/day and fresh apples were fed 3 times/week. Water was available *ad libitum*. Prior to test substance administration, the staff veterinarian conducted a complete physical examination. Only monkeys in good health were selected for the study. The test compound, suspended in 0.5% Methocel®, was administered by gavage at a frequency of 7 days/week for 90 days. All doses were given in a constant volume. The same volume of 0.5% Methocel® was given to the vehicle control group. Individual daily doses were based upon the body weights obtained weekly. The monkeys were observed twice daily for

general physical appearance, behavior, and pharmacotoxic signs. General physical examinations were conducted in the control period and monthly during the study. Blood and urine samples were obtained for analysis from all monkeys once during the control period, then again 1 and 3 months after study initiation. After completion of the test substance administration period, all surviving monkeys were anesthetized with Sernylan® (Phencyclidine HCl), exsanguinated, and necropsied. At necropsy, the heart, liver, adrenals, spleen, pituitary, kidneys, testes/ovaries, and brain were weighed; representative tissues were collected in buffered neutral 10% formalin. Eyes were fixed in Russell's fixative. The thyroid/parathyroid was weighed after fixation. Histopathology was performed on the following organs from all monkeys in the control and treatment groups: adrenals, aorta, bone, brain, esophagus, eyes, gallbladder, heart (with coronary vessels), duodenum, ileum, jejunum, cecum, colon, rectum, kidneys, liver, lung, skin, mesenteric lymph node, retropharyngeal lymph node, mammary gland. nerve (with muscle), spleen, pancreas, prostate/uterus, rib junction (bone marrow), salivary gland, lumbar spinal cord, pituitary, stomach, testes/ovaries, thyroid, parathyroid, thymus, trachea, tonsil, tongue, urinary bladder, vagina, identifying tattoo, and any tissues(s) with lesions. Histopathology was performed on the following organs from all monkeys in the control and treatment groups: adrenals, aorta, bone, brain, esophagus, eyes, gallbladder, heart (with coronary vessels), duodenum, ileum, jejunum, cecum, colon, rectum, kidneys, liver, lung, skin, mesenteric lymph node, retropharyngeal lymph node, mammary gland, nerve (with muscle), spleen, pancreas, prostate/uterus, rib junction (bone marrow), salivary gland, lumbar spinal cord, pituitary, stomach, testes/ovaries, thyroid, parathyroid, thymus, trachea, tonsil, tongue, urinary bladder, vagina, identifying tattoo, and any tissues(s) with lesions.

RESULTS

NOAEL (dose and effect): 0 (control) and 3 mg/kg/day—Soft stool, moderate to marked diarrhea, and frothy emesis were occasionally observed at both of these dosage levels.

LOAEL (dose and effect): 30 mg/kg/day—Clinical signs of toxicity, adverse histologic changes in the adrenals, bone marrow, spleen, and lymph nodes, and deaths.

Toxic response/effects by dose level: There was no mortality at the 0, 3, and 10 mg/kg/day dosage levels. Three monkeys in the 30 mg/kg/day dosage group, and all 4 monkeys in the 100 mg/kg/day dosage group, died during this study.

3 mg/kg/day— Soft stool and/or moderate to marked diarrhea, frothy emesis were noted at this dosage level.

10 mg/kg/day—The following symptoms were noted in one monkey: anorexia during week 4, pale and swollen face during week 7, and black stools during week 12.

30 mg/kg/day—Three monkeys died during weeks 7, 12, and 13 of the study. From week 4, these 3 monkeys were anorexic. All 4 monkeys in this group showed slight to moderate, and sometimes severe, decreased activity. In addition, swollen face, eyes, and vulva, as well as pallor of the face and gums were noted in all 4 monkeys. Other symptoms observed in some/all monkeys included the following: emesis, ataxia, black stools, dehydration, ptosis of the eyelids, and loss of body weight. The surviving male had decreased numbers of erythrocytes, decreased hemoglobin, decreased hematocrit, and increased platelets. Prothrombin time and activated prothrombin time were also increased. These increases were apparent at 1 month but were much more marked at three months. There was a decrease in alkaline phosphatase levels at one month (365 vs 597 in the control) which persisted in the one surviving male (360 vs 851 IU/l in the control) at 3 months. SGOT levels were reduced at one

month (59 vs 29 IU/l in the control) and in the one surviving male at 3 months (88 vs 45 IU/l in the control). SGPT was elevated at 1 month (44 vs a control value of 15 IU/l) and at 3 months (46 vs 31 IU/l in the control group). Cholesterol in the one surviving male was elevated (240 vs 165 mg/100 ml). Total protein and albumin in this animal were reduced; total protein was 5.52 vs a control level of 8.21 g/100 ml and total albumin was 2.00 vs a control level of 4.82 g/100 ml. During the pathological studies, the following compound-related symptoms were observed in male and female monkeys: marked lipid depletion of the adrenals, slight to moderate hypocellularity of the bone marrow, and moderate atrophy of lymphoid follicles of the spleen and lymph nodes.

100 mg/kg/day— All monkeys from the 100 mg/kg/day dosage level died during weeks 2 – 5 of the study. In addition, the monkeys in this group showed the same signs of toxicity as the monkeys dosed 30 mg/kg/day. However, the signs of toxicity observed in the 100 mg/kg/day dosage group (anorexia, frothy emesis, pale face, pale gums, swollen face and eyes, decreased activity, prostration, body weight loss, and body trembling) appeared earlier in the study than was observed in the 30 mg/kg/day dosage group. During the pathological studies, the following compound related symptoms were observed in male and female monkeys: marked diffuse lipid depletion of the adrenals, slight to moderate hypocellularity of the bone marrow, and moderate atrophy of lymphoid follicles of the spleen and lymph nodes.

Statistical results: There were statistically significant decreases in body weight for the male monkeys at the 30 mg/kg/day dosage level during week 13 of the study. The female monkeys of the 30 mg/kg/day dosage level and the monkeys of the 100 mg/kg/day dosage level were already dead at this time. Statistically significant variations in sex-group mean weights of a few organs occurred between the control and experimental groups. The statistically significant variations were the following: decreased absolute and relative heart weight for females in the 10 mg/kg/day dosage group ($p < 0.05$, $p < 0.01$, respectively), decreased absolute brain weight for females in the 10 mg/kg/day dosage group ($p < 0.01$), and increased relative pituitary weight for males in the 3 mg/kg/day dosage group ($p < 0.05$). These variations were of unknown biological significance and were not accompanied by morphologic alterations.

Remarks: Following one month of treatment, glucose was significantly elevated in the 3 mg/kg/day group (117 vs 89 mg/100 ml in the control). The authors of the report attribute this to a single high value for male #7366 who had a value of 131. The other three monkeys in the 3 mg/kg/day group had levels of 112, 105, and 120 mg/100 ml. Glucose levels in the 10 and 30 mg/kg/day groups were 104 and 122 mg/100 ml, respectively, after one month of treatment. At three months of treatment, glucose levels were 81, 96, 88, and 66 mg/100 ml in the control, 3, 10 and 30 mg/kg/day groups respectively. SGPT was elevated in the 10 and 30 mg/kg/day dose groups at 1 month, but were comparable to control at 3 months. There were no treatment related changes in urinalysis studies at any time period studied.

000201

CONCLUSIONS

The test substance caused clinical signs of toxicity, adverse histologic changes in the adrenals, bone marrow, spleen, and lymph nodes, and deaths in male and female rhesus monkeys when administered at dosage levels of 30 and 100 mg/kg/day.

Remarks: The test substance was not sufficiently characterized.

REFERENCE

3M Company, International Research and Development Corporation. 1978. Ninety-Day Subacute Rhesus Monkey Toxicity Study. Laboratory study number 137-090.

000202

REPEAT DOSE DATA

Title: Ninety Day Subacute Rat Toxicity Study

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: White powder, 3M stock no. 98-0211-0008-0 Lot 340; purity of the test substance was not indicated.

METHOD

Method/guideline followed: No guideline number stated

Study duration: 90 days

GLP (Y/N): No

Year study performed: 1977-78

Species/strain: Charles River CD rat

Sex: Male and female

Number of animals per dose group: 5 animals/sex/group

Route of administration: Dietary

Doses tested and frequency: 0, 10, 30, 100, 300, and 1,000 ppm

Post-treatment observation period: None

Statistical methods used: Statistical analysis comparing results of the treatment groups with the control group was performed by analysis of variance (one-way), Bartlett's test for homogeneity of variances and the appropriate t-test (for equal or unequal variances) using Dunnett's multiple comparison tables to judge significance of differences.

Remarks: Initial age of the test animals was not given; however, the mean initial body weight of the males was 222-254 g and the initial weight of the females was 151-179 g. The test chemical was mixed with 500 g of feed at weekly intervals. The treated animals were observed twice daily for clinical signs of toxicity and for mortality. Individual body weights were recorded weekly. Blood and urine samples were collected prior to study initiation and at 1 and 3 months of treatment to evaluate hematology, biochemistry, urinalysis, and serum samples. Food consumption was recorded. At 90 days, the animals were sacrificed and necropsied for macroscopic and microscopic examination. Histopathology was performed on the following organs from rats from the control, 100, 300, and 1,000 ppm dose groups: brain with cervical cord, lumbar spinal cord, peripheral nerve, eyes, pituitary, thyroid with parathyroid, adrenals, lung, heart with coronary vessels, aorta, spleen, mesenteric lymph node, thymus, bone with

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marrow (sternum), salivary gland, small intestines (duodenum, jejunum, ileum) colon, pancreas, liver, kidneys, urinary bladder, testes, ovaries, prostate, uterus, skin (mammary gland), any tissue(s) with gross lesions. Livers from rats from the 10 and 30 ppm dose groups were also examined microscopically and liver samples from all dose groups were frozen and sent to the sponsor for analysis.

RESULTS

NOAEL
Males: 30 ppm
Females: 300 ppm

LOAEL
Males: 100 ppm (decrease in food consumption, liver lesions)
Females: 1,000 ppm (liver lesions, increased liver weight)

Remarks: One female in the 100 and one female in the 300 ppm group died during collection of blood. These deaths were not considered to be treatment related. All other animals survived until scheduled sacrifice.

There was a significant reduction in mean body weight in males in the 1,000 ppm group (362 g vs 466 g in the control group). Food consumption was reduced in males in the 100, 300 and 1000 ppm groups, but the differences were not statistically significant.

Males in the 30, 100, 300 and 1,000 ppm groups had significantly reduced numbers of erythrocytes at the end of the treatment period. The values were 7.95, 7.05, 7.16, 6.72, and 6.94 in the control, 30, 100, 300 and 1000 ppm groups, respectively. Males had reduced leukocyte values compared to the controls in all dose groups, but were statistically significant at the 300 ppm group only; leukocyte values were 10.64, 8.88, 9.33, 9.35, 7.63, and 8.06 in the control, 10, 30, 100, 300 and 1,000 ppm groups, respectively. A similar phenomenon was seen with hemoglobin values which were reduced at all dose levels but were significant at the 10 ppm dose level only. Hemoglobin values were 16.2, 14.7, 15.0, 15.4, 14.9, 13.1 in the control, 10, 30, 100, 300 and 1000 ppm groups, respectively. There was no similar effect upon the hematological parameters of female rats in the study.

Males at the 30, 100, 300, and 1,000 ppm dose levels had increased glucose levels (mg/100 ml) which were statistically significant at all but the 100 ppm dose level. Reported glucose levels were 121, 120, 136, 134, 143 and 135 mg/100 ml for the 0, 10, 30 100, 300 and 1,000 ppm groups, respectively. B.U.N. levels were elevated in males at the 100, 300, and 1,000 ppm dose levels; mean values at 90 days were 20.4, 23.9 and 35.1 mg/100 ml for the three dose groups, respectively, compared to 16.2 mg/100 ml for the controls. Alkaline phosphatase was elevated in males in the 100, 300, and 1,000 ppm groups; the levels were 147, 204 and 212 IU/l for the three groups, respectively, compared to 104 IU/l for the controls. Females showed no similar changes in biochemical measurements.

Neither males nor females showed any treatment related changes in urinalysis parameters although females from all groups showed a higher frequency of occult blood in the urine than did males.

The only gross necropsy observation was noted in males at the 1,000 ppm dose level. These animals had enlarged livers which showed varying degrees of surface discoloration. Neither females from the 1,000 ppm dose level nor males or females from the lower dose levels showed such effects.

Both absolute and relative liver weights were significantly increased in males in the 30, 300 and 1,000 ppm groups and in one female in the 1,000 ppm group. Compound-related liver lesions occurred in all male rats in the 100, 300 and 1,000 ppm groups. These lesions consisted of focal to multifocal, very slight to slight hypertrophy of hepatocytes in centrilobular to midzonal regions of the affected liver lobules. In some instances these lesions were accompanied by increased amount of yellowish-brown pigment resembling lipofuscin in the cytoplasm of hepatocytes and occasionally in sinusoidal lining cells. The incidence and severity of the lesions was more pronounced among male rats at the 1,000 ppm dietary level.

CONCLUSIONS

The test substance resulted in a significant reduction in mean body weight in males at 1000 ppm, a significant reduction in the number of erythrocytes in males at 30, 100, 300 and 1000 ppm, and liver lesions in males at 100, 300 and 1000 ppm.

REFERENCE

Goldenthal, E., D. Jessup, R. Geil, N. Jefferson, and R. Arceo. 1978. Ninety day subacute toxicity study: Fluorad Fluorochemical FC-143. 3M Company. Study no. 137-089.

REPEAT DOSE DATA

Title: 13-Week Dietary Toxicity Study with T-5180, Ammonium Perfluorooctanoate (CAS No. 3825-26-1) in Male Rats

TEST SUBSTANCE

Identity: T-5180, Ammonium perfluorooctanoate (APFO), CAS No. 3825-26-1, Lot No. 115. It is a lightly colored powder.

Remarks: Purity of the test substance was not indicated.

METHOD

Method/guideline followed: Guideline 82-1 (source not specified). The study was conducted in compliance with Hazleton Wisconsin, Inc. (HWI) protocol TP9321 dated November 30, 1990.

Study duration: 13 weeks

GLP (Y/N): Y

Year study performed: 1993

Species/strain: Rat/Sprague-Dawley, Crl:CD®BR

Sex: Male

Number of animals per dose group: 55 in each group, except pair-fed controls, which had 45 animals

Route of administration: Dietary

Doses tested and frequency: 0 (pair-fed and nonpair-fed controls), 1, 10, 30, or 100 ppm (approximate mean compound consumption at week 13 of 0.05, 0.47, 1.44, and 4.97 mg/kg/day) fed *ad libitum*. Fifteen animals per group were sacrificed at 4, 7, and 13 weeks. The remaining 10 animals per group (all groups except pair-fed controls) were sacrificed after 13 weeks of treatment and 8 weeks without treatment.

Post-treatment observation period: 8 weeks

Statistical methods used: Levene's test was used to test for variance homogeneity. In cases of heterogeneity of variance at p . 0.05, transformations were used to stabilize the variance. ANOVA was performed on the homogeneous or transformed data. If the ANOVA was significant, Games and Howell Modified Tukey-Kramer test was used for pairwise comparisons between groups. One-way ANOVA was used to analyze body weights, cumulative body weight gains, food consumption, clinical chemistry, hormone values, organ weights, organ-to-body weight percentages, and organ-to-brain weight ratios. Body weights, cumulative body weight gains, and food consumption values were analyzed for all groups (except pair-fed controls) using the SAS program according to HWI methods. Group comparisons were evaluated at the 5.0% two-tailed probability level. In the analysis of the data, animals in groups exposed to 1, 10, 30, and 100 ppm APFO were compared to the control animals in the nonpair-fed group, while the data from the pair-fed control animals were compared to animals exposed to 100 ppm APFO.

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Remarks: Male rats were used to characterize the effects of the test substance on testicular physiology. At study initiation, the animals were approximately 41 days old and weighed 181 to 229 g. The appropriate amount of the test substance was thoroughly mixed with rodent chow before providing it *ad libitum* to the animals. Control groups of pair-fed and nonpair-fed rats were maintained on a basal diet not containing the test substance. All diets were assayed weekly for the first four weeks to determine the dietary concentration of the test substance; thereafter, weekly analyses were performed on the control diet and one test diet, selected sequentially.

Throughout the study, animals were observed twice daily for signs of toxicity. Individual body weight data were recorded on the first day, weekly thereafter, and on the day of necropsy. Food consumption data were collected daily for pair-fed groups and weekly for nonpair-fed groups. Serum samples collected from 10 animals/group at each scheduled sacrifice were analyzed for estradiol, total testosterone, luteinizing hormones, and test material content. At necropsy, samples of liver, testes, lungs, and subcutaneous adipose tissue were collected from each animal and frozen for possible test material residue analysis. A section of liver was obtained from all animals at each scheduled sacrifice. For 5 animals/group at each scheduled sacrifice, the liver was assayed for the level of palmitoyl CoA oxidase as an indicator of peroxisome proliferation. Fifteen animals/group were necropsied after 4, 7, and 13 weeks of treatment, as well as 10 animals/group at the end of the 8-week recovery period. The macroscopic examinations included the external surface of the body, all orifices, the cranial cavity, the external surfaces of the brain and spinal cord, the nasal cavity and paranasal sinuses, and the thoracic, abdominal, and pelvic cavities and viscera. The brain, liver, lungs, testes, and accessory sex organs (seminal vesicle, prostate, coagulating gland, urethra) were weighed. Organ-to-body weight percentages and organ-to-brain weight ratios were calculated. The following were examined microscopically (when present): lesions, brain, liver, lungs, testes, and accessory sex organs. Electron microscopy was also used to evaluate tissues from the brain, liver, lungs, testes, and accessory sex organs.

RESULTS

NOAEL (dose and effect): 1.0 ppm

LOAEL (dose and effect): 10 ppm

Toxic response/effects by dose level: One animal at the 100 ppm dose level was sacrificed during week 4 due to severe neck sores, but all other animals survived until scheduled sacrifice. No clinical signs of toxicity were observed in any groups during treatment or recovery.

> 10 ppm — higher hepatic palmitoyl CoA oxidase activity, decreased mean body weight gains, increased absolute and relative liver weights, and hepatocellular hypertrophy
>
> 30 ppm — higher hepatic palmitoyl CoA oxidase activity, decreased mean body weight gains, increased absolute and relative liver weights, and hepatocellular hypertrophy
>
> 100 ppm — lower body weights and cumulative body weight gains, lower food consumption, higher hepatic palmitoyl CoA oxidase activity, increased absolute and relative liver weights, hepatocellular hypertrophy, and elevated estradiol levels

Statistical results: High dose animals exhibited consistently significantly lower body weights and cumulative body weight gains compared to those of the nonpair-fed group. They also consumed significantly less food than the nonpair-fed controls at weeks 1 and 2. Animals at dose levels of 30 and 100 ppm exhibited higher hepatic palmitoyl CoA oxidase activities that were statistically significant at

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weeks 5, 8, and 14. Animals fed 10 ppm had transiently higher hepatic palmitoyl CoA oxidase activity that was statistically significant at week 5. Absolute and relative liver weights were significantly higher in the animals of the high dose group than the pair-fed controls at weeks 4, 7, and 13.

Remarks: Though statistically significant increases in hepatic palmitoyl CoA oxidase activities were reported during treatment, no difference was seen after the 8-week recovery period. The effect was dose-dependent and reversible. Increased absolute and relative liver weights and hepatocellular hypertrophy were observed in animals fed 10, 30, or 100 ppm; absolute and relative liver weights were significantly higher in the animals of the high dose group than the pair-fed controls at weeks 4, 7, and 13. The progression of hepatocellular hypertrophy did not appear to be affected by the length of treatment. The changes observed are suggestive of a test material effect on intracellular metabolism and may be associated with peroxisome proliferation. There was no evidence of increased liver weights of animals after the recovery period, which indicates that the effects were reversible. Animals in the high dose group exhibited consistently significantly lower body weights and cumulative body weight gains than those of the nonpair-fed control group. They also consumed significantly less food than the nonpair-fed controls at weeks 1 and 2. Overall, no significant difference in mean food consumption between nonpair-fed and pair-fed groups was noted. Though there was no statistically significant difference, the estradiol levels in the high dose animals appeared to be elevated at week 5.

CONCLUSIONS

The study author concluded that the no observed adverse effect level (NOAEL) for the test substance when fed *ad libitum* to rats for at least 13 weeks was 100 ppm and that the no observed effect level (NOEL) was 1.0 ppm.

Remarks: In animals fed 10, 30, and 100 ppm, the report describes treatment-related liver effects that may be considered adverse, including increased absolute and relative liver weights, hepatocellular hypertrophy, and significantly increased hepatic palmitoyl CoA oxidase activities. On the basis of these findings, the LOAEL was 10 ppm and the NOAEL was 1.0 ppm.

REFERENCE

Palazzolo, M. 1993. 13-Week dietary toxicity study with T-5180, ammonium perfluorooctanoate (CAS No. 3826-1) in male rats. Hazleton Wisconsin, Inc. Madison, WI. 3M Company. St. Paul, MN.

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

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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).

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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

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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.

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-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:

1. 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.
2. 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.
3. 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].
4. 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).

REFERENCE

3M Company/Riker Laboratories, Inc. Two Year Oral (Diet) Toxicity/ Carcinogenicity Study of Fluorochemical FC-143 in Rats. Experiment No. 0281CR0012. St. Paul, MN.; 8EHQ-1087-0394, Oct. 16, 1987.

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Rao, G.N. , Edmondson, J. and Haseman J.K. Influence of viral infections on tumor incidences, body weight and survival of Fischer 344 rats. Toxicologist, 8:166, 1988,

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REPEAT DOSE STUDIES

Title: Mechanisms of Extrahepatic Tumor Induction by Peroxisome Proliferators in Male CD Rats

TEST SUBSTANCE

Identity: Ammonium perfluorooctanoate

Remarks: The substance was 98-100% pure

METHOD

Method/guideline followed: None

Study duration: 2 years

GLP (Y/N): Unknown

Year study performed: 2001 (publication date)

Species/strain: Crl: CD BR rats from Charles River Breeding Laboratories (Raleigh, NC).

Sex: Males

Number of animals per dose group: 156

Route of administration: diet

Doses tested and frequency: 0, 300 ppm

Post-observation period: None

Statistical methods used: One-way analysis of variance. When corresponding F-test for differences among groups was significant, pairwise comparisons were made with Dunnett's test. The Bartlett's test for homogeneity of variance was also performed. Nonparametric procedures included Kruskal-Wallis test for equal medians and Mann-Whitney U test for pairwise comparisons.

Remarks: Hormonal analysis was conducted in 10 rats. Blood was collected from the tail vein about 1,3, 6, 9, 12, 15, 18, and 21 months after initiation of the study. Serum was prepared and frozen and then analyzed for testosterone, estradiol, luteinizing hormone, follicle stimulating hormone, and prolactin concentrations. All samples were analyzed simultaneously in duplicate.

Rats were euthanized at interim time periods (1, 3, 6, 9, 12, 15, 18, and 21 months). Testes, epididymides, accessory sex gland (ASG) unit with fluid, coagulating gland/seminal vesicle (with fluid removed), prostate, and liver were weighed.

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At 24 months, surviving rats were necropsied. Brain, heart, liver, spleen, kidneys, ASG unit, coagulating gland/seminal vesicles with fluid removed, prostate, epididymides, and testes were weighted at necropsy. Liver, testes, epididymides, pancreas, and organs with gross lesions were examined microscopically for lesions.

Six rats/group were selected for evaluations of cell proliferation. For each tissue type, 1000 cells were scored.

Six rats/group were selected for evaluation of peroxisome proliferation. -oxidation activity from liver and Leydig cell peroxisomes was measured at all interim time points. -oxidation activity was determined using the method of Lazarow (1981).

RESULTS

Toxic responses and effects:

Body weight, food consumption, and survival: From test days 8 to 630, body weight was significantly decreased in the C8 group versus the *ad libitum* control group. The decreased body weight was primarily due to reduced 'food efficiency'. On day 714 of the test, survival in the C8 group was increased compared with the control group (statistical significance not indicated). [Hematological changes were discussed in a separate article.]

Liver: Relative liver weights and hepatic -oxidation activity were significantly increased at all times ($p < 0.05$) when compared with one or more control groups. C8 produced a statistically significant increase ($p < 0.05$) in incidence of hepatocellular adenomas (10/76, 13% vs. 2/80, 3% in controls).

Testis: Testis weights were statistically significantly increased ($p < 0.05$) at 24 months in C8-treated rats. Incidences of Leydig cell hyperplasia and adenomas were also statistically significantly increased ($p < 0.05$); the incidence of Leydig cell tumors was 8/76(11%) as compared to 0/80 (0%) in controls.

Pancreas: Pancreatic acinar cell proliferation was statistically significantly increased ($p < 0.05$) at 15, 18, and 21 months. Incidence of acinar cell hyperlpasia and adenomas was significantly increased in C8 rats ($p < 0.05$). Carcinoma was observed in one C8-treated rat (not statistically significant). The combined incidence of acinar cell adenoma and carcinoma was 8/76 (11%) whereas that of the control was 0/80 (0%).

Serum hormone measurements: Serum estradiol concentrations were significantly elevated ($p < 0.05$) at 1, 3, 6, 9, and 12 months compared to control groups. There were no consistent differences in serum testosterone, FSH, prolactin, or LH concentrations in the treated rats when compared to the controls.

Statistical results: Statistically significant results are reported above.

Remarks: A full range of organs and tissues was not examined histologically. Therefore, it is unknown whether the mammary gland tumors observed in the earlier multi-dose study (3M, 1987) was induced in this study.

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CONCLUSIONS

The study demonstrate that C8, a peroxisome proliferator, induces hepatic as well as extrahepatic tumors (testis and pancreas) in CD rats. Data from this study also suggest that the induction of Leydig cell tumors by C8 is a result of a sustained increase in serum estradiol concentration.

Remarks: none
Last Modified: 7/03/01
REFERENCE

Biegel, L.B., Hurtt, M.E., Frame, S.R., O'Connor, J.C., and J.C. Cook. 2001. Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. Toxicological Sciences. 60: 44-55.

3M. Final report of " Two year oral (diet) toxicity and carcinogenicity study of fluorochemical FC-143 (perfluorooctanane ammonium carboxylate) in rats. Vol. 1-4, #M/RIKER Exp. No. 0281Croo12; 8EHQ-1087-0394, Oct. 16, 1987.

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DEVELOPMENTAL TOXICITY

Title: Oral Teratology Study of T-2998CoC in Rats

TEST SUBSTANCE

Identity: Ammonium Perfluorooctanoate (APFO) or T-2998CoC (FC-143)

Remarks: Purity of the test substance was not indicated.

METHOD

Method/Guideline followed: The procedure complies with the general recommendations of the FDA issued in January, 1966 ("Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use"). The study was conducted according to the 1978 Good Laboratory Practice Regulations and Safety Evaluation Laboratory's Standard Operating Procedures.

GLP (Y/N): Yes

Year study performed: 1981

Species/Strain: Rat/Sprague Dawley-derived CD

Number of animals per dose: 22

Route of administration: Oral gavage

Dosing regimen: Five groups of 22 time-mated Sprague-Dawley rats were administered 0, 0.05, 1.5, 5, and 150 mg/kg/day APFO in water by gavage on gestation days (GD) 6-15. A constant dose volume of 5 ml/kg was administered.

Doses: 0, 0.05, 1.5, 5, and 150 mg/kg/day

Statistical methods used: Dunnett's t test for dam and pup weights, number of fetuses, number of resorption sites, number of implantation sites and number of corpora lutea; Chi square for percent abnormalities.

Remarks – Detail and discuss any significant protocol parameters and deviations: Based on the results of a range-finding study, an upper dose level of 150 mg/kg/day was set for the definitive study in which five groups of 22 time-mated Sprague-Dawley rats were administered 0, 0.05, 1.5, 5, and 150 mg/kg/day APFO in distilled water by gavage on gestation days (GD)
6-15. Doses were adjusted according to body weight. Dams were monitored on GD 3-20 for clinical signs of toxicity. Individual body weights were recorded on GD 3, 6, 9, 12, 15, and 20. Animals were sacrificed on GD 20 by cervical dislocation and the ovaries, uteri, and contents were examined for the number of corpora lutea, number of viable and non-viable fetuses, number of resorption sites, and number of implantation sites. Fetuses were weighed and sexed and subjected to external gross necropsy. Approximately one-third of the fetuses were fixed in Bouin=s solution and examined for visceral

abnormalities by free-hand sectioning. The remaining fetuses were subjected to skeletal examination using alizarin red.

RESULTS

NOAEL (dose and effect) – maternal and developmental: The NOAEL for maternal toxicity is 5 mg/kg/day. The NOAEL for developmental toxicity is 150 mg/kg/day, the highest dose tested.

LOAEL (dose and effect) - maternal and developmental: The LOAEL for maternal toxicity is 150 mg/kg/day, based on statistically significant reductions in mean maternal body weight, ataxia, and death. No signs of developmental toxicity were observed at any dose level.

Toxic response/effects by dose level - maternal: Signs of maternal toxicity consisted of statistically significant reductions in mean maternal body weights on GD 9, 12, and 15; and ataxia and death, all at the high-dose group of 150 mg/kg/day.

Toxic response/effects by dose level - developmental: No statistically significant signs of developmental toxicity were seen at any dose level.

Statistical results:

Maternal data: Statistically significant reductions (Dunnett's t test, $p<0.05$) in mean maternal body weight were observed on gestation days 9, 12, and 15. No statistical information was available for other signs of maternal toxicity (ataxia and death).

Fetal data: A statistically significant increase (Chi-square, $p<0.05$) in one sternebrae missing was observed at the highest dosed-group of 150 mg/kg/day.

Remarks – Additional information to adequately assess the data:

Signs of maternal toxicity consisted of statistically significant reductions in mean maternal body weights on GD 9, 12, and 15 at the high-dose group of 150 mg/kg/day. Mean maternal body weight on GD 20 continued to remain lower than controls, although the difference was not statistically significant. Other signs of maternal toxicity occurring only at the high-dose group included ataxia and death observed in three rat dams. No other effects were reported. Administration of APFO during gestation did not appear to affect the ovaries or reproductive tract contact of the dams.

A significantly higher incidence in fetuses with one missing sternebrae was observed at the high-dose group of 150 mg/kg/day; however this skeletal variation also occurred in the controls and the other three dose groups (at similar incidence but lower than the high-dose group) and therefore was not considered to be treatment-related. No significant differences between treated and control groups were noted for other developmental parameters that included the mean number of males and females, total and dead fetuses, the mean number of resorption sites, implantation sites, corpora lutea and mean fetus weights. Likewise, a fetal lens finding initially described as a variety of abnormal morphological changes localized to the area of the embryonal nucleus, was later determined to be an artifact of the free-hand sectioning technique and therefore not considered to be treatment-related.

CONCLUSIONS

Comment on author's conclusions and whether you agree:

Conclusions are summarized above and this reviewer agrees.

REFERENCE

Gortner, E. 1981. Oral Teratology Study of T-2998CoC in Rats. Riker Laboratories, Inc., St. Paul, MN. Experiment #0681TR0110, December 1981.

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DEVELOPMENTAL TOXICITY

Title: Oral Teratology Study of T-3141CoC in Rabbits

TEST SUBSTANCE

Identity: Ammonium Perfluorooctanoate (APFO) or T-3141CoC (FC-143).

Remarks: According to the study authors, the analytical report (Appendix IV) demonstrated that T-3141CoC has an analysis within specifications, is stable and is representative of commercial material. By this analysis, the C_8 acid was 97.6% and 98.4% of the test substance pre-study and post-study, respectively.

METHOD

Method/Guideline followed: The procedure complies with the general recommendations of the FDA issued in January, 1966: AGuidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use.@

Type of study: Developmental Toxicity

GLP (Y/N): Yes

Year study performed: 1981

Species/Strain: New Zealand White/Minikin rabbits

Number of animals per dose: 18

Route of administration: Oral gavage

Dosing regimen: Four groups of 18 pregnant New Zealand White rabbits were administered 0, 1.5, 5, and 50 mg/kg/day APFO in distilled water by gavage on gestation days (GD) 6-18. A constant dose volume of 1 ml/kg was administered.

Doses: 0, 1.5, 5, and 50 mg/kg/day

Statistical methods used: Dunnett=s t test for dam and pup weights, number of fetuses, number of resorption sites, number of implantation sites and number of corpora lutea; Chi-square test with Yates correction for percent abnormalities.

Remarks - Detail and discuss any significant protocol parameters and deviations: Based on the results of a range-finding study, an upper dose level of 50 mg/kg/day was set for the definitive study in which four groups of 18 pregnant New Zealand White rabbits were administered 0, 1.5, 5, and 50 mg/kg/day APFO in distilled water by gavage on gestation days (GD) 6-18. Pregnancy was established in each sexually mature female by i.v. injection of pituitary lutenizing hormone in order to induce ovulation, followed by artificial insemination with 0.5 ml of pooled semen collected from male rabbits; the day of insemination was designated as day 0 of gestation. A constant dose volume of 1 ml/kg was administered.

Individual body weights were measured on GD 3, 6, 9, 12, 15, 18, and 29. The does were observed daily on GD 3-29 for abnormal clinical signs. On GD 29, the does were euthanized and the ovaries, uterus and contents examined for the number of corpora lutea, live and dead fetuses, resorptions and implantation sites. Fetuses were examined for gross abnormalities and placed in a 37°C incubator for a 24 hour survival check. Pups were subsequently euthanized and examined for visceral and skeletal abnormalities. A blood sample was taken from six does prior to dosing and then on GD 18 and 29; a liver sample was taken from the same animals on GD 29. All samples were sent to the sponsor for analysis. This information was unavailable at the time of this review.

RESULTS

NOAEL (dose and effect) - maternal and developmental: A NOAEL of 50 mg/kg/day, the highest dose tested, for maternal toxicity was indicated. A NOAEL for developmental toxicity could not be established since signs of developmental toxicity were seen at all doses, with statistical significance at the highest dose.

LOAEL (dose and effect) - maternal and developmental: No signs of maternal toxicity were observed at any dose level. The LOAEL for developmental toxicity is 50 mg/kg/day, based on dose-related increases in a skeletal variation, with statistical significance at the high-dose group.

Toxic response/effects by dose level B maternal: Signs of maternal toxicity consisted of statistically significant transient reductions in body weight gain on GD 6-9 when compared to controls; body weight gains returned to control levels on GD12-29.

Toxic response/effects by dose level B developmental: Signs of developmental toxicity consisted of a dose-related increase in a skeletal variation, extra ribs or 13th rib, with statistical significance at the high-dose group of 50 mg/kg/day.

Statistical results:

Maternal data: Statistically significant reductions in mean body weight gains (Dunnett=s test, $p<0.05$) between gestation day 6-9 were observed in the highest dosed-group of 50 mg/kg/day. After gestation 9, mean body weight gains were comparable to control animals for all dosed-groups.

Fetal data: Dose-related increases in a skeletal variation, extra ribs or 13th rib, with statistical significance (Dunnett=s test, p,0.05) at the high-dose group (38% at 50 mg/kg/day, 30% at 5 mg/kg/day, 20% at 1.5 mg/kg/day, and 16 % at 0 mg/kg/day). A statistically significant increase (Dunnett=s test, p,0.05) in 13th ribs-spurred occurred in the mid-dose group of 5 mg/kg/day.

Remarks - Additional information to adequately assess the data:

Signs of maternal toxicity consisted of statistically significant transient reductions in body weight gain on GD 6-9 when compared to controls; body weight gains returned to control levels on GD12-29. Administration of APFO during gestation did not appear to affect the ovaries or reproductive tract contents of the does. Six deaths occurred during the study; however, five of the six deaths were attributed to gavage errors. No clinical or other treatment-related signs were reported.

No significant differences were noted between controls and treated groups for the number of males and females, dead or live fetuses, and fetal weights. Likewise, there were no significant differences reported for the number of resorption and implantation sites, corpora lutea, the conception incidence, abortion rate,

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or the 24 hour mortality incidence of the fetuses. Gross necropsy and skeletal/visceral examinations were unremarkable. The only sign of developmental toxicity consisted of a dose-related increase in a skeletal variation, extra ribs or 13th rib, with statistical significance at the high-dose group (38% at 50 mg/kg/day, 30% at 5 mg/kg/day, 20% at 1.5 mg/kg/day, and 16 % at 0 mg/kg/day). A statistically significant increase in 13th ribs-spurred occurred in the mid-dose group of 5 mg/kg/day; however, the biological significance of this effect is uncertain since in both the high- and low-dose groups, this effect occurred at the same rate and was not statistically significantly different from controls.

CONCLUSIONS

Comment on author=s conclusions and whether you agree: This reviewer does not agree with the conclusions of the authors that the finding of extra ribs, or 13th rib, in this particular study are not a sign of developmental toxicity. While it is agreed that the biological significance of an altered incidence of anatomical variations is difficult to assess, the incidence of 13th rib in this study showed a dose-related increase with statistical significance at the highest dosed-group, in the absence of maternal toxicity, and therefore is evaluated as a possible indication of developmental toxicity.

REFERENCE

Gortner, E.G., E.G. Lamprecht, M.T. Case. 1982. Oral teratology study of T-3141CoC in rabbits. 3M Company. Riker Laboratories, Inc., St. Paul, MN. Experiment No. 0681TB0398, February 1982.

DEVELOPMENTAL TOXICITY

Title: The Embryo-Fetal Toxicity and Teratogenic Potential of Ammonium Perfluorooctanoate in the Rat

TEST SUBSTANCE

Identity: Ammonium perfluorooctanoate (APFO), pentadecafluorooctanoic acid ammonium salt, ammonium perfluorooctanoate, ammonium perfluorocaprylate, PFO, FC-116, FC-126, FC-169, FC-143, or as a major component of FX-1003 (CASRN 3825-26-1)

Remarks: The test substance, APFO [$CF_3(CF_2)_6COONH_4$], was obtained from 3M (St. Paul, MN 55144). Its purity was >95%; the contaminants present were [$CF_3(CF_2)_4COONH_4$] and PFOA isomers. No inhibitor, carriers, or additives were present. Degradation of APFO was considered insignificant unless the temperature was to exceed 250°C (unpublished Du Pont data).

METHOD

Method/Guideline followed: Not specified

GLP (Y/N): Not specified

Year study performed: 1984

Species/Strain: Rat/Sprague-Dawley derived, Crl:CD (SD)BR strain.

Number of animals per dose: For the inhalation portion of the study, two trials with 12 mated female rats/group/trial. Two additional, pair-fed groups (6 dams/group), were added to Experiment 1 (teratology), and two groups (6 dams/group) were added to Experiment 2 (dams allowed to litter). For the gavage-exposure portion of the study, 25 and 12 mated female rats at each exposure concentration were included in Experiment 1(teratology) and Experiment 2 (dams allowed to litter), respectively.

Route of administration: Inhalation and oral

Dosing regimen (list all with units): For the inhalation portion of the study, the two trials consisted of 12 pregnant Sprague-Dawley rats per group exposed to APFO by whole-body vapor inhalation to 0, 0.1, 1, 10, and 25 mg/m^3 6 hours/day, on GD 6-15; two additional groups (6 dams per group) that were pair-fed to the 10 and 25 mg/m^3 groups, were added to each trail. In the oral portion of the study, 25 and 12 Sprague-Dawley rats for the first and second trials, respectively, were administered 0 and 100 mg/kg/day APFO in corn oil by gavage on GD 6-15.

Doses: For inhalation exposures: 0, 0.1, 1, 10, and 25 mg/m^3, with two additional groups pair-fed to the 10 and 25 mg/m^3 groups; for oral exposures: 0 and 100 mg/kg/day.

Statistical methods used: The litter was used as the experimental unit for the purpose of statistical evaluation (Staples and Haseman, 1974; Haseman and Hogan, 1975). The significance of differences in the incidence of pregnancy, clinical signs, and maternal death was determined by use of the Fisher-exact probability test (Siegel, 1956). A two-way analysis of variance was used to detect differences in feed consumption among breeding lots and between groups. Dunnett's test (Steel and Torrie, 1960) was used to test the statistical significance of differences between the control and APFO groups in maternal body

weight, in body weight gain, and in feed consumption when the one-way analysis of variance was significant. The presence of concentration-related responses for the inhalation portion of the study was determined by Jonckheere's test (Jonckheere, 1954). The significance of differences in incidence of structural alterations between the control group and the APFO group was determined by application of the Mann-Whitney U test (Mann and Whitney, 1947). When more than 75% ties occurred in the data, the Fisher's exact probability test was applied (Haseman and Hoel, 1974). The level of significance selected was p 0.05. Variability about means was expressed as standard error of the mean (SE). In addition, several reproductive indices were calculated for some results from Experiment 2 (dams allowed to litter).

Remarks – Detail and discuss any significant protocol parameters and deviations: The study design consisted of an inhalation and an oral portion, each with two trials or experiments. The first trial was the teratology portion of the study, in which the dams were sacrificed on GD 21; while in the second trial, the dams were allowed to litter and the pups were sacrificed on day 35 post-partum. For the inhalation portion of the study, the two trials consisted of 12 pregnant Sprague-Dawley rats per group exposed to APFO by whole-body vapor inhalation to 0, 0.1, 1, 10, and 25 mg/m^3 6 hours/day, on GD 6-15. In the oral portion of the study, 25 and 12 Sprague-Dawley rats for the first and second trials, respectively, were administered 0 and 100 mg/kg/day APFO in corn oil by gavage on GD 6-15. For both routes of administration, females were mated on an as-needed basis and when the number of mated females were bred, they were ranked within breeding days by body weight and assigned to groups by rotation in order of rank. Finally, two additional groups (six dams per group) that were pair-fed to the 10 and 25 mg/m^3 groups, were added to each trail.

For the teratology portion of the study (trial one), dams were weighed on GD 1, 6, 9, 13, 16, and 21 and observed daily for abnormal clinical signs. On GD 21, the dams were sacrificed by cervical dislocation and examined for any gross abnormalities, liver weights were recorded and the reproductive status of each animal was evaluated. The ovaries, uterus and contents were examined for the number of corpora lutea, live and dead fetuses, resorptions and implantation sites. Pups (live and dead) were counted, weighed and sexed and examined for external, visceral, and skeletal alterations. The heads of all control and high-dose group fetuses were examined for visceral alterations as well as macro- and microscopic evaluation of the eyes.

For trial two, in which the dams were allowed to litter, the procedure was the same as that for trial one up to GD 21. Two days before the expected day of parturition, each dam was housed in an individual cage. The date of parturition was noted and designated Day 1 post-partum (PP). Dams were weighed and examined for clinical signs on Days 1, 7, 14, and 22 PP. On Day 23 PP all dams were sacrificed. Pups were counted, weighed, and examined for external alterations. Each pup was subsequently weighed and inspected for adverse clinical signs on Days 4, 7, 14, and 22 PP. The eyes of the pups were also examined on Days 15 and 17 PP for the inhalation portion and on Days 27 and 31 PP for the gavage portion of the study. Pups were sacrificed on Day 35 PP and examined for visceral and skeletal alterations.

RESULTS

NOAEL (dose and effect) – maternal and developmental:

Inhalation: For trial one - the NOAEL for maternal toxicity is 1 mg/m^3; the NOAEL for developmental toxicity is 10 mg/m^3. For trial two – the NOAEL for maternal toxicity is 1mg/m^3; the NOAEL for developmental toxicity is 10 mg/m^3.

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Oral: A NOAEL could not be determined for either maternal or developmental toxicity since this portion of the study used only one dose level.

LOAEL (dose and effect) – maternal and developmental:

Inhalation:

For trial one – the LOAEL for maternal toxicity is 10 mg/m^3, based on treatment-related clinical signs (consisting of wet abdomens, chromodacryorrhea, chromorhinorrhea, and a general unkept appearance), and significant reductions in food consumption and body weight; the LOAEL for developmental toxicity is 25 mg/m^3, based on reductions in mean fetal body weights and a statistically significant increased incidence of fetuses with partially ossified sternebrae.

For trial two – the LOAEL for maternal toxicity is 10 mg/m^3, based on treatment-related clinical signs consisting of wet abdomens, chromodacryorrhea, chromorhinorrhea, and a general unkept appearance; the LOAEL for developmental toxicity is 25 mg/m^3, based on statistically significant reductions in pup body weight.

Oral: A LOAEL could not be determined for either maternal or developmental toxicity since this portion of the study used only one dose level.

Toxic response/effects by dose level – maternal:

Inhalation

At 10 and 25 mg/m^3, treatment-related clinical signs of maternal toxicity, and reductions in food consumption and body weight; at 25 mg/m^3, statistically significant increases in liver weights; lethargy and death.

Oral

At 100 mg/kg/day, the only dose tested: clinical signs of toxicity, reductions in food Consumption, reductions in body weight gains, and deaths.

Toxic response/effects by dose level – developmental:

Inhalation

At 25 mg/m3, reductions in mean fetal and pup body weights and statistically significant increases in the incidence of fetuses with partially ossified sternebrae.

Oral

At 100 mg/kg/day, the only dose tested, no signs of developmental toxicity were observed.

Statistical results:

Inhalation - maternal

At 10 and 25 mg/m^3: treatment-related clinical signs of maternal toxicity (no statistical significance assigned), significant reductions in food consumption (21.8 $\pm$ 0.46 vs 23.4 $\pm$ 0.38 in controls), and significant reductions in body weight with statistical significance at 25 mg/m^3 (Dunnett's test, 0<0.05); at 25 mg/m^3: statistically significant increases in mean liver weights (two-tailed Mann-Whitney *U* test, p<0.05); lethargy (4 out of 12) and death (3 out of 12 dams).

Inhalation - developmental

At 25 mg/m3, reductions in mean fetal ($p = 0.002$) and pup body weights ($p = 0.02$), and statistically significant increases ($p = 0.04$ by the two-tailed Mann-Whitney U test) in the incidence of fetuses with partially ossified sternebrae.

Oral - maternal

At 100 mg/kg/day, the only dose tested: clinical signs of toxicity (no statistical significance assigned), reductions in food consumption (no statistical significance assigned), reductions in body weights gains ($p \leq 0.05$), and deaths (3 out of 12 dams).

Oral – developmental

No statistically significant differences were noted between treated and control groups for any of the parameters measured.

Remarks – Additional information to adequately assess the data:

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Inhalation Exposure

Trial One:

Treatment-related clinical signs of maternal toxicity for trial one (teratology) occurred at 10 and 25 mg/m^3 and consisted of wet abdomens, chromodacryorrhea, chromorhinorrhea, a general unkept appearance, and lethargy in four dams at the end of the exposure period (high-concentration group only). Three out of 12 dams died during treatment at 25 mg/m^3 (on GD 12, 13, and 17). Food consumption was significantly reduced at both 10 and 25 mg/m^3; however, no significant differences were noted between treated and pair-fed groups. Significant reductions in body weight were also observed at these concentrations, with statistical significance at the high-concentration only. Likewise, statistically significant increases in mean liver weights were seen at the high-concentration group. Under the conditions of the study, a NOAEL and LOAEL for maternal toxicity of 1 and 10 mg/m^3, respectively, was indicated.

No effects were observed on the maintenance of pregnancy or the incidence of resorptions. Mean fetal body weights were significantly decreased in the 25 mg/m^3 group and in the control group pair-fed 25 mg/m^3. A detailed microscopic visceral and eye examination of the fetuses did not reveal any treatment-related effects; however in the control group that was pair-fed 25 mg/m^3, a statistically significant increased incidence of fetuses with partially ossified sternebrae was observed. Under the conditions of the study, a NOAEL and LOAEL for developmental toxicity of 10 and 25 mg/m^3, respectively, was indicated.

Trial Two:

Clinical signs of maternal toxicity seen at 10 and 25 mg/m^3 were similar in type and incidence as those described for trial one. Maternal body weight gain during treatment at 25 mg/m^3 was less than controls, although the difference was not statistically significant. In addition, 2 out of 12 dams died during treatment at 25 mg/m^3. No other treatment-related effects were reported, nor were any adverse effects noted for any of the measurements of reproductive performance. Under the conditions of the study, a NOAEL and LOAEL for maternal toxicity of 1 and 10 mg/m^3, respectively, were indicated.

Signs of developmental toxicity in this group consisted of statistically significant reductions in pup body weight on Day 1PP (6.1 g at 25 mg/m^3 vs. 6.8 g in controls) . On Days 4 and 22 PP, pup body weights continued to remain lower than controls, although the difference was not statistically significant (Day 4 PP: 9.7 g at 25 mg/m^3 vs.10.3 in controls; Day 22 PP: 49.0 g at 25 mg/m^3 vs. 50.1 in controls). No significant effects were reported following external examination of the pups or with ophthalmoscopic examination of the eyes. Under the conditions of the study, a NOAEL and LOAEL for developmental toxicity of 10 and 25 mg/m^3, respectively, were indicated.

Oral Exposure

Trial One:

Three out of 25 dams died during treatment of 100 mg/kg APFO during gestation (one death on GD 11; two on GD 12). Clinical signs of maternal toxicity in the dams that died were similar to those seen with inhalation exposure. Food consumption and body weights were reduced in treated animals compared to controls. No adverse signs of toxicity were noted for any of the reproductive parameters such as maintenance of pregnancy or incidence of resorptions. Likewise, no significant differences between

treated and control groups were noted for fetal weights, or in the incidences of malformations and variations; nor were there any effects noted following microscopic examination of the eyes.

Trial Two:

Similar observations for clinical signs were noted for the dams as in trial one. Likewise, no adverse effects on reproductive performance or in any of the fetal observations were noted.

CONCLUSIONS

Comment on author's conclusions and whether you agree: The author's conclusions appear to be supported by the data.

REFERENCE

Provide full citation of study reviewed: Staples, R.E. et al. 1984. The Embryo-Fetal Toxicity and Teratogenic Potential of Ammonium Perfluorooctanoate (PFOA) in the Rat. Fundamental and Applied Toxicology. 4:429-440. This study was performed at Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE 19711.

TOXICITY TO AQUATIC PLANTS (SELENASTRUM CAPRICORNUTUM)
TEST SUBSTANCE
Identity: Perfluorooctanoic acid, tetrabutylammonium salt; may also be referred to as PFOA tetrabutylammonium salt, tetrabutylammonium perfluorooctanoate, N2803-2, or as a major component of L-13492. (Octanoic acid, pentadecafluoro-, tetrabutylammonium salt, CAS # 95658-53-0)
Remarks: The 3M production lot number was 2327. The test sample is referred to by the testing laboratory as L-13492. The T.R. Wilbury study number is 841-TH. The purity of the sample was not sufficiently characterized, although current information indicates it is a solution of 44.9% tetrabutylammonium perfluorooctanoate, 27.9% water, and 27.2% isopropanol.
The following summary applies to the test sample as a mixture of the test substance in an isopropanol/ water solution with incompletely characterized concentrations of impurities. Data may not accurately relate toxicity of the test sample with that of the test substance.
METHOD
Method: U.S. EPA-TSCA Guideline 797.1050
Test: Acute static
GLP: Yes
Year completed: 1995
Species: *Selenastrum capricornutum*
Source: Originally from The Culture Collection of Algae at the University of Texas at Austin, maintained in culture medium at T.R. Wilbury, Inc., Marblehead, MA.
Element basis: growth rate.
Exposure period: 96-hours
Test organisms laboratory culture: Algae cultures were growing in U.S. EPA-recommended sterile enriched medium for at least 14 days prior to test initiation.
Statistical methods: The average specific growth rate was calculated as the natural log of the number of cells/mL at the exposure period minus the natural log of the number of cells/mL at 0 hours divided by the exposure period. The percent change from the control was calculated by subtracting the treatment average specific growth rate from the control average specific growth rate, dividing the difference by the average specific growth rate in the control, and multiplying that value by 100. The EC50 values were calculated based on a nonlinear regression estimation procedure (Bruce and Versteeg, 1992). The NOEC was determined using a parametric one-way analysis of variance and the average specific growth in each test vessel at the end of the test.
Test Conditions:
Dilution water source: The algae medium was prepared to U.S. EPA recommended concentrations by spiking deionized water with nutrient stocks. The pH of the synthetic algal medium at test initiation was 7.5.
Stock and test solutions preparation: A 16 mg/L primary stock

solution was prepared in sterile enriched media. Appropriate amounts of this stock solution were added directly to dilution water to formulate the test media. A 1,000 mg/L isopropyl alcohol stock solution was also prepared and evaluated.
Exposure vessels: 250 mL glass Erlenmeyer flasks containing 100 mL of test solution.
Agitation: Continuous at 100 rpm
Number of replicates: 3
Initial algal cell loading: 1.0 X 10 4 cells/mL
Number of concentrations: Five plus a negative control
Lighting: ~400 ft-c from continuous cool-white fluorescent lighting
Water chemistry:
pH range: (0 – 96 hours)
7.5 – 10.4 (control exposure)
7.4 – 7.6 (16 mg/L exposure)
7.4 – 10.4 (1,000 mg/L isopropyl alcohol exposure)
Test temperature range: (0 – 96 hours)
23.4 – 23.7°C
RESULTS
Nominal concentrations: Blank control, 1.0, 2.0, 4.0, 8.0, 16.0 mg/L.
A test was performed simultaneously with isopropyl alcohol, a component that represents 27% of L-13492, at 4.4 and 1,000 mg/L.
Element value and 95% confidence interval:
24-hour ErC10 (growth rate) = <1.0 mg/L (CI not calculable)
24-hour ErC50 (growth rate) = 15 (9.8 - >16) mg/L
24-hour ErC90 (growth rate) = >16 (7.7 - >16) mg/L
48-hour ErC10 (growth rate) = <1.0 mg/L (CI not calculable)
48-hour ErC50 (growth rate) = 14 (8.2 – >16) mg/L
48-hour ErC90 (growth rate) = >16 mg/L (CI not calculable)
72-hour ErC10 (growth rate) = <1.0 mg/L (CI not calculable)
72-hour ErC50 (growth rate) = 7.1 (4.1 – 11) mg/L
72-hour ErC90 (growth rate) = >16 mg/L (CI not calculable)
96-hour ErC10 (growth rate) = <1.0 mg/L (CI not calculable)
96-hour ErC50 (growth rate) = 4.9 (3.5 – 6.7) mg/L
96-hour ErC90 (growth rate) = >16 mg/L (CI not calculable)
96-hour NOEC: 1.0 mg/L
Algal growth was not affected by isopropyl alcohol concentrations of 4.4 or 1,000 mg/L.
Element values are based on nominal concentrations.

Biological observations after 96-hours:

Nominal Concentration, mg/L	Mean Number of Cells per mL	Percent Inhibition via Density	Percent Inhibition via Growth Rate
Control	1,249,000	-	-
1.0	1,311,000	-5	0
2.0	275,000	88	30
4.0	113,000	91	50
8.0	84,000	93	56
16	27,000	98	80

.

Control response: Satisfactory

Biological observations after 96-hours for isopropyl alcohol:

Nominal Concentration, mg/L	Mean Number of Cells per mL	Percent Inhibition via Density
Control	1,249,000	-
4.4	1,190,000	5
1,000	1,347,000	–8

Observations: Algal cell counts in each test vessel were determined by means of direct microscope counts with a hemocytometer. After 96 hours of exposure, there were no signs of aggregation, flocculation or adherence of the algae to the flasks in the control or any test treatment group. In addition, there were no noticeable changes in cell size, color or morphology when compared to the control.

Reversibility of Growth Inhibition: Effect of the test substance was determined to be algistatic based on the results of the post-definitive test exposure.

Remarks: Testing was conducted on the mixture as described in the Test Substance Remarks field. The values reported apply to that mixture and not the fluorochemical component alone.

CONCLUSIONS

The test sample 96-hour EC50 and 95% confidence interval for *Selenastrum capricornutum* was determined to be 4.9 mg/L with a 95% confidence interval of 3.5 – 6.7 mg/L. The 96-hour no observed effect concentration (NOEC) for the test substance in solution was 1.0 mg/L. Algae growth in the vessels containing isopropyl alcohol was not affected at 4.4 or 1,000 mg/L, indicating that the concentration of isopropyl alcohol in L-13492 can not, by itself, account for the toxicity of L-13492 to algae. No signs of aggregation, flocculation, or adherence were noted in any of the test solutions. This test substance was determined to be algistatic.

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking 2. The study lacks analytical measurement of test substance concentrations in the test solutions and sample purity is not sufficiently characterized.

000235

REFERENCES

This study was conducted at T.R. Wilbury Laboratories, Inc., Marblehead, MA, at the request of the 3M Company, Lab Request number N2332, 1995.

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Purity of the test material also is a major concern and was not sufficiently characterized in this test. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. In fact, 3M in their summary of each test state: *"Data may not accurately relate toxicity of the test sample with that of the test substance."* I agree with this concern. In addition, if this was a "typical" TSCA section 4 review, I would reject these studies, pending receipt of additional information on purity and studies on analytical measurements of the test chemical in the test medium.

000236

TOXICITY TO AQUATIC PLANTS (SELENASTRUM CAPRICORNUTUM)
TEST SUBSTANCE
Identity: Perfluorooctanoic acid, tetrabutylammonium salt; may also be referred to as PFOA tetrabutylammonium salt, tetrabutylammonium perfluorooctanoate, N2803-2, or as a major component of L-13492. (Octanoic acid, pentadecafluoro-, tetrabutylammonium salt, CAS # 95658-53-0)
Remarks: The 3M production lot number was 2327. The test sample is referred to by the testing laboratory as N2803-2. The T.R. Wilbury study number is 890-TH. The purity of the sample was not sufficiently characterized, although current information indicates it is a solution of 44.9% tetrabutylammonium perfluorooctanoate, 27.9% water, and 27.2% isopropanol
The following summary applies to the test sample as a mixture of the test substance in an isopropanol/water solution with incompletely characterized concentrations of impurities. Data may not accurately relate toxicity of the test sample with that of the test substance.
METHOD
Method: U.S. EPA-TSCA Guideline 797.1050
Test: Acute static
GLP: Yes
Year completed: 1995
Species: *Selenastrum capricornutum*
Source: Originally from The Culture Collection of Algae at the University of Texas at Austin, maintained in culture medium at T.R. Wilbury, Inc., Marblehead, MA.
Element basis: Algal cell count (cells/mL), and specific growth rate.
Exposure period: 96-hours
Test organisms laboratory culture: Algae cultures were growing in U.S. EPA-recommended sterile enriched medium for at least 14 days prior to test initiation.
Statistical methods: The average specific growth rate was calculated as the natural log of the number of cells/mL at the exposure period minus the natural log of the number of cells/mL at 0 hours divided by the exposure period. The percent change from the control was calculated by subtracting the treatment average specific growth rate from the control average specific growth rate, dividing the difference by the average specific growth rate in the control, and multiplying that value by 100. The EC50 values were calculated by probit analysis. The NOEC was determined using a parametric one -way analysis of variance and the average specific growth rate and the number of cells/mL in each test vessel at the end of the test.
Test Conditions:
Dilution water source: The algae medium was prepared to U.S. EPA recommended concentrations by spiking deionized water with nutrient stocks. The pH of the synthetic algal medium at test initiation was 7.5.
Stock and test solutions preparation: A 160 mg/L primary stock

solution was prepared in sterile enriched media. Appropriate amounts of this stock solution were added directly to dilution water to formulate the test media.
Exposure vessels: 250 mL glass Erlenmeyer flasks containing 50 mL of test solution.
Agitation: Continuously at 100 rpm
Number of replicates: 3
Initial algal cell loading: 1.0 X 10^4 cells/mL
Number of concentrations: Five plus a negative control
Lighting: Continuous lighting at ~380 ft-c using cool-white fluorescent lamps
Water chemistry:
pH range: (0 – 96 hours)
7.5 – 10.8 (control exposure)
7.5 – 8.4 (16 mg/L exposure)
Test temperature range: (0 – 96 hours)
23.4 – 23.7°C
RESULTS
Nominal concentrations: Blank control, 0.99, 2.0, 4.0, 8.0, 16.0 mg/L.
Element value and 95% confidence interval:
24-hour EC10 (cell density) = 4.1 (0 – 9.0) mg/L
24-hour EC50 (cell density) = >16 (7.7 - >16) mg/L
24-hour ErC50 (growth rate) = >16 mg/L (CI not calculable)
24-hour EC90 (cell density) = >16 mg/L (CI not calculable)
48-hour EC10 (cell density) = 1.2 (<0.99 – 1.6) mg/L
48-hour EC50 (cell density) = 7.1 (6.0 – 8.6) mg/L
48-hour ErC50 (growth rate) = >16 mg/L (CI not calculable)
48-hour EC90 (cell density) = >16 mg/L (CI not calculable)
72-hour EC10 (cell density) = <0.99 (<0.99 – 1.9) mg/L
72-hour ErC10 (growth rate) = 2.2 (1.6 – 2.8) mg/L
72-hour EC50 (cell density) = 2.8 (1.1 – 5.8) mg/L
72-hour ErC50 (growth rate) = 11 (9.4 - 15) mg/L
72-hour EC90 (cell density) = 8.4 (4.5 – >16) mg/L
72-hour ErC90 (growth rate) = >16 mg/L (CI not calculable)
96-hour EC10 (cell density) = 1.4 (<0.99 – 2.4) mg/L
96-hour ErC10 (growth rate) = 2.3 (<0.99 – 3.6) mg/L
96-hour EC50 (cell density) = 2.9 (1.0 – 7.7) mg/L
96-hour ErC50 (growth rate) = 8.4 (5.9 - 14) mg/L
96-hour EC90 (cell density) = 6.0 (3.5 - >16) mg/L
96-hour ErC90 (growth rate) = >16 mg/L (CI not calculable)
96-hour NOEC (cell density): 0.99 mg/L
96-hour NOEC (growth rate): 2.0 mg/L
Element values were based on nominal concentrations.
Control response: Satisfactory

Biological observations after 96-hours:

Nominal Concentration, mg/L	Mean Number of Cells per mL	Percent Inhibition via Density	Percent Inhibition via Growth Rate
Control	3,227,000	-	-
0.99	3,633,000	-13	-2
2.0	2,440,000	24	5
4.0	581,000	82	30
8.0	169,000	95	52
16	61,000	98	68

Observations: Algal cell counts in each test vessel were determined by means of direct microscope counts with a hemocytometer. After 96 hours of exposure, there were no signs of aggregation, flocculation or adherence of the algae to the flasks in the control or any test treatment group. In addition, there were no noticeable changes in cell size, color or morphology when compared to the control.
Reversibility of Growth Inhibition: Effect of the test substance was determined to be algistatic based on the results of the post-definitive test exposure.
Remarks: Testing was conducted on the mixture as described in the Test Substance Remarks field. The values reported apply to that mixture and not the fluorochemical component alone.

CONCLUSIONS
The test sample 96-hour EC50 and 95% confidence interval for *Selenastrum capricornutum* was determined using two calculation methods. The test substance 96-hour EC50 for *Selenastrum capricornutum* was determined to be 2.9 mg/L with a 95% confidence interval of 1.0 – 7.7 mg/L, when calculated using cell density. The 96-hour EC50 for *Selenastrum capricornutum* was determined to be 8.4 mg/L with a 95% confidence interval of 5.9 – 14 mg/L using growth rate. No signs of aggregation, flocculation, or adherence were noted in any of the test solutions. This test substance was determined to be algistatic.
Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133
DATA QUALITY
Reliability: Klimisch ranking 2. The study lacks analytical measurement of test substance concentrations in the test solutions and sample purity is not sufficiently characterized.
REFERENCES
This study was conducted at T.R. Wilbury Laboratories, Inc., Marblehead, MA, at the request of the 3M Company, Lab Request number N2803-2, 1995.
OTHER

000239

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Purity of the test material also is a major concern and was not sufficiently characterized in this test. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. In fact, 3M in their summary of each test state: *"Data may not accurately relate toxicity of the test sample with that of the test substance."* I agree with this concern. In addition, if this was a "typical" TSCA section 4 review, I would reject these studies, pending receipt of additional information on purity and studies on analytical measurements of the test chemical in the test medium.

TOXICITY TO AQUATIC PLANTS (SELENASTRUM CAPRICORNUTUM)
TEST SUBSTANCE
Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)
Remarks: The 3M production lot number was 427. The test sample is FC-143, referred to by the test laboratory as N2803-4. The T.R. Wilbury study number is 895-TH. The purity of the sample was not sufficiently characterized, although current information indicates it is a mixture of 96.5 - 100% test substance and 0 – 3.5% C6, C7, and C9 perfluoro analogue compounds.
METHOD
Method: U.S. EPA-TSCA Guideline 797.1050
Test: Acute static
GLP: Yes
Year completed:1996.
Species: *Selenastrum capricornutum*
Source: Originally from The Culture Collection of Algae at the University of Texas at Austin, maintained in culture medium at T.R. Wilbury, Inc., Marblehead, MA.
Element basis: Algal cell counts (cells/ml), and specific growth rates.
Exposure period: 96-hours
Statistical methods: Cell densities, growth rates and percent inhibition values used to estimate the EC10, EC50, and EC90 values and 95% confidence limits were calculated using the computer software of C.E. Stephan. The no observed effect concentration (NOEC) was calculated using one-way analysis of variance (ANOVA).
Analytical monitoring: pH and temperature
Test Conditions:
Algal nutrient medium: U.S. EPA-recommended sterile enriched medium, prepared by spiking deionized water with nutrient stocks. The pH of the synthetic algal medium at test initiation was 7.5.
Stock and test solutions preparation: A 1,000 mg/L primary stock solution was prepared in sterile enriched media. Appropriate amounts of this stock solution were added directly to dilution water to formulate the test media.
Exposure vessels: 250 mL glass Erlenmeyer flasks containing 50 mL of test solution.
Agitation: Shaken continuously at 100 rpm
Number of replicates: 3
Initial algal cell loading: 1.0 X 10 4 cells/mL
Number of concentrations: Five plus a negative control
Lighting: ~400 ft-c from continuous cool-white fluorescent lighting
Water chemistry:
pH range: (0 – 96 hours)
7.5 – 9.6 (control exposure)
5.4 – 7.4 (1,000 mg/L exposure)

Test temperature range: (0 – 96 hours)
23.5 – 23.7°C
RESULTS
Nominal concentrations: Bk control, 62, 130, 250, 500, 1,000 mg/L.
Element value and 95% confidence interval:
72-hour EC10 (cell density) = 310 (110 – 440) mg/L
72-hour ErC10 (growth rate) = 470 (380 - 550) mg/L
72-hour EC50 (cell density) = 520 (250 – 1,000) mg/L
72-hour ErC50 (growth rate) = >1,000 mg/L (C.I. not calculable)
72-hour EC90 (cell density) = >1,000 mg/L (C.I. not calculable)
72-hour ErC90 (growth rate) = >1,000 mg/L (C.I. not calculable)
96-hour EC10 (cell density) = 97 (77 – 120) mg/L
96-hour ErC10 (growth rate) = 220 (160 - 280) mg/L
96-hour EC50 (cell density) = 310 (280 - 350) mg/L
96-hour ErC50 (growth rate) = >1,000 mg/L (C.I. not calculable)
96-hour EC90 (cell density) = 1,000 (830 - >1,000) mg/L
96-hour ErC90 (growth rate) = >1,000 mg/L (C.I. not calculable)
96-hour NOEC (cell density): 62 mg/L
96-hour NOEC (growth rate): 500 mg/L
Element values based on nominal concentrations

Biological observations after 96-hours:

Nominal Concentration, mg/L	Mean Number of Cells per mL	Percent Inhibition via Density	Percent Inhibition via Growth Rate
Control	1,407,000	-	-
62	1,445,000	-3	0
130	1,129,000	20	6
250	753,000	46	13
500	443,000	69	25
1,000	181,000	87	42

Control response: Satisfactory
Observations: Algal cell counts in each test vessel were determined by means of direct microscope counts with a hemocytometer. After 96 hours of exposure, there were no signs of aggregation, flocculation or adherence of the algae to the flasks in the control or any test treatment group. In addition, there were no noticeable changes in cell size, color or morphology when compared to the control.
Reversibility of Growth Inhibition: Effect of the test substance was determined to be algistatic based on the results of the post-definitive test exposure.
CONCLUSIONS
The test sample 96-hour EC50 and 95% confidence interval for *Selenastrum capricornutum* was determined using two calculation methods. By cell density, it was 310 (280 - 350) mg/L, and by growth rate

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>1,000 mg/L. The 96-hour NOEC was determined to be 62 mg/L using cell density and 500 mg/L using growth rate. No signs of aggregation, flocculation, or adherence were noted in any of the test solutions. This test substance was determined to be algistatic.
Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133.
DATA QUALITY
Reliability: Klimisch ranking = 2. The study lacks analytical measurement of test substance concentrations in the test solutions and the sample purity is not sufficiently characterized.
REFERENCES
This study was conducted at T.R. Wilbury Laboratories, Inc., Marblehead, MA, at the request of the 3M Company, Lab Request number N2803-4.
OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Ecotoxicity Study

Title: Multi-Phase Exposure/Recovery Algal Assay Test Method

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)

Remarks: The 3M production lot number was 37. The test sample was FC-143. The purity was not completely characterized, although information indicates it is a mixture of 96.5-100 percent test substance and 0-3.5% C_6, C_7, and C_9 perfluoro analogue compounds. The chemical is soluble in water at ambient room temperature.

METHOD

Method/guideline followed: Modified from and modeled after ASTM-E-35.23 Draft No. 2, OECD; A.G. Payne. (as described in USEPA 600/9-78-018)

Test type: Static

GLP (Y/N): No

Year study performed: 1981

Species: *Selenastrum capricornutum* (7 day old stock)

Supplier: USEPA – ERL in Corvallis, Oregon.

Measure of growth used: biomass in cell dry-weight (mg/L); cell count (no./mL)

Concentrations used: Range-finding: 0, 100, 250, 500, 750, 1000, 1500 mg/L;
Main study: 0, 100, 180, 320, 560, 1000, 1800 mg/L (nominal values).

Exposure period: 4, 7, 10, and 14 days

Analytical monitoring: There was no information on the measurement of the chemical during the test. There was no information on detection limits of the chemical or impurities.

Statistical methods: EC50 values and 95% confidence limits were calculated using the linear regression model 3M Sixcur

000244

Test conditions:
- The algal culture was stored in the dark at 4C before use. The initial algal cell count in the stock culture was 277,000 cells/mL.
- Mineral (inorganic) standard nutrient medium was used for culturing/testing algae. This was prepared with all mineral nutrients essential for algal growth. The pH was adjusted to 7.5 $\pm$ 0.1 prior to use in the assays. This nutrient medium was the diluent for all operations that used algae including the preparation of stock solutions.
- Exposure vessels were 250 mL Erlenmeyer flasks with 50 mL of test solution and stoppered with autoclaved foam plugs.
- During the test, the temperature was 23 $\pm$ 2C. The light was by fluorescent illumination of 400 ft candles $\pm$ 10%, and cultures were agitated with a continuous shaking platform at 100 rpm.
- Initial algal loading was 1.0 x 10^4 cells/mL
- Algal recovery response was evaluated following the exposure periods.
- There was no information on dilution water source, contaminants, or chemistry of the water.
-Three replicates were taken at each dose.

Remarks: pH values were acceptable according to OPPTS Harmonized Guidelines. Water hardness was not presented.

RESULTS

Dose associated with each endpoint (as mg/L):

EC50s

Number of Days Exposed	Cell Dry Weight (mg/L)	Cell-Count (no.cells/mL)
4	149 (57-340)[1]	49 (28-75)
7	70 (34-118)	30 (21-40)
10	49 (15-96)	27 (8-50)
14	73 (25-147)	43 (14-81)

[1]95% Confidence Limits

EC10s

Number of Days Exposed	Cell-Count (no. cells/mL)
4	5.3 (3-7)[1]
7	3.3 (2-4)
10	2.9 (1-5)
14	5 (2-8)

[1]95% Confidence Limits

EC90s

Number of Days Exposed	Cell-Count (no. cells/mL)
4	624 (C.I. not calculated)[1]

000245

7	283 (150-590)
10	386 (C.I. not calculated)
14	307 (C.I. not calculated)

[1]95% Confidence Limits

Was control response satisfactory (yes/no/unknown): unknown

Statistical results, as appropriate: No p-values were reported

Remarks: After exposure ceased, the algal cells recovered and resumed logarithmic growth when resuspended in fresh nutrient medium in the absence of the test substance.

CONCLUSIONS

Ammonium perfluorooctanoate exhibits a 14-day EC50 (cell count) value of 43 mg/L with a 95% confidence interval of 14 to 81 mg/L.

Submitters' remarks: The authors indicate a Klimisch ranking of 2. The study meets the criteria for quality testing at the time it was conducted. However, the study lacked information on test substance purity and actual measurements of the test substance in solution.

REFERENCE

Elnabarawy, M.T. 1981. 3M Technical Report Summary, Multi-Phase Exposure/Recovery Algal Assay Test Method. Report Number 006. Project Number 9970030000. October 16.

OTHER

General remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.
The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations. As it is we are operating in the dark on this issue.

INVERTEBRATE TOXICITY

Title: Acute Toxicity to Aquatic Invertebrates (Summary of three 48-hour studies in diet)

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; also referred to as PFOA ammonium salt, ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS# 3825-26-1)

Remarks: The 3M product lot number used was 37. The test sample was FC-143. It's purity was not completely characterized, although information indicated it was a mixture of 96.5-100% test substance and 0-3.5% C6, C7, and C9 perfluoro-analogue compounds.

METHODS

Method/guideline followed: **Not stated**

Test type: **Static**

GLP (Y/N): No

Year study performed: 1982

Species: *Daphnia magna*
age = First-Instar young
length = 0.5 – 1.5 mm

Supplier: USEPA-ERL Duluth, MN

Concentrations tested: 0, 10, 30, 100, 300, 1000 mg/L in two tests; 0, 10, 100, 500, 1000 in third study; nominal concentrations

Exposure period: 48 hours

Analytical monitoring: No monitoring of the test substance concentrations was done

Statistical methods: Chi-square test was used

Test conditions:
-Dilution water used was carbon-filtered well water (all three tests), with a temperature of 24C in two tests (the temp. in the third test was not given).
-Test solutions were made from a common stock solution with a concentration of 5g/L.
-Two replicates were taken

000247

-Number of *Daphnia* were 10-20 per replicate
-Exposure vessels were 250 mL glass beakers containing 200 mL test solution (6 cm in depth)
-Water chemistry during the test was as follows:
Temp ranged from 22-24C
pH was 8.7 for the tests in which it was reported (measured at 48 hours)
DO was 8.2-8.5 ppm for tests in which it was reported and for which it could be read (measured at 48 hours)

Remarks: pH and hardness measurements were not presented for dilution water; hardness during the test was not presented. Information on diet was also not presented.

RESULTS

Dose of each endpoint (as mg/L): 48-hour EC50 ranged from 126 mg/L (with C.I. 86 to 183 mg/L) to > 1000 mg/L

Remarks:
- Lowest test substance concentration causing 100% mortality was 500 mg/L (from both replicates of a single test)
- Mortality of controls was 10% in one replicate of one test
- Abnormal responses included marked reduction in locomotion at 1000 mg/L in one test

Was control response satisfactory (yes/no/unknown): It appears that the control response was satisfactory, given the information provided.

Statistical results, as appropriate: Some statistically significant results are presented (p values associated with Chi-square tests), but it is unclear what they refer to.

CONCLUSIONS

The test sample results (i.e., the large range in EC50 values) were inconsistent. The authors suggest that this difference may be due to differences in diet.

Submitters' remarks: The Klimisch ranking for the study was 3. Several reasons were indicated, including: the method was not described, sample purity was not properly characterized and lacked analytical confirmation of test substance concentrations. They also note the values from multiple tests contradict each other. The authors also note that they conducted the studies specifically to evaluate diet.

Reviewers' remarks: Although the authors indicate that diet may be the cause of the differences in EC50s, no information on diets is presented.

REFERENCE

Not enough information except what submitters include in their reference section, which states: "These studies were conducted by the 3M Company, Environmental Laboratory, St. Paul, MN, completed from May to June, 1982"

OTHER

General remarks: This summary was based on a report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

000249

INVERTEBRATE TOXICITY

Title: Chronic Toxicity to Freshwater Invertebrates (*Daphnia magna*)

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; also referred to as PFOA ammonium salt, ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS# 3825-26-1)

Remarks: The 3M product lot number used was 264. The test sample was FC-143. It's purity was not completely characterized, although information indicated it was a mixture of 96.5-100% test substance and 0-3.5% C6, C7, and C9 perfluoro analogue compounds.

METHODS

Method/guideline followed: USEPA-1982, OECD 1981

Test type: Semi-static life-cycle toxicity

GLP (Y/N): No

Year study performed: 1984

Species: *Daphnia magna*
Age = < 24-hour neonates

Supplier: 3M Environmental Laboratory St. Paul, MN

Concentrations tested: Acute test: 25, 40, 63, 100, 160, 250, 400, 630 mg/L
Chronic test: 0, 5, 8, 13, 22, 36, and 60 mg/L, nominal concentrations.
(Results, however, were based on mean measured concentrations – see below.)

Exposure period: 21 days

Analytical monitoring: Not stated

Statistical methods: Acute test: Probit analysis. Chronic test: moving average angle method. Ascl Corp, Duluth, MN recalculated statistics in 1998. NOECs and LOECs were generated using Toxstat. Reproduction was normal and homogenous therefore Dunnett's Test was used. Fisher's Test was used for survival. IC50s (for reproduction) were generated using ICp program. Survival EC50s were generated using 'Trimmed Spearman-Karber'.

000250

Test conditions:

-Dilution water source was aerated carbon-filtered well water
-Dilution water chemistry was:
hardness: 240 mg/L as CaCO3
alkalinity: 230 mg/L as CaCO3
pH: 7.8
COD: <0.4 mg/L

-Media renewal information/rationale: medium was changed once every two days
-Exposure vessels were 250 ml glass beakers (containing 200 mL of solution to a depth of about 5 cm; 1 animal per 40 ml, no aeration during the test)
-Ambient laboratory lighting was used (cool-white fluorescent, at ambient levels, 16 hours per day)
-Four replicate beakers were used
-There were 20 animals per concentration (5 per replicate)
-Water temperature during the test was 22 $\pm$ 2C
-A suspension of fish food and yeast containing 5 mg dry solids per 1 ml mixture was fed on a daily basis

Remarks: pH and hardness measurements of water during the test were not presented. Also, the OPPTS harmonized guidelines state that hardness should be a maximum of 180 mg/L as CaCO3 during the test; if chemistry prior to the test was indicative of during the test, the hardness value of 240 mg/L of the dilution water was too high.

RESULTS

Dose of each endpoint (as mg/L)*:

14 day NOEC (survival) = 60 mg/L
14 day NOEC (reproduction) = 8 mg/L
21 day NOEC (survival) = 22 mg/L
21 day NOEC (reproduction) = 22 mg/L
14 day IC50 (reproduction) = 40 (28-48) mg/L
21 day IC50 (reproduction) = 43 (35-46) mg/L

***All element concentrations were based on mean measured concentrations and the reported endpoint values are from the reanalysis of the data by Ascl Corp.**

Remar ks:

- All surviving first generation daphnids appeared normal at the end of the test. In the 36 and 60 mg/L treatments, survival was statistically different from the negative control group.
- Neonates started to be produced on Day 7. Reproduction was statistically significantly different from the control (Dunnett's) at the 13, 22, 36, and 60 mg/L concentrations after 14 days, and in the 36 and 60 mg/L test solutions at 21 days.
- 100% mortality was not observed at any dose
- No mortality was seen in the controls

000251

Was control response satisfactory (yes/no/unknown): Yes, based on the fact that there was no mortality.

Statistical results, as appropriate: It appears some results are statistically significant, based on computer printouts. However, lack of information made these difficult to interpret.

CONCLUSIONS

There were no adverse effects on survival or reproduction at concentrations $\leq$ 22 mg/L for 21 days.

Submitters' remarks: The Klimisch ranking was 3. The study was apparently well conducted according to the methodology available at the time of the start of the test.

Reviewers' remarks: none

REFERENCE

3M Company. Chronic toxicity to freshwater invertebrates. [No other information available]

OTHER

General remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

Also, an acute test was conducted, possibly as a range-finding study. However, the purpose of the acute test was not very clear from the study.

The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

INVERTEBRATE TOXICITY

Title: Acute toxicity to aquatic invertebrates (*Daphnia magna*)

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; also referred to as PFOA ammonium salt, ammonium perfluorooctanoate, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS# 3825-26-1)

Remarks: The 3M production lot number was 390. The test sample was FC-126, a white powdery solid. Its purity ws not sufficiently characterized, although current information indicates it is a mixture of 78-93% test substance and 7-22% C5, C6, and C7 perfluoro analogue compounds.

METHODS

Method/guideline followed: Not stated

Test type: Static

GLP (Y/N): No

Year study performed: 1987

Species: *Daphnia magna*
Age = < 24 hour neonates

Supplier: 3M Environmental Labs

Concentrations tested: 0, 100, 180, 320, 560, and 1000 mg/L, nominal concentrations

Exposure period: 48 hours

Analytical monitoring: None because concentrations were nominal.

Statistical methods: Probit analysis

Test conditions:

-Dilution water source was carbon-filtered well water with the following chemical characteristics:
Temp was 21C
DO was 9.4 ppm
pH was 7.9

-Test solutions were prepared by direct weights addition
-Stability of the test chemical solution was not noted

-Exposure vessel was a 250 mL Pyrex glass beaker containing 200 mL test solution (to a 6 cm depth)
-Number of daphnids per replicate was 10
-Water chemistry during test:

DO
control: 9.0 mg/L
1000 mg/L exposure: 8.8 mg/L
pH
control: 8.0
1000 mg/L: 8.1

Temp
21C

Remarks: No other details of the test conditions were presented, including water hardness.

RESULTS

Dose of each endpoint (as mg/L): 48-hour EC50 was 221 mg/L (95% CI: 186-261)

Remarks:
- Lowest test substance concentration causing 100% mortality was 560 mg/L at 48 hours (both replicates)
- There was no mortality in the controls

Was control response satisfactory (yes/no/unknown): Yes, based on the fact that there was no mortality

Statistical results, as appropriate: Not presented

CONCLUSIONS

The 48-hour EC50 was determined to be 221 mg/L (with a 95 % C.I. of 186-261)

Submitters' remarks: The data quality Klimisch ranking was 2. Testing met the criteria for quality testing. However, sample purity was not properly characterized and it lacked analytical confirmation of test substance concentrations.

Reviewers' remarks: none

REFERENCE

3M Company. 1987. [Report title not given.] Lab Request Number E1282-1. Completed on April 30.

OTHER

General remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

000255

TOXICITY TO INVERTEBRATES

Title: Static Acute Toxicity of FX-1003 to the Daphnid, *Daphnia magna*

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOS 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 3M production lot number was 2327. The purity of the test sample, FX-1003, was not sufficiently characterized, though available information indicated it was a solution of <45% ammonium perfluorooctanoate, 50% water, <3% inert perfluorinated compound, and 1 – 2% C_5 and C_7 perfluoro-analogue compounds.

METHODS

Method/guideline followed: OECD 202

Test type: Static

GLP (Y/N): Yes

Year study performed: 1990

Species: Daphnids used in the test were less than 24 hours old at the start of the test. They were produced from an in-house culture that was maintained under test conditions for at least 14 days. Prior to testing, daphnids were maintained in 100% dilution water (collected from wells at EnviroSystems in Hampton, New Hampshire) under static conditions. During acclimation, daphnids were not treated for disease and were free of apparent sickness, injuries, and abnormalities at the beginning of the test. Daphnids were fed yeast, trout chow, and the freshwater alga *Selenastrum capricornutum* once daily before the test. Daphnids were not fed during the test.

Supplier: EnviroSystems-daphnids, 3M-test substance

Concentrations tested: A screening test was performed prior to the definitive toxicity test. Nominal concentrations of the test substance were 0.1, 1, 100 and 1000 mg/L; the number of replicates was not indicated. For the definitive test, four replicates of each of the following test substance concentrations were used: 0 (blank control), 150, 250, 400, 600, and 1000 mg/L.

Exposure period: 48 hours

Analytical monitoring: Test substance concentrations were not measured during the study. Dissolved oxygen, pH, conductivity, and temperature were measured and recorded daily in each test chamber containing live test animals.

000256

Statistical methods: **Results of the toxicity test were interpreted by standard statistical techniques (Stephan, 1983). The probit, moving average, or linear interpolation method was used to calculate the 48-hour EC_{50} (based on immobilization) using nominal concentrations of the test substance.**

Test conditions: Water used for acclimation of the test organisms, and for all toxicity testing, was collected from wells at EnviroSystems in Hampton, New Hampshire. Water was adjusted to a hardness of 180 mg/L as $CaCO_3$ and aerated in 500-gallon polyethylene tanks prior to test initiation. During the acclimation period (24-hours prior to the test initiation), the dilution water temperature was 19.8 C. No stock solution was prepared, as the test substance was added directly to dilution water contained in the test vessels without the use of a solvent. The stability of the test solutions was not indicated. Nominal concentrations of the test substance were 0 (blank control), 150, 250, 400, 600, and 1000 mg/L. Four replicates of each test concentration were used. Twenty daphnids were randomly and equally distributed among four replicates of each treatment. Exposure vessels were 250 mL glass beakers containing 200 mL of the test solution (approximately 7 cm depth). Test vessels were randomly arranged in an incubator during the 48-hour test. During the test, the following water chemistry ranges (0 – 48 hours) were determined: Conductivity 800 – 900 mhos/cm (control exposure and 1000 mg/L exposure); pH 8.1 – 8.2 (control exposure), 8.3 – 8.4 (1000 mg/L exposure); Temperature 19.5 – 20.1 C (control exposure and 1000 mg/L exposure); Dissolved O_2 9.0 – 9.5 mg/L (control exposure), 9.1 – 9.5 mg/L (1000 mg/L exposure). Aeration was not required during the study to maintain dissolved oxygen concentrations above acceptable levels. A 16-hour light and 8-hour dark photoperiod was automatically maintained with cool-white fluorescent lights that provided an intensity of 45 $Es^{-1}m^{-2}$. Loading rate during the toxicity test was approximately 0.018 g/L.

Remarks: No additional comments

RESULTS

Dose of each endpoint (as mg/L):

Screening test: LC_{40} = 1000 mg/L, NOEC = 100 mg/L

Definitive test (based on immobilization): 24-hour EC_{50} >1000 mg/L, 48-hour EC_{50} = 584 mg/L (95% confidence level = 400 – 1000 mg/L)

Remarks: For the screening test, after 48 hours of exposure, there was 60% survival at 1000 mg/L and 100% survival at all other tested concentrations. For the definitive test, all test vessels maintained a clear appearance throughout the study. 100% survival occurred in the control exposure. Control daphnids had an average wet weight (blotted dry) of 0.0007 g at the end of the test. Loading rate during the toxicity test was approximately 0.018 g/L. Exposure of daphnids to the reference toxicant, sodium dodecyl sulfate, resulted in a 48-hour LC_{50} of 33 mg/L and a 48-hour EC_{50} of 16 mg/L.

Was control response satisfactory (yes/no/unknown): yes

Statistical results, as appropriate: No additional comments

CONCLUSIONS

The test sample 48-hour EC50 for *Daphnia magna* was determined to be 584 mg/L, with a 95% confidence interval of 400 – 1000 mg/L.

000257

Submitters' remarks: Klimisch ranking 2. Testing meets the criteria for quality testing. However, sample purity was not properly characterized and no attempt was made to confirm test substance concentrations.

Reviewers' remarks: The submitters' conclusions appear to be accurate, based on the data.

REFERENCE

EnviroSystems, Inc. 1990. Static Acute Toxicity of FX-1003 to the Daphnid, *Daphnia magna.* Hampton, NH. EnviroSystems study number 9013-3.

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Purity of the test material also is a major concern and was not sufficiently characterized in this test. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. In fact, 3M in their summary of each test state: *"Data may not accurately relate toxicity of the test sample with that of the test substance."* I agree with this concern. In addition, if this was a "typical" TSCA section 4 review, I would reject these studies, pending receipt of additional information on purity and studies on analytical measurements of the test chemical in the test medium.

AQUATIC PLANTS TOXICITY

Title: Growth and Reproduction Toxicity Test with N2803-3 and the Freshwater Alga, *Selenastrum capricornutum*

TEST SUBSTANCE

Identity: Perfluorooctanoic acid; may also be referred to as PFOA, FC-26, or FX-1001. (Octanoic acid, pentadecafluoro-, CASRN 335-67-1)

Remarks: The 3M production lot number was 269. The test sample was FC-26, referred to by the test laboratory as N2803-3. The purity of the sample was not sufficiently characterized, although current information indicated it was a mixture of 96.5 – 100% test substance and 0 – 3.5% C_6, C_7, and C_9 perfluoro-homologue compounds. The test sample was a white powder. As stated by the submitter, the sample preparation directions given to the laboratory were to dissolve the test material in a 50:50 water:isopropanol solution. In the protocol amendment, it was stated that the sample was combined with isopropanol in a 50:50 ratio prior to use.

METHODS

Method/guideline followed: USEPA-TSCA Guideline 797.1050

Test type: Static

GLP (Y/N): Y

Year study performed: 1995

Species: The algae were acclimated in sterile, enriched media and maintained at test conditions for at least 14 days prior to the definitive test. The sub-sample of algae used to inoculate media at the start of the definitive test was from a 10-day old culture.

Supplier: The supplier of the algae culture was the Culture Collection of Algae at the U of Texas at Austin.

Measure of growth used: number of cells/mL, growth rate

Concentrations tested: For the range-finding test, the following concentrations of test substance were used: 0.050, 0.50, 5.0, and 50 mg/L (the number of replicates was not specified). For the definitive test, one dilution water control and five nominal concentrations were used in the study. The 50:50 mixture of isopropanol and test substance (N2803-3) was considered to be 100% test substance during the performance of the toxicity test. All results were reported both on the basis of test substance as-tested (50% N2803-3 and 50% isopropanol) and test substance as-received (N2803-3 without isopropanol). The as-tested concentrations were: 0, 63, 125, 250, 500, and 1000 mg/L; the as-received concentrations were: 0, 32, 63, 130, 250, and 500 mg/L. Three replicates of each control and test concentration were utilized under static test conditions.

Exposure period: 96 hours

000259

Analytical monitoring: Concentrations of the test substance were not measured during the study. The pH in each test vessel was measured at the beginning and end of the test. Incubator temperature was measured and recorded daily. The temperature in a representative vessel of water, which was incubated with the test vessels, was continuously recorded.

Statistical methods: Cell densities, growth rate, and percent inhibition values, which were used to estimate 72- and 96-hour EC_{50} values and 95% confidence limits, were calculated using the binomial/interpolation method (Stephan, 1984). All calculations were performed using the number of cells/mL, the average specific growth rates, and the nominal concentrations of the test substance. The no-observed-effect-concentration (NOEC) was calculated using a parametric one-way analysis of variance (ANOVA), the number of cells/mL, and the average specific growth rate in each test vessel at the end of the test.

Test conditions: The algal medium was prepared according to USEPA recommended concentrations by combining de-ionized water and nutrient stocks. Water used for the acclimation of the test organisms and for all toxicity testing was sterile, enriched media, adjusted to a target pH of 7.5 with 0.1 M HCl prior to use (the pH of the synthetic algal medium at test initiation was 7.4). Algal medium was used for culturing and as the diluent. A chemical characterization of a representative sample of test media, and water used to formulate test media, was performed. Phosphorous, nitrate, and chloride were detected at the following concentrations: 0.46, 0.08, and 14 mg/L, respectively. Heavy metals detected in the diluent and test medium included cadmium (0.0002 mg/L) and lead (0.08 mg/L). Other potential contaminants were at or below the level of detection. The test substance (as-received) was assumed to have a purity of 100% active ingredient and to be stable under storage and testing conditions. A 1000 mg/L primary stock solution of the test substance (as-tested) was prepared by combining N2803-3 (as-tested) and sterile, enriched media. Appropriate amounts of this stock solution were added directly to dilution water to formulate the test media. Algae were distributed among three replicates of each treatment at the rate of 10,000 cells/mL. Exposure vessels consisted of 250 mL glass Erlenmeyer flasks containing 50 mL of test solution. Test vessels were randomly arranged on a rotary shaker adjusted to 100 rpm in an incubator during the test. The pH of the test solutions for the 250, 500, and 1000 mg/L exposure concentrations were in the range of 2.9-4.0 at test initiation. As stated by the submitter, this low pH may have adversely affected the survival and subsequent growth of the algae. The measured water chemistry values (0-96 hours) were as follows: pH range = 7.4 – 10.3 (control exposure), 2.9 – 3.0 (1000 mg/L exposure), mean temperature range = 23.5 - 24 C. A 24-hour light and 0-hour dark photoperiod was automatically maintained with cool-white fluorescent lights that provided a light intensity of approximately 380 footcandles. Algal cell counts in each test vessel were determined daily by means of direct microscope counts with a hemocytometer.

Remarks: Water hardness was not indicated. The pH for many exposure concentrations was outside the accepted range for *S. capricornutum* toxicity testing (7.5 ± 0.1). As stated by the

submitter, there appears to be a discrepancy between the sample preparation directions given to the laboratory and the procedure conducted by the laboratory to prepare the test solutions.

RESULTS

Dose of each endpoint (as mg/L): The NOEC for the range-finding study was 50 mg/L. For the definitive study, the 96-hour EC_{50} (and associated 95% confidence limits) was 180 mg/L (125 -250 mg/L), based on test substance as-tested (50% N2803-3 and 50% isopropanol) and 90 mg/L (63 - 130

000260

mg/L), based on test substance as-received. The 96-hour NOEC and LOEC values were 125 and 250 mg/L, respectively, based on test substance as-tested. The 96-hour NOEC and LOEC values were 63 and 130 mg/L, respectively, based on the test substance as-received. The cell growth data is presented are table 1.

Table 1: Cell growth data of acute toxicity test with N2803-3 and the freshwater algae, *Selenastrum capricornutum*

Nominal concentration (as-tested), mg/L	**Number of cells/mL x 10^3 (hour)** Hours					**Percent Inhibition via Density**	**Percent Inhibition via Growth Rate**
	0	**24**	**48**	**72**	**96**		
Control	10	21	156	424	1,671	-	-
63	10	24	149	487	1,732	-4	-2
125	10	21	109	513	1,560	7	0
250	10	<10	<10	<10	<10	100	100
500	10	<10	<10	<10	<10	100	100
1,000	10	<10	<10	<10	<10	100	100

Remarks: After 96 hours of exposure, there were no signs of aggregation, flocculation, or adherence of the algae to the flasks in the control or any test treatment group. In addition, there were no noticeable changes in cell size, color, or morphology when compared to the control. The effect of N2803-3 was determined to be algistatic, not algicidal, based on the results of the post-definitive test exposure. As stated by the submitter, biological data generated by a previous exposure of algae to isopropyl alcohol (Ward, et al. 1995) demonstrated that 1000 mg/L of isopropyl alcohol did not inhibit growth. This finding indicated that the concentration of isopropyl alcohol in the test chemical did not account for the toxicity of N2803-3. Test substance concentrations above 125 mg/L reduced pH values to 4.0 and lower; the low pH may have caused inhibition of algae growth at higher concentrations of test substance. The lowest concentration of test substance observed to cause 100% mortality was 250 mg/L.

Was control response satisfactory (yes/no/unknown): yes

Statistical results, as appropriate: No additional comments

CONCLUSIONS

The NOEC for the range-finding study was 50 mg/L. For the definitive study, the 96-hour EC_{50} was 180 mg/L, based on test substance as-tested, and 90 mg/L, based on test substance as-received. The 96-hour NOEC and LOEC values were 125 and 250 mg/L (as-tested) and 63 and 130 mg/L (as-received), respectively.

Submitters' remarks: Klimich ranking 3. The study lacks analytical measurement of test substance concentrations in the test solutions. Sample purity is not sufficiently characterized. Additionally, there appears to be a discrepancy between the sample preparation directions given to the laboratory and the

procedure conducted by the laboratory to prepare the test solutions. Survival and growth of the algae may have been adversely affected by initial low pH values in the higher test substance concentrations.

Reviewers' remarks: The conclusions appear to be supported by the data. The low pH, which may have further increased growth inhibition, was clearly stated as a possible source of error.

REFERENCE

T.R. Wilbury Laboratories, Inc. 1995. Growth and Reproduction Toxicity Test with N2803-3 and the Freshwater Alga, *Selenastrum capricornutum*. Marblehead, MA. Study number B93-TH.

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

TOXICITY TO INVERTEBRATES

Title: Acute Toxicity of N2803-3 to the Daphnid, *Daphnia magna*

TEST SUBSTANCE

Identity: Perfluorooctanoic acid; may also be referred to as PFOA, FC-26, or FX-1001. (Octanoic acid, pentadecafluoro-, CASRN 335-67-1)

Remarks: The 3M production lot number was 269. The test substance was a white powder. The test sample, FC-26, was referred to by the test laboratory as N2803-3. The purity of the sample was not sufficiently characterized, although available information indicated it was a mixture of 96.5 – 100% test substance and 0 – 3.5% C_6, C_7, and C_9 perfluoro- homologue compounds.

METHODS

Method/guideline followed: U.S. EPA-TSCA Guideline 797.1300

Test type: Static

GLP (Y/N): Yes, with one exception: the stability of the test substance was assumed, but not verified.

Year study performed: 1996

Species: Water used for acclimation of the test organisms was deionized water collected at T.R. Wilbury Laboratories in Marblehead, MA. Daphnids employed in the study were less than 24 hours old. Test specimens were produced by adult daphnids that were maintained under test conditions for more than 7 days. The original culture was obtained from Aquatic Research Organisms, Hampton, NH. During acclimation, daphnids were not treated for disease, they were free of apparent sickness, injuries, and abnormalities at test initiation. There was no mortality during the 48 hours preceding the start of the test. The culture was supplied with a yeast/trout chow mix and the freshwater alga *Selenastrum capricornutum* daily during acclimation. Daphnids were not fed during the test.

Supplier: T.R. Wilbury Laboratories supplied the test organisms. 3M, the sponsor, supplied the test substance.

Concentrations tested: For the static screening test, the nominal concentrations of the test substance (as-received) were: 0.050, 0.50, 5.0, 50, and 500 mg/L. For the static definitive test, two replicates of each concentration were used. The following nominal concentrations were utilized: 0 (blank control), 130, 200, 360, 600, and 1000 mg/L (tested as a 50:50 mixture of test substance and isopropanol). The nominal concentration of test substance (as-received) in solution was 0 (blank control), 65, 110, 180, 300, and 500 mg/L.

000263

Exposure period: 48 hours

Analytical monitoring: Test substance concentrations were not measured during the study. All toxicity tests were based on nominal concentrations of test substance. Dissolved oxygen, pH, conductivity, and temperature were measured and recorded daily in each test chamber that contained live animals. The temperature in a beaker of water incubated among the test vessels was recorded continuously during the test.

Statistical methods: LC_{50} and EC_{50} values were calculated, when possible, by probit analysis, moving average method, or binomial probability with non-linear interpolation (Stephan, 1983).

Test conditions:

Water used for acclimation of the test organisms and for all toxicity testing was deionized water collected at T.R. Wilbury Laboratories in Marblehead, MA. Water was adjusted to a hardness of 160 – 180 mg/L as $CaCO_3$ and stored in 500-gallon polyethylene tanks, where it was aerated and continuously passed through a particle filter, ultraviolet sterilizer, and activated carbon. A chemical characterization of a representative sample of dilution water detected iron at 0.03 mg/L; all other potential contaminants were below the level of detection or not present. The test substance was prepared by combining N2803-3, as-received from the sponsor, with isopropanol in a 50:50 ratio. However, the test substance preparation directions given to the laboratory were to dissolve the test material in a 50:50 water:isopropanol solution. This 50:50 mixture was then considered to be 100% test substance during the toxicity test, but all results are reported on an active ingredient basis (active ingredient is the test substance as-received minus the isopropanol). The test substance was assumed to have a purity of 100% active ingredient and to be stable under storage and testing conditions. For the test organisms, measurements made during the 7 days prior to the start of the definitive test with N2803-3 indicated a culture temperature range of 19.5 – 20.5 C and a dissolved oxygen concentration of at least 9.1 mg/L. A 1000 mg/L stock solution was prepared by combining 2.0 g of the test substance (50:50 mixture of N2803-3 and isopropanol) and dilution water to a final volume of 2000 mL. The test vessels were 300 mL glass beakers that contained 250 mL of test solution (approximate depth was 9 cm) during the test. Appropriate amounts of the stock solution were added to dilution water in test vessels to formulate test media with the use of a solvent. Twenty daphnids were indiscriminately and equally distributed among two replicates of each test concentration. Test vessels were randomly arranged in an incubator and loosely covered during the 48-hour test. During the definitive test, the following ranges were estimated from measurements: dissolved oxygen = 8.7 – 8.8 mg/L, temperature = 19.4 – 20.7 C, conductivity = 570 – 610 mhos/cm, pH = 8.3 – 8.5. A 16-hour light and 8-hour dark photoperiod with a 15-minute transition period was automatically maintained with cool-white fluorescent lights that provided a light intensity of 58 footcandles. Aeration was not required to maintain dissolved oxygen concentrations above an acceptable level.

A static test was conducted with 390 mg/L isopropanol and a dilution water control. Two replicates of each concentration and 10 daphnids/replicate were used. This test was conducted in a manner similar to the test with N2803-3. Measurements made during the 7 days prior to

initiation of the isopropanol toxicity test. These measurements indicated a culture temperature range of 20.1 – 20.9 and a dissolved oxygen concentration of at least 8.3 mg/L. The test vessels were randomly arranged in a water bath and the light intensity was 13 – 25 footcandles. The following ranges were estimated from measurements during the test: dissolved O_2 = 8.1 – 8.5 mg/L, temperature = 20.4 – 20.9 C, conductivity = 530 – 540 mhos/cm, pH = 8.3 – 8.5.

000264

Remarks: No additional comments

RESULTS

Dose of each endpoint (as mg/L): LC_{50} and EC_{50} values and 95% confidence intervals

Based on test substance as-received:

24-hour LC_{50} = 500 (300 - >500) mg/L
24-hour EC_{50} = 420 (370 – 490) mg/L
48-hour LC_{50} = 400 (350 – 460) mg/L
48-hour EC_{50} = 360 (300 – 500) mg/L
48-hour NOEC = 180 mg/L

Based on test substance as-tested (50:50 ratio of N2803-3:isopropanol):

24-hour LC_{50} = 1000 (600 - >1000) mg/L
24-hour EC_{50} = 840 (740 – 970) mg/L
48-hour LC_{50} = 800 (700 – 920) mg/L
48-hour EC_{50} = 720 (660 – 780) mg/L
48-hour NOEC = 360 mg/L

Remarks: During the toxicity test with 390 mg/L isopropanol, no mortality or sublethal effects were observed and the 48-hour EC_{50} and LC_{50} values were > 390 mg/L. For the screening test (48-hours post-exposure), there was at least 95% survival at 0 (blank control), 0.050, 0.5, and 5.0 mg/L. Also, there was 15% survival at 500 mg/L (surviving daphnids exposed to 500 mg/L were immobilized). During the definitive toxicity test with N2803-3, no insoluble material was noted during the test. After 48 hours of exposure, the control daphnids had an average wet weight (blotted) of 0.53 mg. The test substance (N2803-3) did not cause 100% mortality at any concentration tested. No mortality was observed in the controls during the definitive test or screening test. Table 1 depicts cumulative percent mortality of the definitive test.

Table 1. Cumulative Percent Mortality

Nominal as-tested concentration (mg/L)	24 hours	48 hours
Blank control	0	0
130	0	0
200	0	0
360	0	0
600	10	15
1000	50	80

Was control response satisfactory (yes/no/unknown): Yes

Statistical results, as appropriate: No additional comments

CONCLUSIONS

The as-tested test substance (N2803-3) 48-hour LC50 for *Daphnia magna* was determined to be 800 mg/L with a 95% confidence interval of 700 – 920 mg/L. The as-tested test substance 48-hour EC50 for *Daphnia magna* was determined to be 720 mg/L with a 95% confidence interval of

000265

660 – 780 mg/L. The as-tested test substance 48-hour no-observed-effect-concentration (NOEC) was 360 mg/L. The 48-hour LC50 for *Daphnia magna*, based on the concentration of test substance in solution was determined to be 400 mg/L, with a 95% confidence interval of 350 – 460 mg/L. The 48-hour EC_{50} for *Daphnia magna*, based on the concentration of test substance in solution was determined to be 360 mg/L, with a 95% confidence interval of 300 – 500 mg/L. The 48-hour no- observed-effect-concentration (NOEC) for the test substance in solution was 180 mg/L.

Submitters' remarks: Klimisch ranking 3. The study lacks analytical measurement of test substance concentrations in the test solutions and sample purity is not sufficiently characterized. Additionally, there appears to be a discrepancy between the sample preparation directions given to the laboratory and the procedure conducted by the laboratory to prepare the test solutions.

Reviewers' remarks: The author's conclusions appear to be supported by the data.

REFERENCE

T.R. Wilbury Laboratories, Inc. 1996. Acute Toxicity of N2803-3 to the Daphnid, Daphnia magna. *Marblehead, MA. Study number 892-TH.*

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

AQUATIC PLANTS TOXICITY

Title: Growth and reproduction toxicity test with FC-1015 and the freshwater alga, *Selenastrum capricornutum*

TEST SUBSTANCE

Identity: Perfulorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, FC-143 or as the major component of FC-1015. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS #3825-26-1)

Remarks: The test sample is FC-1015. Its purity was not sufficiently characterized, though current information indicates it is a 30% straight carbon chain version of FC-143 in 80% water. The 3M product lot number was "HOGE 205." Data may not accurately relate toxicity of the test sample with that of the test substance. Data were used to compare toxicity of the branched/straight chain ammonium perfluorooctanoate homolog mixture in FC-143 v. what is supposed to be the 100% straight carbon chain ammonium perfluorooctanoate in FC-1015.

METHODS

Method/guideline followed: OECD 201, USEPA-TSCA, Guideline 797.1050

Test type: static

GLP (Y/N): N

Year study performed: 1996

Species: *Selenastrum capricornutum*

Supplier: University of Texas at Austin

Measure of growth used: algal cell counts (cells/mL), cell dry weights

Concentrations used: 0, 210, 430, 830, 1670, and 3330 mg/L. The concentrations were nominal. Three replicates at each concentration were tested.

Exposure period: 96 hours

Analytical monitoring: none

Statistical methods: Probit analysis

Test Conditions: The algae were grown in sterile enriched nutrient medium per USEPA 1978 guideline and were allowed 14 days for acclimation to the conditions. The nutrient medium provided all mineral nutrients essential for algal growth and also served as the diluent for all algal operations. The pH of this synthetic algal medium was adjusted to 7.5 prior to use in assays. For preparation of the test solution, a 3330 mg/L stock solution was prepared by diluting 3.33 g of test substance in 1 liter of water. Aliquots

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were then added directly to dilution water in test vessels to create test solutions. The exposure vessels used were 250 mL Erlenmeyers containing 100 mL test solution and capped with inverted glass beakers. The vessels were loaded with an initial concentration of 1.0×10^4 cells/mL and shaken continuously at 100 rpm. Four hundred foot-candles of lighting were provided by continuous cool-white fluorescent lighting. The water chemistry parameters measured during the test included: pH = 7.5-10.8 (control) and 7.4-7.6 (3330 mg/L exposure) and temperature = 24.0-24.2 (incubator). Three replicates of the experiment were performed. Measurements of dilution water chemistry were also performed (see Appendix for parameters and detection limits). The only chemical detected was iron at 0.03 mg/L.

Remarks: The pH range for the control was outside acceptable limits, but the other tested sample was within the acceptable range. Water hardness, during the study, was not indicated.

RESULTS

Dose of each endpoint (as mg/L): Values calculated using cell count (cells/mL):
24 h EC50 = 2510 (1340-3330) mg/L
48 h EC50 > 3330 mg/L
72 h EC50 = 2040 (1190-3330) mg/L
96 h EC50 = 1980 (1710-2360) mg/L
96 h NOEC = 210 mg/L
96 h LOEC = 430 mg/L

Values calculated using the average specific growth rate:
24 h EC50 = 1700 (673-3300) mg/L
48 h EC50 > 3330 mg/L
72 h EC50 > 3330 mg/L
96 h EC50 > 3330 mg/L
96 h NOEC = 430 mg/L
96 h LOEC = 830 mg/L

Remarks: Aliquots of the 3330 mg/L test solution were diluted with algal medium and cultured for 72 hours. Based on growth observed in the recovery phase, the effect on algal growth was found to be algistatic.

Was control response satisfactory (yes/no/unknown): yes

Statistical results, as appropriate: none

CONCLUSIONS

FC-1015 exhibits a 96 hour EC50 cell count value of 1980 (1710-2360 mg/L) and a 96 hour EC50 growth rate value of >3330 mg/L. The 96 hour No Observed Effect Concentration (NOEC) is 210 mg/L for cell count and 430 mg/L for growth rate. The Lowest Observed Effect Concentration (LOEC) is 430 mg/L for cell count and 830 mg/L for growth rate. This test substance was determined to be algistatic.

Submitters' remarks: For data reliability, this study was assigned a Klimisch rating of 2. The study meets criteria for quality testing. However, it lacks information on the purity of the test substance and actual measurements of the amount of test substance in solution. Also, no explanation is given as to why the 48 hour values were in excess of 24 hour values.

Reviewers' remarks: none

REFERENCE

Ward, T., Nevius, J. and R. Boeri. 1996. Growth and reproduction toxicity test with FC-1015 and the freshwater alga, *Selenastrum capricornutum*. T.R. Wilbury Laboratories, Inc. Lab Request number P1624. 3M Company, St. Paul, MN.

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Purity of the test material also is a major concern and was not sufficiently characterized in this test. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. In fact, 3M in their summary of each test state: *"Data may not accurately relate toxicity of the test sample with that of the test substance."* I agree with this concern. In addition, if this was a "typical" TSCA section 4 review, I would reject these studies, pending receipt of additional information on purity and studies on analytical measurements of the test chemical in the test medium.

APPENDIX

Chemical measurements of dilution water

Parameter:	Detection Limit:
Metals	
Aluminum	0.1 mg/L
Arsenic	0.01 mg/L
Boron	0.5 mg/L
Cadmium	0.0002 mg/L
Chromium	0.01 mg/L
Cobalt	0.03 mg/L
Copper	0.005 mg/L
Iron	0.03 mg/L
Lead	0.005 mg/L
Mercury	0.0003 mg/L
Nickel	0.03 mg/L
Silver	0.02 mg/L
Zinc	0.02 mg/L

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Nitrate	0.05 mg N/L
Chloride	1 mg/L
Fluoride	0.1 mg/L
Total organic carbon	1 mg/L
Total phosphorous	0.03 mg/L
Organochlorine Pesticides	0.5 µg/L
Toxaphene	2 µg/L
Organophosphorous Pesticides	0.5 µg/L
Dimethoate	2.0 µg/L
TEPP	2.0 µg/L
Monocrotophos	2.0 µg/L
PCBs	0.5 µg/L

000270

INVERTEBRATE TOXICITY

Title: Acute toxicity of FC-1015 to Daphnid, *Daphnia magna*

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, FC-143 or as the major component of FC-1015. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS #3825-26-1)

Remarks: The test sample is FC-1015. Its purity was not sufficiently characterized, though current information indicates it is a 30% straight carbon chain version of FC-143 in 80% water. The 3M product lot number was "HOGE 205."

METHODS

Method/guideline followed: USEPA-TSCA, Guideline 797.1300

Test type: static

GLP (Y/N): N

Year study performed: 1996

Species: *Daphnia magna*, less than 24 hours old, wet weight = 0.35 mg.

Supplier: initial brood stock from Aquatic Biosystems

Concentrations used: 0, 430, 730, 1200, 2000, and 3300 mg/L. The concentrations were nominal. Two replicates at each concentration were tested.

Exposure period: 48 hours

Analytical monitoring: none

Statistical methods: Probit analysis

Test Conditions: The deionized dilution water used in the test was adjusted to a hardness of 160-180 mg/L (as $CaCO_3$), which is within the acceptable range. The water was passed through a particulate filter, ultraviolet sterilizer, and activated carbon. Water used for the test had a hardness of 176 mg/L and an alkalinity of 108 mg/L as $CaCO_3$, and it contained <0.01 mg/L particulate matter and <1 mg/L total organic carbon. Further measurements of dilution water chemistry were also performed (see Appendix for parameters and detection limits). The only chemical detected was iron at 0.03 mg/L. Test solutions were created by direct weights addition. Glass beakers (300 mL) containing 250 mL test solution (9 cm depth) were used as exposure vessels. They were lightly covered during the experiment. Two replicates, each of 10 daphnids, were tested at each concentration. Cool-white fluorescent lights at 130 foot-candles

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were used for lighting. A daily photoperiod of 16 hours light and 8 hours dark with a 15 minute transition period was maintained throughout the testing period.

The water chemistry parameters measured during the study included: conductivity range = 610-620 µmhos/cm (control) and 710-720 µmhos/cm (2000 mg/L exposure), pH = 8.2-8.3 (control) and 8.1-8.2 (2000 mg/L exposure), temperature = 20.4-20.7 °C (control) and 20.3-20.8 °C (2000 mg/L exposure), and dissolved oxygen = 8.1-9.1 mg/L (control) and 8.1-9.1 mg/L (2000 mg/L exposure). The 2000 mg/L (second highest) concentration was used because the highest concentration resulted in total mortality by 48 hours.

Remarks: Water hardness, during the study, was not indicated.

RESULTS

Dose of each endpoint (as mg/L): 24 hour EC50 = 1790 (1550-2070) mg/L
48 hour EC50 = 1200 (730-2000) mg/L
48 hour NOEC = 730 mg/L

Remarks: none

Was control response satisfactory (yes/no/unknown): yes

Statistical results, as appropriate: none

CONCLUSIONS

The FC-1015 48 hour EC50 was determined to be 1200 mg/L with a 95% confidence interval of 730-2070 mg/L. The 48 hour No Observed Effect Concentration (NOEC) was 730 mg/L.

Submitters' remarks: For data reliability, the study was assigned a Klimisch rating of 2. The study meets the criteria for quality testing. However, the study lacks information on purity of the test substance and actual measurements of the amount of test substance in solution.

Reviewers' remarks: none

REFERENCE

Ward, T., Nevius, J., and R. Boeri. 1996. Acute toxicity of FC-1015 to Daphnid, *Daphnia magna*. T.R. Wilbury Laboratories, Inc. Lab request number P1624. 3M Company, St. Paul, MN.

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis,

volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Purity of the test material also is a major concern and was not sufficiently characterized in this test. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. In fact, 3M in their summary of each test state: *"Data may not accurately relate toxicity of the test sample with that of the test substance."* I agree with this concern. In addition, if this was a "typical" TSCA section 4 review, I would reject these studies, pending receipt of additional information on purity and studies on analytical measurements of the test chemical in the test medium.

APPENDIX

Chemical measurements of dilution water

Parameter:	Detection Limit:
Metals	
Aluminum	0.1 mg/L
Arsenic	0.01 mg/L
Boron	0.5 mg/L
Cadmium	0.0002 mg/L
Chromium	0.01 mg/L
Cobalt	0.03 mg/L
Copper	0.005 mg/L
Iron	0.03 mg/L
Lead	0.005 mg/L
Mercury	0.0003 mg/L
Nickel	0.03 mg/L
Silver	0.02 mg/L
Zinc	0.02 mg/L
Nitrate	0.05 mg N/L
Chloride	1 mg/L
Fluoride	0.1 mg/L
Total organic carbon	1 mg/L
Total phosphorous	0.03 mg/L
Organochlorine Pesticides	0.5 µg/L
Toxaphene	2 µg/L
Organophosphorous Pesticides	0.5 µg/L
Dimethoate	2.0 µg/L
TEPP	2.0 µg/L
Monocrotophos	2.0 µg/L
PCBs	0.5 µg/L

000273

ACUTE TOXICITY TO AQUATIC INVERTEBRATES (DAPHNIA MAGNA)
TEST SUBSTANCE
Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)
Remarks: The 3M production lot number was 427. The test sample is FC-143, referred to by the test laboratory as N2803-4. The T.R. Wilbury study number is 895-TH. The purity of the sample was not sufficiently characterized, although current information indicates it is a mixture of 96.5 - 100% test substance and 0 – 3.5% C6, C7, and C9 perfluoro analogue compounds.
METHOD
Method: U.S. EPA-TSCA Guideline 797.1300
Test type: Acute static
GLP: Yes
Year Completed: 1995
Species: *Daphnia magna*
Supplier: Obtained from cultures maintained by T.R. Wilbury Laboratories Inc, Marblehead, MA from an original culture from Aquatic Research Organisms, Hampton, NH.
Analytical monitoring: DO, conductivity, pH, and temperature
Exposure period: 48-hours
Test organism age: < 24-hours
Statistical methods: Interpreted by standard statistical techniques. Computer methods (Stephan, 1983) were used to calculate LC50s and EC50s.
Test conditions
Dilution water: Deionized water adjusted to a hardness of 160-180 mg/L as CaCO3/L
Dilution water chemistry:
Hardness: 164 mg/L as CaCO3
Alkalinity: 106 mg/L as CaCO3
TOC: < 1.0 mg/L
Residual chlorine: <0.1 mg/L
Lighting: Cool-white fluorescent bulbs with an intensity of 110 ft-c. Photoperiod of 16-hours light, 8 -hours dark with a 15 minute transition period.
Stock and test solutions preparation: A 1,000 mg/L primary stock solution was prepared in dilution water. After mixing, the primary stock was proportionally diluted with dilution water to prepare the test concentrations. No insoluble material was noted during the test.
Exposure vessels: 300 mL glass beakers containing 250 mL of test solution. The approximate depth of test solution was 9 cm.
Number of replicates: 2
Number of daphnids per replicate: 10
Number of concentrations: five plus a negative control

Water chemistry during the study:
Dissolved oxygen range (0 – 48 hours):
8.4 – 8.7 mg/L (control exposure)
8.1 – 8.7 mg/L (1,000 mg/L exposure)
Conductivity range (0 – 48 hours)
540 – 560 µmhos/cm (control exposure)
710 – 720 µmhos/cm (1,000 mg/L exposure)
pH range (0 – 48 hours)
8.0 – 8.3 (control exposure)
8.0 – 8.2 (1,000 mg/L exposure)
Test temperature range (0 – 48 hours)
19.1 – 20.5°C (control exposure)
19.4 – 20.5°C (1,000 mg/L exposure)
RESULTS
Element basis: mortality and immobilization
Nominal concentrations: Blank control, 130, 220, 360, 600, 1,000 mg/L.
Element value and 95% confidence interval:
24-hour EC50 = 780 (600 – 1,000) mg/L
24-hour LC50 = >1,000 mg/L (C.I. not calculable)
48-hour EC50 = 720 (600 – 1,000) mg/L
48-hour LC50 = 720 (600 – 1,000) mg/L
48-hour NOEC = 360 mg/L
Element values based on nominal concentrations.

Cumulative percent immobilization (includes mortality)

Nominal Test Conc., mg/L	24-hours	48-hours
Control	0	0
13	0	0
22	0	0
36	0	0
60	0	15
100	100	100

Note: At 1000 mg/L, all daphnids were immobilized at 24 hours and dead at 48 hours.
Control response: satisfactory
CONCLUSIONS
The test substance 48-hour LC50 for *Daphnia magna* was determined to be 720 mg/L with a 95% confidence interval of 600 – 1,000 mg/L. The test substance 48-hour EC50 for *Daphnia magna* was also determined to be 720 mg/L with a 95% confidence interval of 600 – 1,000 mg/L. The 48-hour no observed effect concentration (NOEC) was 360 mg/L.
Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133
DATA QUALITY
Reliability: Klimisch ranking 2. The study lacks analytical measurement of test substance concentrations in the test solutions and sample purity is not sufficiently characterized.

REFERENCES

This study was conducted at T.R. Wilbury Laboratories, Inc., Marblehead, MA, at the request of the 3M Company, Lab Request number N2803-4

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

000276

Ecotoxicity Study

ACUTE TOXICITY TO AQUATIC INVERTEBRATES (DAPHNIA MAGNA)

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, tetrabutylammonium salt; may also be referred to as PFOA tetrabutylammonium salt, tetrabutylammonium perfluorooctanoate, or as a major component of L -13492. (Octanoic acid, pentadecafluoro-, tetrabutylammonium salt, CAS # 95658-53-0)

Remarks: The 3M production lot number was 2327 The test sample is referred to by the testing laboratory as L -13492. The T.R. Wilbury study number is 840-TH. The 3M Environmental Laboratory Request Number is N2332. The purity of the sample was not sufficiently characterized, although current information indicates it is a solution of 44.9% tetrabutylammonium perfluorooctanoate, 27.9% water, and 27.2% isopropanol

The following summary applies to the test sample as a mixture of the test substance in water solution with incompletely characterized concentrations of impurities. Data may not accurately relate toxicity of the test sample with that of the test substance.

METHOD

Method: U.S. EPA-TSCA Guideline 797.1300

Test type: Acute static

GLP: Yes

Year Completed: 1995

Species: *Daphnia magna*

Supplier: Obtained from cultures maintained by T.R. Wilbury Laboratories Inc, Marblehead, MA from an original culture from Aquatic Research Organisms, Hampton, NH.

Analytical monitoring: DO, conductivity, pH, and temperature were monitored daily.

Exposure period: 48-hours

Test organism age: < 24-hours

Statistical methods: LC50 and EC50 values calculated, when possible, by probit analysis, moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.

Test conditions

Dilution water: Deionized water adjusted to a hardness of 160-180 mg/L as CaCO3/L

Dilution water chemistry: Not given.

BACK TO MAIN.Lighting: Cool-white fluorescent bulbs with an intensity of 20 ft-c. Photoperiod of 16-hours light, 8 -hours dark with a 15 minute transition period.

Stock and test solutions preparation: A 100 mg/L primary stock solution was prepared in dilution water. After mixing, the primary stock was proportionally diluted with dilution water to prepare the test concentrations. No insoluble material was noted during the test.

Exposure vessels: 300 mL glass beakers containing 250 mL of test solution. The approximate depth of test solution was 9 cm.

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The vessels were loosely covered during the test.
Number of replicates: 2
Number of daphnids per replicate: 10
Number of concentrations: five plus a negative control
Water chemistry during the study:
Dissolved oxygen range (0 – 48 hours):
8.5 – 8.7 mg/L (control exposure)
8.5 – 8.7 mg/L (100 mg/L exposure conc.)
Conductivity range (0 – 48 hours)
620 – 630 µmhos/cm (control exposure)
620 – 640 µmhos/cm (100 mg/L exposure conc.)
pH range (0 – 48 hours)
8.4– 8.6 (control exposure)
8.5 - 8.6 (100 mg/L exposure conc.)
Test temperature range (0 – 48 hours)
20.5 – 20.7°C (control exposure)
20.5 – 20.6°C (100 mg/L exposure conc.)
Element basis: mortality and immobilization
RESULTS
Nominal concentrations: Blank control, 13, 22, 36, 60, 100 mg/L.
Element value: 24-hour EC50 = 89 (60 - >100) mg/L
24-hour LC50 = >100 mg/L (CI not calculable)
48-hour EC50 = 34 (30 – 39) mg/L
48-hour LC50 = 77 (60 – 100) mg/L
48-hour NOEC = 13 mg/L
Element values based on nominal concentrations.
Remarks: Testing was conducted on a mixture as described in the Test Substance Remarks field. The values reported apply to that mixture and not the fluorochemical component alone.

Cumulative percent immobilization (includes mortality):

Nominal Test Conc., mg/L	24-hours	48-hours
Control	0	0
13	0	0
22	5	5
36	5	5
60	0	100
100	70	100

Control response: satisfactory
CONCLUSIONS
The test substance 48-hour LC50 for *Daphnia magna* was determined to be 77 mg/L with a 95% confidence interval of 60 - 100 mg/L. The test substance 48-hour EC50 for *Daphnia magna* was determined to be 34 mg/L with a 95% confidence interval of 30 – 39 mg/L. The test substance 48-hour no observed effect concentration (NOEC) was 13 mg/L.
Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St.

Paul, Minnesota, 55133
DATA QUALITY
Reliability: Klimisch ranking 2. The study lacks analytical measurement of test substance concentrations in the test solutions and sample purity is not sufficiently characterized.
REFERENCES
This study was conducted at T.R. Wilbury Laboratories, Inc., Marblehead, MA, at the request of the 3M Company, Lab Request number N2332, 1995.
OTHER

> **General remarks:** The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Purity of the test material also is a major concern and was not sufficiently characterized in this test. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. In fact, 3M in their summary of each test state: *"Data may not accurately relate toxicity of the test sample with that of the test substance."* I agree with this concern. In addition, if this was a "typical" TSCA section 4 review, I would reject these studies, pending receipt of additional information on purity and studies on analytical measurements of the test chemical in the test medium.

000279

ACUTE TOXICITY TO AQUATIC INVERTEBRATES (DAPHNIA MAGNA)
TEST SUBSTANCE
Identity: Perfluorooctanoic acid, tetrabutylammonium salt; may also be referred to as PFOA tetrabutylammonium salt, tetrabutylammonium perfluorooctanoate, N2803-2 or as a major component of L-13492. (Octanoic acid, pentadecafluoro-, tetrabutylammonium salt, CAS # 95658-53-0)
Remarks: The 3M production lot number was 2.
The test sample is referred to by the testing laboratory as N2803-2. The T.R. Wilbury study number is 889-TH. The purity of the sample was not sufficiently characterized, although current information indicates it is a solution of 44.9% tetrabutylammonium perfluorooctanoate, 27.9% water, and 27.2% isopropanol.
The following summary applies to the test sample as a mixture of the test substance in an isopropanol/water solution with incompletely characterized concentrations of impurities. Data may not accurately relate toxicity of the test sample with that of the test substance.
METHOD
Method: U.S. EPA-TSCA Guideline 797.1300
Test type: Acute static
GLP: Yes
Year Completed: 1996.
Species: *Daphnia magna*
Supplier: Obtained from cultures maintained by T.R. Wilbury Laboratories Inc, Marblehead, MA from an original culture from Aquatic Research Organisms, Hampton, NH.
Analytical monitoring: DO, conductivity, pH, and temperature were monitored daily.
Exposure Period: 48-hours
Test organism age: < 24-hours
Statistical methods: LC50 and EC50 values calculated, when possible, by probit analysis, moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.
Test conditions
Dilution water: Deionized water adjusted to a hardness of 160-180 mg/L as CaCO3/L
Dilution water chemistry:
Hardness: Not noted
Alkalinity: Not noted
pH: Not noted
Lighting: Cool-white fluorescent bulbs with an intensity of 110 ft-c. Photoperiod of 16-hours light, 8 -hours dark with a 15 minute transition period.
Stock and test solutions preparation: A 200 mg/L primary stock solution was prepared in dilution water. After mixing, the primary stock was proportionally diluted with dilution water to prepare the test concentrations. No insoluble material was noted during the test.

Exposure vessels: 300 mL glass beakers containing 250 mL of test solution. The approximate depth of test solution was 9 cm. The vessels were loosely covered during the test.
Number of replicates: 2
Number of daphnids per replicate: 10
Number of concentrations: five plus a negative control
Water chemistry during the study:
Dissolved oxygen range (0 – 48 hours):
8.6 – 9.0 mg/L (control exposure)
8.7 – 9.0 mg/L (100 mg/L exposure conc.)
Conductivity range (0 – 48 hours)
670 – 690 µmhos/cm (control exposure)
670 – 720 µmhos/cm (100 mg/L exposure conc.)
pH range (0 – 48 hours)
8.3– 8.5 (control exposure)
8.4 - 8.6 (100 mg/L exposure conc.)
Test temperature range (0 – 48 hours)
19.3 – 20.2°C (control exposure)
19.3 – 20.4°C (100 mg/L exposure conc.)
Element basis: mortality and immobilization
RESULTS
Nominal concentrations: Blank control, 13, 22, 36, 60, 100 mg/L.
Element value and 95% confidence interval:
24-hour EC50 = 72 (63-83) mg/L
24-hour LC50 = >100 mg/L (CI not calculable)
48-hour EC50 = 62 (36 – 100) mg/L
48-hour LC50 = 93 (74 – >100) mg/L
48-hour NOEC = 13 mg/L
Element values based on nominal concentrations.
Remarks: Testing was conducted on a mixture as described in the Test Substance Remarks field. The values reported apply to that mixture and not the fluorochemical component alone.

Cumulative percent immobilization (includes mortality)

Nominal Test Conc., mg/L	24-hours	48-hours
Control	0	0
13	0	0
22	0	5
36	0	5
60	30	45
100	85	100

Note: at 48-hours, more organisms than noted above were affected at 22, 36, and 60 mg/L (noted as "less active than control daphnids), but were not considered immobilized.
Control response: satisfactory

CONCLUSIONS

The test substance 48-hour LC50 for *Daphnia magna* was determined to be 93 mg/L with a 95% confidence interval of 74 - >100 mg/L. The test substance 48-hour EC50 for *Daphnia magna* was determined to be 62 mg/L with a 95% confidence interval of 36 – 100 mg/L. The test substance 48-hour no observed effect concentration (NOEC) was 13 mg/L.

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking 2. The study lacks analytical measurement of test substance concentrations in the test solutions and sample purity is not sufficiently characterized.

REFERENCES

This study was conducted at T.R. Wilbury Laboratories, Inc., Marblehead, MA, at the request of the 3M Company, Lab Request Number N2803-2, 1996.

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Purity of the test material also is a major concern and was not sufficiently characterized in this test. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. In fact, 3M in their summary of each test state: *"Data may not accurately relate toxicity of the test sample with that of the test substance."* I agree with this concern. In addition, if this was a "typical" TSCA section 4 review, I would reject these studies, pending receipt of additional information on purity and studies on analytical measurements of the test chemical in the test medium.

TOXICITY TO FISH

Title: Acute Toxicity to Fish

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CASRN 3825-26-1)

Remarks: The 3M product lot number was 83. The test sample was FC-143. The purity of the sample was not sufficiently characterized, though current information indicates it is a mixture of 96.5 – 100% test substance and 0 – 3.5% C_6, C_7 and C_9 perfluoro analog compounds.

METHODS

Method/guideline followed: Not stated

Test type: Static

GLP (Y/N): No

Year study performed: 1974

Species: Fathead minnow (*Pimephales promelas*)
Average weight = 1.6 g
Average length = 2 inches

Supplier: Not stated

Concentrations used: 0, 10, 20, 30, 40, 50 mg/L nominal

Exposure period: 96 hours

Analytical monitoring: The test substance concentrations were not measured.

Statistical methods: There were no statistical methods given by the study authors; the LC_{50} was determined graphically.

Test Conditions:
-One replicate was performed.
-Dissolved oxygen range (24-96 hours) = 4.7 - 5.7 mg/L (control) and 4.0 - 4.9 mg/L (50 mg/L)
-pH range (24-96 hours) = 7.0 - 7.1 (control) and 6.4 - 6.5 (50 mg/L exposure)
-Temperature range = 70 - 72 degrees Fahrenheit
-Water hardness was not indicated.
-Dilution water was carbon-filtered city water from St. Paul, MN.

Remarks: Further details on the test conditions and procedures were not provided.

RESULTS

Dose of each endpoint (as mg/L): 96 hour LC_{50} = 70 mg/L (C.I. not calculated) based on nominal concentrations

Remarks: Observed mortality of the controls was 10% at both 72 and 96 hours. There was no other mortality with the exception of 20% mortality in the 50 mg/L group.

Was control response satisfactory (yes/no/unknown): unknown, see Remarks above

Statistical results, as appropriate: Percent survival versus concentration (mg/L) was plotted to obtain a calculated LC_{50} value.

CONCLUSIONS

The study authors concluded that the acute LC_{50} to fathead minnow was equal to 70 mg/L (C.I. not calculated) based on nominal concentrations.

Submitter's Remarks: Reliability - Klimisch ranking 3. There was insufficient documentation of the methodology. The LC_{50} was extrapolated from an insufficient number of data points. The general pre-test health and age of fish were not noted. Sample purity was not properly characterized and it lacks analytical confirmation of test substance concentrations.

Reviewer's Remarks: The pH levels during the test period were within the acceptable range; however, the water hardness was not given. Testing should have been done with two replicates. The number of organisms per dose and the loading rate were not indicated.

REFERENCE

3M Company. [No title given]. Lab Request number 2340. St. Paul, MN.

OTHER

Remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations. As it is we are operating in the dark on this issue.

TOXICITY TO FISH

Title: Acute Toxicity to Fish

TEST SUBSTANCE

Identity: Perfluorooctanoic acid; may also be referred to as PFOA, FC-26, or FX-1001. (Octanoic acid, pentadecafluoro-, CASRN 335-67-1)

Remarks: 3M production lot number 269. The test sample was FC-26. The purity of the sample was not sufficiently characterized, though current information indicates it is a mixture of 96.5-100% test substance and 0-3.5% C_6, C_7, and C_9 perfluoro homologue compounds.

METHODS

Method/guideline followed: Not stated

Test type: Static

GLP (Y/N): No

Year study performed: 1974

Species: Fathead minnow (*Pimephales promelas*)
Average length = 2 inches
Average weight = 1.5 g

Supplier: Not stated

Concentrations used: 0, 50, 125, 250, 375, 500 mg/L nominal

Exposure period: 96 hours

Analytical monitoring: Nominal concentrations were not measured

Statistical methods: There were no statistical methods given by the study authors; the LC_{50} was determined graphically.

Test Conditions:
-One replicate was performed.
-Temperature 69-70 degrees Fahrenheit
-Dissolved oxygen range (24-96 hours) was between 4.7 and 5.7 mg/L for the control exposure and between 3.8 and 5.2 mg/L for the 375 mg/L test exposure.
-Water hardness was not given.
-pH range (24-96 hours) was 7.0 to 7.2 for the control exposure and 6.0 to 6.7 for the 375 mg/L exposure.

Remarks: No further details were provided on the test conditions or methods.

RESULTS

Dose of each endpoint (as mg/L): 96-hour LC_{50} = 440 mg/L (C.I. not calculated) based on nominal concentrations

Remarks: For concentrations of 0 to 375 mg/L, survival was 100%. For the 500mg/L concentration, survival was 100% at 24 hours, but 0% at 48-96 hours.

Was control response satisfactory (yes/no/unknown): Yes

Statistical results, as appropriate: Concentration (mg/L) versus percent survival was graphed to obtain an LC_{50}.

CONCLUSIONS

Submitter's Remarks: Reliability - Klimisch ranking of 3. This study lacks documentation and information on the methodology. Sample purity was not properly characterized. Test concentrations were not characterized. Condition of the fish prior to study initiation is not known.

Reviewer's Remarks: Testing should have been performed in two replicates. Water hardness during the study period was not given. The number of organisms per dose and the loading rate were not indicated.

REFERENCE

3M Company. [No title given]. Lab Request Number 2485. St. Paul, MN.

OTHER

Remarks: This summary was based on a summary report submitted by 3M, therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations. As it is we are operating in the dark on this issue.

TOXICITY TO FISH

Title: Acute Toxicity to Fish

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CASRN 3825-26-1)

Remarks: The 3M production lot number was 83. The test sample was FC-143. The purity of the sample was not sufficiently characterized, though current information indicates it is a mixture of 96.5-100% test substance and 0-3.5% C_6, C_7, and C_9 perfluoro analogue compounds.

METHODS

Method/guideline followed: Not stated

Test type: Static

GLP (Y/N): No

Year study performed: 1978

Species: Bluegill sunfish (*Lepomis machrochirus*)
Average length = 4 cm
Average weight = 0.20 g

Supplier: Not stated

Concentrations used: 0, 135, 180, 240, 320, 420 mg/L nominal

Exposure period: 96 hours

Analytical monitoring: Concentrations were not measured.

Statistical methods: Not stated

Test Conditions:
-Dilution: carbon-filtered well water,
-Dilution water chemistry:
temperature = 19 degrees Celsius
dissolved oxygen = 9.1 ppm
pH = 7.9
-One replicate
-Exposure vessels were tanks containing 16 liters of test solution
-20 fish per replicate
-Loading rate = 0.25 g/L

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-Water chemistry during study (24-96 hours):
dissolved oxygen = 5.3-6.9 mg/L (control), 5.1-7.3 mg/L (420 mg/L exposure)
pH range = 7.9 (control), 7.8-8.0 (420 mg/L exposure)
test temperature = 18-19 degrees Celsius
-Water hardness was not stated

Remarks: No further details were provided on the test methods or conditions.

RESULTS

Dose of each endpoint (as mg/L): 96-hour LC50 > 420 mg/L based on nominal concentrations

Remarks: No mortalities occurred in the controls or 135-240 mg/L groups. Mortalities in the 320 and 420 mg/L groups both consisted of one in twenty fish dead at 72 hours

Was control response satisfactory (yes/no/unknown): Yes

Statistical results, as appropriate: Not stated

CONCLUSIONS

Submitter's Remarks: Reliability – Klimisch ranking 3. Testing lacks information on the method followed. No information on stock or test solution preparations was provided. Average fish weight is suspect. Sample purity was not properly characterized and it lacks analytical confirmation of test substance concentrations.

Reviewer's Remarks: Only one replicate was performed. Water hardness during the study period was not indicated.

REFERENCE

3M Company. [No title given]. Lab Request number 3844. St. Paul, MN.

OTHER

Remarks: This summary was based on a summary report submitted by 3M, therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.
The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

TOXICITY TO FISH

Title: Acute Toxicity to Fish

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CASRN 3825-26-1)

Remarks: The 3M production lot number was 83. The test sample was FC-143. The purity of the sample was not sufficiently characterized, though current information indicates it is a mixture of 96.5 – 100% test substance and 0 – 3.5% C_6, C_7, and C_9 perfluoro analogue compounds.

METHODS

Method/guideline followed: Not stated

Test type: Static

GLP (Y/N): No

Year study performed: 1978

Species: Bluegill sunfish (*Lepomis machrochirus*)
Average length = 3.0 cm
Average weight = 1.2 g

Supplier: Not stated

Concentrations used: 0, 420, 560, 750, 1000, 1350 mg/L nominal

Exposure period: 96 hours

Analytical monitoring: Concentrations were not measured.

Statistical methods: Not stated

Test Conditions:
-Dilution water source: carbon-filtered well water
-Dilution water chemistry: temperature = 19 degrees C, dissolved oxygen = 9.9 ppm, pH = 7.6
-One replicate, 20 fish per replicate
-Test performed in tanks containing 16 liters test solution
-Loading rate = 1.5 g/L
-Water chemistry during test (24 – 96 hours):
Dissolved oxygen range = 5.6 to 6.5 mg/L (control) and 4.9 to 5.9 mg/L (750 mg/L exposure)
pH range = 7.8 (control) and 7.6 to 7.8 (750 mg/L exposure)

Temperature = 19 to 20 degrees C
-Water hardness not given

Remarks: No further details were provided on the testing methods or conditions.

RESULTS

Dose of each endpoint (as mg/L): 96-hour LC50 = 569 mg/L, based on nominal concentrations

Remarks: The 95% confidence interval was 500 –636 mg/L. 100% mortality occurred by 48 hours in the 1000 and 1350 mg/L concentration groups.

Was control response satisfactory (yes/no/unknown): One out of twenty fish died at 48 hours in the control group.

Statistical results, as appropriate: Not stated

CONCLUSIONS

Submitter's Remarks: Reliability – Klimish ranking 3. Testing lacks information on the method followed. No information on stock or test solution preparations was provided. Sample purity was not properly characterized and the study lacks analytical confirmation of test substance concentrations.

Reviewer's Remarks: Only one replicate was performed and water hardness during the test period was not given.

REFERENCE

3M Company. [No title given]. Lab Request number 3844. St. Paul, MN.

OTHER

Remarks: This summary was based on a summary report submitted by 3M, therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.
The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

TOXICITY TO FISH

Title: Chronic Toxicity to Fish

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as 78.03, PFOA ammonium salt, ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)

Remarks: The 3M production lot number was 83. The test sample was FC-143. The testing laboratory refers to it as "78.03." The purity of the sample was not sufficiently characterized, though current information indicates it being a mixture of 96.5% - 100% test substance and 0 – 3.5% C_6, C_7, and C_9 pefluoro analogue compounds.

METHODS

Method/guideline followed: The methodology for the egg and fry exposure closely followed that presented in "Proposed recommended bioassay procedure for egg and fry stages of freshwater fish," U.S. EPA, 1972.

Test type: Flow-through

GLP (Y/N): No

Year study performed: 1978

Species: Fathead minnow (*Pimephales promelas*)
Pre-treatment: Eggs were placed in a 60 mg/L malachite green solution for 15 seconds to eliminate possible fungus growth.

Test fish age: Eggs within 48-hours after fertilization

Supplier: U.S. Environmental Protection Agency's Environmental Research Laboratory in Duluth, MN.

Concentrations tested: Blank control, 6.2, 12.5, 25, 50, 100 mg/L

Exposure period: 30 days post hatch

Analytical monitoring: Temperature, dissolved oxygen concentration, and pH were monitored daily. Weekly samples were taken from each aquarium for determination of ammonium perfluorooctanoate concentration. All samples taken during the test were stored in polyethylene bottles and shipped on May 31, 1978 to the 3M Company.

Statistical methods: Means of measured biological parameters from duplicate aquaria were subjected to analysis of variance (Steele and Torrie, 1960, completely randomized block design, P=0.05). Data for percentage survival and percentage hatch were transformed to arc sin square root of percentage prior to analysis.

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Test conditions:

-Dilution water source and contaminants: Well water pumped to a concrete reservoir where it was aerated before flowing to the exposure system through aged PVC pipe

-Dilution water chemistry (0-30 days):

total hardness = 31-38 mg/L as $CaCO_3$
alkalinity = 26 –32 mg/L as $CaCO_3$
pH = 7.0 – 7.4
Specific conductance = 149 – 170 µmhos/cm

-Stock and test solution preparation: A modified, proportional diluter with a 0.50 dilution factor was used. The diluter delivered five nominal concentrations ammonium perfluorooctanoate ranging from 100 to 6.2 mg/L and control water to duplicate test aquaria. A 4 liter glass Mariotte bottle toxicant delivery system was used to deliver 6.6 mL of a nominal ammonium perfluorooctanoate stock concentration of 29.4 mg/mL in distilled water to the mixing chamber of the diluter.

-Fry exposure vessel type: Glass test aquarium measuring 30.5x30.5x30.5 cm with a 17.5 cm high standpipe drain, water volume of 16 liters.

-Egg cups: Acrylic tubes (3 cm O.D., 7 cm long) with 40 mesh Nitex screen on one end. An egg cup rocker arm apparatus, as described by Mount (1968), was used to gently oscillate the egg cups in the test water.

-Diluter: Delivered 0.50 liters of test water to each aquarium 195 times per day, yielding a 90% test water replacement time of approximately 10 hours.

-Feeding: Fry were fed live brine shrimp nauplii three times daily on weekdays and twice daily on weekends throughout the exposure period.

-Number of replicates: Two replicates. There were 60 eggs for the hatchability test and 40 fry from each egg cup.

-Water chemistry during test:

Dissolved oxygen: >95% saturation
pH: 7.0 – 7.3
Temperature: 25 ± 1 degrees Celsius, maintained by water bath

Remarks: At the termination of the test, the fry from the control and the high concentration (100 mg/L) were preserved in 10% buffered formalin while the fry from the other test aquaria were frozen. Ten formalin-preserved fry (5 from each replicate) from the control and the high concentration underwent histopathological examination of a transverse section of the nares and cephalic extension of the lateral line. The remaining preserved fry and frozen fry were analyzed at a later date (by 3M Company) for ammonium perfluorooctanoate concentrations.

RESULTS

Dose of each endpoint (as mg/L): 30-day NOAEL ≥ 100 mg/L

Nominal Concentration (mg/L)			30 Days Post Hatch		
	Replicate	Hatch %	Survival %	Mean length in mm (± SD)	Mean weight in mg
Control	A	98	92	20(2)	62
	B	95	95	21(3)	75
6.2	A	95	98	20(2)	59

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	B	94	88	22(2)	79
12.5	A	93	95	21(3)	70
	B	88	100	21(2)	72
25	A	98	90	21(2)	74
	B	100	95	21(2)	70
50	A	90	90	20(2)	60
	B	95	98	20(3)	65
100	A	95	88	19(2)	59
	B	97	82	20(2)	60

Test Material	Number of Observations	Histopathological Findings
Control	10	3/10 Normal 6/10 Liver fatty change 3/10 Gill hyperplasia (Epithelium) I
100 mg/L Ammonium Perfluorooctanoate	10	5/10 Normal 5/10 Liver fatty change 2/10 Gill hyperplasia (Epithelium) I

Remarks: Submitter's Note - Only those tissues which were missing or contained demonstrable change are listed. The only tissue changes observed were hyperplasia of gill lamellar epithelium and fatty change of the liver. These changes were judged to be minimal and consistent with changes seen routinely in healthy fish. Autolipis of gill tissue was observed in several fish. This change was probably due to the poor penetration of the buffered formalin to the posterior dorsal portion of the gill space.

Was control response satisfactory (yes/no/unknown): Yes

Statistical results, as appropriate: No statistically significant findings were reported.

CONCLUSIONS

Biological data generated in this study indicate that the nominal concentration of 100 mg/L had no adverse effect upon the hatchability or eggs or upon the survival and growth of fathead minnow fry through 30 days post-hatch.

Submitters' remarks: Klimisch ranking 2. This study meets all the criteria for quality testing at the time it was conducted, but has several deficiencies. It lacks information on purity of the test substance, and the production lot number from which the test sample was taken. There is no information available on the analysis of the test solution concentrations or on the preserved fry and frozen fry samples.

Reviewers' remarks: Water hardness was not monitored during the study period or at study completion as part of the analysis of test conditions.

REFERENCE

The effects of continuous exposure to 78.03 on hactchability of eggs and growth and survival of fry of fathead minnow (*Pimephales promelas*). 1978. Report #BW-78-6-175. Research report submitted to 3M Company, St. Paul, MN by EG&G Bionomics Aquatic Toxicology Laboratory, Wareham, MA.

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations. As it is we are operating in the dark on this issue.

FISH ECOTOXICITY

Title: 96-hour acute static toxicity to fathead minnow – FX-1001

TEST SUBSTANCE

Identity: Perfluoroctanoic acid, also referred to as PFOA, FC-26, or FX-1001. (Octanoic acid, pentadecafluoro-, CAS # 335-67-1)

Remarks: The 3M production lot number was not noted. The test sample was FX-1001. Its purity was not completely characterized, although information indicated it was a mixture of 95-98 percent test substance and 1-5 percent perfluorochemical inerts.

METHODS

Method/guideline followed: Not noted

Test type: Static

GLP (Y/N): No

Year study performed: 1985

Species: *Pimephales promelas,*
average length = 4.1 cm;
average weight = 0.50 g;
age not noted

Supplier: Dale Fattig of Brady, NB

Concentrations used: 0, 690, 750, 810, 870, 930 mg/L (nominal values).

Exposure period: 96 hours

Analytical monitoring: No measurements were taken. Also, there was no information on detection limits of the chemical or impurities in the sample.

Statistical methods: Probit analysis

Test Conditions:
- Fish were pretreated with 25.0 mg/L tetracycline HCl, 5 months prior to study to fight diseases
- Dilution water chemistry:

Carbon-filtered well water
DO 9.5 ppm
pH 7.8
Temp 19C

- Water chemistry during the test:

Dissolved oxygen range (24-96 hours):
7.3 – 7.7 mg/L (control)
6.1 – 6.7 mg/L (870 mg/L)*
pH range (24-96 hours):
7.5 – 7.7 (control)
7.5 – 7.5 (870 mg/L)*

*870 mg/L test group (second highest concentration) data given because total mortality occurred in the highest test concentration by 48 hours.

Test temperature (24-96 hours):
19-20 C

- Stock solution was prepared by dissolving 45 g test substance (neutralized with NaOH to pH 7.5) in 3 liters water. Test solutions were prepared by transferring stock solution aliquots to make 5 liters at the selected test concentrations.
- Loading rate: 0.50 g fish/L
- Stability of the test chemical solutions was not noted
- Exposure vessels were glass beakers with a 24 cm inside diameter and a 26 cm depth, and contained 5 liters test solution.
-Two replicates were taken at each dose, with 5 fish per replicate

Remarks: Water hardness was not presented. The pH values are acceptable according to the OPPTS harmonized guidelines.

RESULTS

Dose of each endpoint (as mg/L): 96-hour LC50: 843 mg/L (C.I.: 811-878), based on nominal concentrations.

Remarks:
- 930 mg/L was the lowest test substance concentration causing 100% mortality (seen at 48 hours)
- There was no mortality in controls
- Surfacing of the fish occurred at doses of 810, 870, and 930 mg/L

Was control response satisfactory (yes/no/unknown): Yes, based on zero mortality.

Statistical results, as appropriate: No p-values were reported.

CONCLUSIONS

The test sample 96-hour LC50 for fathead minnow was determined to be 843 mg/L with a 95% C.I. of 811-878 mg/L.

Submitter remarks: The data quality ranking was a Klimisch ranking of 2 because testing met criteria for quality testing. However, sample purity was not properly characterized and it lacked analytical confirmation of test substance concentrations.

Reviewer remarks: none

REFERENCE

3M Company. 1985. 96-hour acute static toxicity to fathead minnow – FX-1001. Environmental Laboratory, St. Paul, MN. Lab Request Number C1006. February 2.

OTHER

General remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.
The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

FISH ECOTOXICITY

Title: Acute Toxicity to Fish

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; also referred to as PFOA ammonium salt, ammonium perfluorooctanoate, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS# 3825-26-1)

Remarks: The 3M product lot number used was 390. The test sample was FC-126, a white powdery solid. It's purity was not completely characterized, although information indicated it was a mixture of 78-93% test substance and 7-22% C6, C7, and C9 perfluoro analogue compounds.

METHODS

Method/guideline followed: Not stated

Test type: Static

GLP (Y/N): No

Year study performed: 1987

Species: *Pimephales promelas*
Average length = 3.5 cm
Average weight = 0.30 g

Supplier: Dale Fattig of Brady, NB

Concentrations tested: 0, 100, 180, 320, 560, and 1000 mg/L

Exposure period: 96 hours

Analytical monitoring: None because nominal concentrations were used.

Statistical methods: Probit analysis

Test conditions:

-Dilution water source carbon-filtered well water
-Dilution water chemistry:
Temp of 21C
DO of 9.3 ppm
pH of 7.9

-Test solutions were prepared by direct weights addition

-Stability of the test chemical solutions was not noted
-Exposure vessels were 4 Liter Pyrex glass beakers containing 3 liters test solution
-Two replicates were used
- Six fish per replicate were used
-Loading rate was 0.6 g/L
-Water chemistry during test:

DO range (24-96 hours):
6.6-7.4 mg/L (control)
5.6-6.6 mg/L (320 mg/L*)

pH range (24-96 hours):
7.7-7.9 (control)
7.7-8.0 (320 mg/L*)

Temp (24-96 hours): 20C
*This group was used because total mortality occurred at higher doses

Remarks: No other details on test conditions were given, including water hardness. The pH values are within acceptable ranges according to OPPTS Harmonized guidelines.

RESULTS

Dose of each endpoint (as mg/L): The 96-hour LC50 for fathead minnow was determined to be 301 mg/L (95% CI: 244-370).

Remarks:

- Surfacing of fish was observed at 180 and 320 mg/L (both replicates)
- Lowest test substance concentration causing 100% mortality was 560 mg/L
- No mortality was observed in the controls

Was control response satisfactory (yes/no/unknown): Yes, based on the fact that there was no mortality

Statistical results, as appropriate: Not presented

CONCLUSIONS

Rounding the results to 2 significant figures, the test sample 96-hour LC50 for fathead minnow was determined to be 300 mg/L with a 95% confidence interval of 240-370 mg/L.

Submitters' remarks: Data quality was given a Klimisch ranking of 2. Testing met the criteria for quality testing. However, the sample purity was not properly characterized and it lacked analytical confirmation of the test substance concentrations.

Reviewers' remarks: none

REFERENCE

3M Company. 1987. St. Paul, MN. Lab Request Number E128201, completed on May 1.

OTHER

General remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.
The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

TOXICITY TO FISH

Title: Static Acute Toxicity of FX-1003 to the Fathead Minnow, *Pimephales promelas*

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be 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 3M production lot number was 2327. The test sample was FX-1003. The purity of FX-1003 was not sufficiently characterized; however, available information indicated it was a solution of <45% ammonium perfluorooctanoate, 50% water, <3% inert perfluorinated compound and 1 – 2% C_5 and C_7 perfluoro- analogue compounds.**

METHODS

Method/guideline followed: OECD 203

Test type: Static

GLP (Y/N): Yes

Year study performed: 1990

Species: Prior to testing, juvenile fathead minnows were acclimated for 63 days in 100% dilution water under flow-through conditions in an all glass aquarium. Fish were acclimated to the target test temperature (23 2 C) for 14 days before test initiation. During acclimation, fish were not treated for disease and were free of apparent sickness, injuries, and abnormalities at test initiation. Fish were fed a commercial fish food, once or twice daily, for the acclimation period. The average length and weight values of the fish were 3.8 cm and 0.45 grams (wet), respectively.

Supplier: The fish used in the toxicity test were purchased from a commercial supplier (Aquatic Research Organisms, Hampton, NH). The sponsor, 3M, provided the test substance.

Concentrations tested: A screening toxicity test was performed with the following five nominal concentrations: 0.1, 1, 10, 100, and 1000 mg/L. For the definitive toxicity test, five nominal concentrations and one blank dilution water control were used. Two replicates of each concentration were used in the definitive toxicity test and the nominal concentrations tested were: 0, 150, 250, 400, 600, and 1000 mg/L. Both tests were performed under static conditions.

Exposure period: 96 hours

Analytical monitoring: Dissolved oxygen, pH, conductivity, and temperature were measured and recorded daily in each test chamber that contained live fish. Concentrations of the test substance were not measured during the test.

Statistical methods: Non-linear interpolation, moving average, and/or probit analysis; however, results of the toxicity test could not be interpreted by standard statistical techniques due to 100% survival at the highest tested concentration.

Test conditions:

The screening test was performed using nominal concentrations (0.1, 1.0, 10, 100, and 1000 mg/L) of the test substance under similar conditions as those of the definitive test.

Water used for acclimation of the test organisms, and for all toxicity testing, was well water collected from wells at EnviroSystems in Hampton, NH. Water was adjusted to a hardness of 88 mg/L as $CaCO_3$ and stored in tanks, where it was aerated. A chemical characterization of a representative sample of the natural well water used as the dilution water for the toxicity test was performed; the following values were determined: pH = 7.4, conductivity = 1500 mhos/cm. Organochlorine pesticides, organophosphorous pesticides, and PCBs were below the level of detection, or not present. The test vessels were 19.6 L glass aquaria that contained 15 L of test solution (approximate water depth was 17 cm). Stability of the test substance was not indicated. No stock solution was prepared as test material was added directly to dilution water contained in the test vessels without the use of a solvent. The following nominal concentrations were used in the definitive test: 0, 150, 250, 400, 600, and 1000 mg/L. Twenty fish were randomly and equally distributed among two replicates of each treatment. The loading rate was determined to be approximately 0.30 g/L. Test vessels were randomly arranged in a water bath during the 96-hour test. Static conditions were maintained throughout the study. A 16-hour light and 8-hour dark photoperiod was automatically maintained with cool-white fluorescent lights that provided a light intensity of 35 $Es^{-1}m^{-2}$. Aeration was employed after 48 hours to maintain dissolved oxygen concentrations above acceptable levels. Fish were not fed during the test. The following water chemistry ranges (0 – 96 hours) were determined: conductivity = 1200 – 1500 mhos/cm (control exposure), = 1300 – 1600 mhos/cm (1000 mg/L exposure); pH range = 7.4 – 8.4 (control exposure), = 7.8 – 8.2 (1000 mg/L exposure); temperature range = 21.0 - 22 C (control and 1000 mg/L exposure); dissolved O_2 = 6.1 – 9.2 mg/L (control exposure), = 6.2 – 9.1 (1000 mg/L exposure). The pH and hardness were within the accepted ranges (6.0 < pH <8.5; 40<hardness<180 mg/L) for the duration of the study.

Remarks: No additional comments

RESULTS

Dose of each endpoint (as mg/L):

Screening test:

96-hour LC50 > 1000 mg/L, 96-hour NOEC > 1000 mg/L

Definitive test:

96-hour LC50 > 1000 mg/L, 96-hour NOEC > 1000 mg/L

Remarks: All test vessels remained clear throughout the test. 100% survival occurred in the control exposure and all other test exposures in the screening test and in the definitive test.

Was control response satisfactory (yes/no/unknown): Yes

Statistical results, as appropriate: Results of the toxicity test could not be interpreted by standard statistical methods due to 100% survival at the highest tested concentration.

CONCLUSIONS

The 96-hour LC50 and 96-hour NOEC were determined to be >1000 mg/L.

Submitters' remarks: Klimisch ranking 2. Testing meets the criteria for quality testing. However, sample purity was not properly characterized and the test lacked analytical confirmation of test substance concentrations.

Reviewers' remarks: The conclusions appear to be supported by the data.

REFERENCE

EnviroSystems, Inc. 1990. Static Acute Toxicity of FX-1003 to the Fathead Minnow, *Pimephales promelas*. Hampton, NH. Study number was 9014-3.

OTHER

General remarks:

The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

TOXICITY TO FISH

Title: Acute toxicity of N2803-3 to the Fathead Minnow, *Pimephales promelas*

TEST SUBSTANCE

Identity: Perfluorooctanoic acid; may also be referred to as PFOA, FC-26, or FX-1001. (Octanoic acid, pentadecafluoro-, CASRN 335-67-1)

Remarks: The 3M production lot number was 269. The test substance was a white powder. The test sample, FC-26, was referred to by the laboratory as N2803-3. The purity of the sample was not sufficiently characterized; however, available information indicated it was a mixture of 96.5 – 100% test substance and 0 – 3.5% C_6, C_7, and C_9 perfluoro- homologue compounds. The test substance ("as-received") was combined with isopropanol in a 50:50 ratio prior to use. The substance resulting from this mixture was referred to as the "as-tested" substance.

METHODS

Method/guideline followed: U.S. EPA-TSCA Guideline 797.1400

Test type: Static

GLP (Y/N): Yes

Year study performed: 1996

Species: Juvenile fathead minnows were acclimated under flow-through conditions in a 270 L fiberglass tank. During acclimation, the fish were not treated for disease. The fish were free of apparent sickness, injuries, and abnormalities at test initiation. Mortality during the final 48 hours of acclimation was <3%. During the 14-day period before test initiation, the acclimation temperature range was 21.0 °C – 22.3 °C and the dissolved oxygen concentration was at least 8.0 mg/L. During acclimation, fish were fed daily, except during the 48 hours immediately preceding the test. The average total length and wet weight of the test organisms were 33.4 mm and 0.35 grams, respectively.

Supplier: Juvenile fathead minnows were procured from Aquatic Biosystems, Fort Collins, CO. The sponsor, 3M, supplied the test substance.

Concentrations tested: For the static screening test, the following nominal concentrations, as-received, were used: 0, 0.05, 0.50, 5.0, 50, and 500 mg/L. For the static definitive test, the following nominal concentrations, as-tested, were used: 0, 130, 220, 360, 600, and 1000 mg/L. For the static test with isopropanol, nominal concentrations of 0 (dilution water control) and 500 mg/L isopropanol were used.

Exposure period: 96 hours

Analytical monitoring: Dissolved oxygen, pH, temperature, and conductivity were measured and recorded daily in each test chamber. The temperature in one test vessel was recorded at least hourly during the test. Concentrations of the test substance were not measured during the study.

Statistical methods: LC_{50} values were calculated by non-linear interpolation (Stephan, 1983), when possible, using probit analysis, moving average method, or binomial probability.

Test conditions:

For the static screening test, nominal concentrations of the test substance, as-received, were 0 (dilution water control), 0.05, 0.50, 5.0, 50, and 500 mg/L. The screening test was performed under similar conditions as those of the definitive test.

Water used for acclimation of the test organisms, and for all toxicity testing, was deionized water collected at T.R. Wilbury Laboratories in Marblehead, MA. Water was adjusted to a hardness of 40 – 48 mg/L as $CaCO_3$ and stored in polyethylene tanks. In the tanks, the water was aerated and continuously passed through a particle filter, ultraviolet sterilizer, and activated carbon. In a chemical characterization of a representative sample of dilution water, iron was detected at 0.03 mg/L. Other metals and potential contaminants were either below the level of detection, or not present. The test substance, as-received, was combined with isopropanol in a 50:50 ratio prior to use. This 50:50 mixture was then considered to be 100% test substance during the toxicity test. The test substance was assumed to have a purity of 100% active ingredient and to be stable under storage and testing conditions. The test vessels were 20 L glass aquaria, which contained 15 L of test solution (approximate water depth was 18 cm). Twenty fathead minnows were indiscriminately and equally distributed among two replicates of each treatment. Appropriate amounts of test substance were added directly to dilution water in the test vessels to formulate the media. The test concentrations, as-tested, for the definitive test were: 0, 130, 220, 360, 600, and 1000 mg/L. The test vessels were loosely covered and randomly arranged in a water bath during the 96-hour test. A 16-hour light and 8-hour dark photoperiod, with a 15-minute transition period, was automatically maintained with cool-white fluorescent lights that provided a light intensity of 31 footcandles. Aeration was initiated after 72 hours to maintain dissolved oxygen concentrations above acceptable levels. Measured water chemistry during the test provided the following ranges (0 – 96 hours): Dissolved O_2 = 6.4 – 8.7 mg/L (control exposure), = 5.2 – 9.0 mg/L (220 mg/L exposure); Conductivity = 110-370 µmhos/cm (control and 220 mg/L exposure); pH = 7.1 – 7.6 (control exposure), = 3.0 – 7.4 (220 mg/L exposure); Temperature = 21.8 – 22.6 °C (control and 220 mg/L exposure). The pH values of the test solutions for the 360, 600, and 1000 mg/L exposure concentrations were in the range of 3.0 – 4.3 at test initiation. These low pH values were outside the acceptable range for aquatic toxicity studies (6.0 <pH<8.5). Hardness was within the acceptable range for toxicity studies (40 – 180 mg/L, as $CaCO_3$).

For the static toxicity test performed with isopropanol, a similar protocol was followed as that for the screening and definitive toxicity tests with N2803-3. 0 (dilution water control) and 500 mg/L isopropanol (nominal concentration) were used. Test vessels were randomly arranged in a water bath and the light intensity was 28 footcandles.

Remarks: No additional comments

RESULTS

Dose of each endpoint (as mg/L) in the definitive test:

Test substance concentration, as-tested, and associated 95% confidence limits:

24-hour LC_{50} = 280 (220 – 360) mg/L
48-hour LC_{50} = 280 (220 – 360) mg/L
72-hour LC_{50} = 280 (220 – 360) mg/L
96-hour LC_{50} = 280 (220 – 360) mg/L

96-hour NOEC = 220 mg/L

Test substance concentration, as-received, and associated 95% confidence limits:

24-hour LC_{50} = 140 (110 - 180) mg/L
48-hour LC_{50} = 140 (110 - 180) mg/L
72-hour LC_{50} = 140 (110 - 180) mg/L
96-hour LC_{50} = 140 (110 - 180) mg/L
96-hour NOEC = 110 mg/L

Remarks: For the screening test, the following survival rates were observed: 100% survival in the control, 90% survival at 0.05 mg/L, 100% survival at 0.50, 5.0, and 50 mg/L, and 0% survival at 500 mg/L. For the definitive test, no insoluble material was observed in any of the test vessels during the study. 100% survival occurred in the control exposure and these fathead minnows did not exhibit any sublethal effects. Fish in the 130 and 220 mg/L exposure concentrations appeared normal. Total mortality was observed within 24 hours in the 360, 600, and 1000 mg/L exposure concentrations; therefore, the lowest concentration, which caused 100% mortality, was 360 mg/L. During the toxicity test with 500 mg/L isopropanol, no mortality or sublethal effects were observed; the 96-hour LC_{50} value was >500 mg/L.

Was control response satisfactory (yes/no/unknown): Yes

Statistical results, as appropriate: No additional comments

CONCLUSIONS

The as-tested 96-hour LC_{50} was determined to be 280 mg/L, with a 95% confidence interval of 220 – 360 mg/L. The as-tested 96-hour no-oberved-effect-concentration (NOEC) was 220 mg/L. The 96-hour LC_{50}, based on the test substance as received, was determined to be 140 mg/L, with a 95% confidence interval of 110 – 180 mg/L. The 96-hour NOEC for the test substance, as-received, was 110 mg/L.

Submitters' remarks: Klimisch ranking 3. The study lacked analytical measurement of test substance concentrations in the test solutions and sample purity was not sufficiently characterized. Additionally, there appeared to be a discrepancy between the sample preparation directions given to the laboratory and the procedure conducted by the laboratory to prepare the test solutions. The absence of partial mortality at intermediate doses resulted in a sharp dose-response curve. As a result, the LC_{50} values determined in this study may not accurately reflect the true toxicity of the solution. The low pH values observed in the high concentrations tested may have had an adverse effect on survival.

Reviewers' remarks: The conclusions appear to be supported by the data.

REFERENCE

T.R. Wilbury Laboratories, Inc. 1996. Acute toxicity of N2803-3 to the Fathead Minnow, *Pimephales promelas*. Marblehead, MA. Study number 891-TH.

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort

had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Purity of the test material also is a major concern and was not sufficiently characterized in this test. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. In fact, 3M in their summary of each test state: *"Data may not accurately relate toxicity of the test sample with that of the test substance."* I agree with this concern. In addition, if this was a "typical" TSCA section 4 review, I would reject these studies, pending receipt of additional information on purity and studies on analytical measurements of the test chemical in the test medium.

000307

FISH ECOTOXICITY

Title: Acute toxicity of FC-1015 to the fathead minnow, *Pimephales promelas*

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, FC-143 or as the major component of FC-1015. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS #3825-26-1)

Remarks: The test sample is FC-1015. Its purity was not sufficiently characterized, though current information indicates it is a 30% straight carbon chain version of FC-143 in 80% water. The 3M product lot number was "HOGE 205." Data may not accurately relate toxicity of the test sample with that of the test substance. Data were used to compare toxicity of the branched/straight chain ammonium perfluorooctanoate homolog mixture in FC-143 vs. what is supposed to be the 100% straight carbon chain ammonium perfluorooctanoate in FC-1015.

METHODS

Method/guideline followed: USEPA–TSCA 1993. 797.1400

Test type: static

GLP (Y/N): N

Year study performed: 1996

Species: *Pimephales promelas*. The fish were juveniles with an average length of 35 mm and average weight of 0.36g (wet).

Supplier: Aquatic Biosystems

Concentrations used: 0, 530, 830, 1330, 2100, and 3300 mg/L. The concentrations were nominal. Two replicates at each concentration were performed.

Exposure period: 96 hours

Analytical monitoring: none

Statistical methods: LC50 values were calculated using the Stephan computer program, 1983

Test Conditions: The dilution water used was deionized water adjusted for hardness and passed through a particulate filter, ultraviolet sterilizer, and activated carbon. The dilution water chemistry was measured as follows: hardness = 44 mg/L as $CaCO_3$, alkalinity = 29-30 mg/L as $CaCO_3$, and TOC < 1 mg/L. For lighting, cool-white fluorescent lights at 30 foot-candles were used. A daily photoperiod of 16 hours light and 8 hours dark with a 15 minute transition period was maintained throughout the testing period. The test solutions were created by direct individual weight additions. After a 14 day acclimation period, the fish were exposed, at a loading of 0.24g fish/L, in 20 L glass aquaria containing 15 liters test solution at

an approximate depth of 18 cm. Two replicates were tested at each concentration. Twenty fish were used in each replicate.

The water chemistry parameters measured during the study included: conductivity range = 180-190 µmhos/cm (control) and 300-320 µmhos/cm (2100 mg/L exposure), pH = 7.3-7.9 (control) and 7.2-7.7 (2100 mg/L exposure), temperature = 22.0-22.4 °C (control) and 21.8-22.5 °C (2100 mg/L exposure), and dissolved oxygen = 5.8-9.2 mg/L (control) and 5.7-9.2 mg/L (2100 mg/L exposure). The 2100 mg/L (second highest) concentration was used because the highest concentration resulted in total mortality at 72 hours. The pH range was within the acceptable range. Measurements of dilution water chemistry were also performed (see Appendix for parameters and detection limits). The only chemical detected was iron at 0.03 mg/L.

Remarks: Water hardness, during the study, was not indicated.

RESULTS

Dose of each endpoint (as mg/L): 96 hr LC50 = 2470 (2100-3330) mg/L
96 hr NOEC = 830 mg/L

Remarks: none

Was control response satisfactory (yes/no/unknown): yes

Statistical results, as appropriate: none

CONCLUSIONS

The test sample 96 hour LC50 for fathead minnow was determined to be 2470 mg/L with a 95% confidence interval of 2100-3330 mg/L.

Submitters' remarks: For data reliability, the study was assigned a Klimisch rating of 2. Testing meets the criteria for quality testing. However, sample purity was not properly characterized and it lacks analytical confirmation of test substance concentrations.

Reviewers' remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Purity of the test material also is a major concern and was not sufficiently characterized in this test. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. In fact, 3M in their summary of each test state: *"Data may not accurately relate toxicity of the test sample with that of the test substance."* I agree with this concern. In addition, if this was a "typical" TSCA section 4 review, I would

reject these studies, pending receipt of additional information on purity and studies on analytical measurements of the test chemical in the test medium.

REFERENCE

Ward, T., Nevius, J. and R. Boeri. 1996. Acute toxicity of FC-1015 to the fathead minnow, *Pimephales promelas*. T.R. Wilbury Laboratories, Inc. Lab Request number P1624. 3M Company, St. Paul, MN.

OTHER

General remarks: none

APPENDIX

Chemical measurements of dilution water

Parameter:	Detection Limit:
Metals	
Aluminum	0.1 mg/L
Arsenic	0.01 mg/L
Boron	0.5 mg/L
Cadmium	0.0002 mg/L
Chromium	0.01 mg/L
Cobalt	0.03 mg/L
Copper	0.005 mg/L
Iron	0.03 mg/L
Lead	0.005 mg/L
Mercury	0.0003 mg/L
Nickel	0.03 mg/L
Silver	0.02 mg/L
Zinc	0.02 mg/L
Nitrate	0.05 mg N/L
Chloride	1 mg/L
Fluoride	0.1 mg/L
Total organic carbon	1 mg/L
Total phosphorous	0.03 mg/L
Organochlorine Pesticides	0.5 µg/L
Toxaphene	2 µg/L
Organophosphorous Pesticides	0.5 µg/L
Dimethoate	2.0 µg/L
TEPP	2.0 µg/L
Monocrotophos	2.0 µg/L
PCBs	0.5 µg/L

ACUTE TOXICITY TO FISH (FATHEAD MINNOW)
TEST SUBSTANCE
Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)
Remarks: The 3M production lot number was 427. The test sample is FC-143, referred to by the test laboratory as N2803-4. The T.R. Wilbury study number is 895-TH. The purity of the sample was not sufficiently characterized, although current information indicates it is a mixture of 96.5 - 100% test substance and 0 – 3.5% C6, C7, and C9 perfluoro analogue compounds.
METHOD
Method: U.S. EPA-TSCA Guideline 797.1400
Type: Acute static
GLP: Yes
Year completed: 1995
Species: *Pimephales promelas*
Supplier: Aquatic Biosystems, Fort Collins, CO
Analytical monitoring: DO, conductivity, pH and temperature
Exposure period: 96-hours
Statistical methods: LC50 values calculated, when possible, by probit analysis, moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.
Test fish age: Not noted.
Length and weight: Average length = 28.6 cm
Average weight = 0.19g (wet)
Loading: 0.13 g/L
Pretreatment: None
Test conditions:
Dilution Water: Deionized water adjusted to a hardness of 40-48 mg/L as CaCO3/L
Dilution water chemistry: Not noted.
Lighting: Cool-white fluorescent bulbs with an intensity of 30 ft-c. Photoperiod of 16-hours light, 8 -hours dark with a 15 minute transition period.
Stock and test solutions preparation: Test substance added directly to the dilution water in the test vessels.
Concentrations dosing rate: Once
Stability of the test chemical solutions: No insoluble material was noted during the test.
Exposure vessels: 20 L glass aquaria containing approximately 15 L of test solution, water depth approximately 18 cm.
Number of replicates: 2
Number of fish per replicate: 10
Number of concentrations: five plus a negative control
Water chemistry during the study:
Dissolved oxygen range (0 – 96 hours):
7.2 – 8.5 mg/L (control exposure)
6.7 – 8.5 mg/L (1,000 mg/L exposure)

Conductivity range (0 – 96 hours)
140 – 180 µmhos/cm (control exposure)
280 – 380 µmhos/cm (1,000 mg/L exposure)
pH range (0 – 96 hours)
7.3 – 7.6 (control exposure)
7.4 – 7.5 (1,000 mg/L exposure)
Test temperature range (0 – 96 hours)
22.0 – 22.6°C (control exposure)
21.8 – 22.5°C (1000 mg/L exposure)
RESULTS
Nominal concentrations: Bk control, 160, 250, 400, 630, 1,000 mg/L
Element value and 95% confidence interval:
24-hour LC50 = > 1,000 mg/L (C.I. not calculable)
48-hour LC50 = 790 (630 – 1,000) mg/L
72-hour LC50 = 760 (630 – 1,000) mg/L
96-hour LC50 = 740 (660 - 830) mg/L
96-hour NOEC = 400 mg/L
Element values based on nominal concentrations
Statistical Evaluation of Mortality: The 48 and 72-hour LC50 values were determined by binomial interpolation. Probit was used to calculate the 96-hour LC50.
Biological observations after 96-hours: Fish in the control and the 160, 250, and 400 mg/L exposure concentrations appeared normal. Dark discoloration and erratic swimming of two fish was observed at the 24-hour observation period in the 1,000 mg/L exposure concentration.

Cumulative percent mortality:

Nominal Test Conc., mg/L	24-hours	48-hours	72-hours	96-hours
Control	0	0	0	0
160	0	0	0	0
250	0	0	0	0
400	0	0	0	0
630	0	0	0	0
1,000	15	90	90	90

Lowest concentration causing 100% mortality: None
Mortality of controls: None

CONCLUSIONS

The test sample 96-hour LC50 for fathead minnow was determined to be 740 mg/L with a 95% confidence interval of 660 –830 mg/L. The 96-hour no observed effects concentration (NOEC) was 400 mg/L.

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking = 2. The study lacks analytical measurement of test substance concentrations in the test solutions and sample purity is not sufficiently characterized.

REFERENCES

This study was conducted at T.R. Wilbury Laboratories, Inc., Marblehead, MA, at the request of the 3M Company, Lab Request number N2803-4.

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

ACUTE TOXICITY TO FISH (FATHEAD MINNOW)

TEST SUBSTANCE
Identity: Perfluorooctanoic acid, tetrabutylammonium salt; may also be referred to as PFOA tetrabutylammonium salt, tetrabutylammonium perfluorooctanoate, N2803-2, or as a major component of L-13492. (Octanoic acid, pentadecafluoro-, tetrabutylammonium salt, CAS # 95658-53-0)
Remarks: The 3M production lot number was 2. The test sample is referred to by the testing laboratory as L-13492. The T.R. Wilbury study number is 839-TH. The 3M Environmental Laboratory Request Number is N2332. The purity of the sample was not sufficiently characterized, although current information indicates it is a solution of 44.9% tetrabutylammonium perfluorooctanoate, 27.9% water, and 27.2% isopropanol
The following summary applies to the test sample as a mixture of the test substance in an isopropanol/water solution with incompletely characterized concentrations of impurities. Data may not accurately relate toxicity of the test sample with that of the test substance.
METHOD
Method: U.S. EPA-TSCA Guideline 797.1400
Type: Acute static
GLP: Yes
Year completed: 1995
Species: *Pimephales promelas*
Supplier: Aquatic Biosystems, Fort Collins, CO
Analytical monitoring: DO, conductivity, pH and temperature were monitored daily.
Exposure period: 96-hours
Statistical methods: LC50 values calculated, when possible, by probit analysis, moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.
Test fish age: Not noted.
Length and weight of fish in control: Average length = 32.8 mm
Average weight = 0.28 g
Loading: 0.19 g fish/L
Pretreatment: None
BACK TO MAIN
Test conditions:
Dilution Water: Deionized water adjusted to a hardness of 40 - 48 mg/L as CaCO3/L
Dilution water chemistry: Not noted.
Lighting: Cool-white fluorescent bulbs with an intensity of 28 ft-c. Photoperiod of 16-hours light, 8 -hours dark with a 15 minute transition period.
Aeration: Initiated after the 72-hour DO measurements.
Stock and test solutions preparation: Test substance was added directly to the dilution water in the test vessels on a weight/volume basis. No insoluble material was noted during the

test.
Concentrations dosing rate: Once
Exposure vessels: 20 L glass aquaria containing approximately 15 L of test solution, water depth approximately 18 cm. Vessels were loosely covered during the test.
Number of replicates: 2
Number of fish per replicate: 10
Number of concentrations: five plus a negative control
Water chemistry during the study (from a representative sample of dilution water):
Hardness: 44 mg/L as CaCO3
Alkalinity: 38 mg/L as CaCO3
pH 7.9
Dissolved oxygen range (0 – 96 hours):
6.1 – 8.8 mg/L (control exposure)
5.1 –8.9 mg/L (1,000 mg/L exposure)
Note: aeration was supplied to test vessels at 72-hours
Conductivity range (0 – 96 hours)
170 – 180 µmhos/cm (control exposure)
200 – 210 µmhos/cm (1,000 mg/L exposure)
pH range (0 – 96 hours)
7.2 – 7.9 (control exposure)
7.3 – 7.8 (1,000 mg/L exposure)
Test temperature range (0 – 96 hours)
22.1 – 22.2°C (control exposure)
21.9 – 22.2°C (1,000 mg/L exposure)
BACK TO MAIN

RESULTS
Nominal concentrations: Blank control, 130, 220, 360, 600, 1,000 mg/L.
Element value and 95% confidence interval:
24-hour LC50 = 1,000 (600 - >1,000) mg/L
48-hour LC50 = 940 (600 - >1,000) mg/L
72-hour LC50 = 890 (600 - >1,000) mg/L
96-hour LC50 = 890 (600 - >1,000) mg/L
96-hour NOEC = 600 mg/L
Element values based on nominal concentrations.
Remarks: Testing was conducted on the mixture as described in the Test Substance Remarks field. The values reported apply to that mixture and not the fluorochemical component alone.

Cumulative percent mortality:

Nominal Test Conc., mg/L	24-hours	48-hours	72-hours	96-hours
Control	0	0	0	0
130	0	0	0	0

220	0	0	0	0
360	0	0	0	0
600	0	0	0	0
1,000	50	60	70	70

Lowest concentration causing 100% mortality: None
Mortality of controls: None
CONCLUSIONS
The test substance 96-hour LC50 for fathead minnow was determined to be 890 mg/L with a 95% confidence interval of 600 - >1,000 mg/L. The test substance 96-hour no observed effect concentration (NOEC) was 600 mg/L.
BACK TO MAIN
Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133
DATA QUALITY
Reliability: Klimisch ranking 2. The study lacks analytical measurement of test substance concentrations in the test solutions and sample purity is not sufficiently characterized.
REFERENCES
This study was conducted at T.R. Wilbury Laboratories, Inc., Marblehead, MA, at the request of the 3M Company, Lab Request number N2332, 1995.
OTHER

> **General remarks:** The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Purity of the test material also is a major concern and was not sufficiently characterized in this test. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. In fact, 3M in their summary of each test state: *"Data may not accurately relate toxicity of the test sample with that of the test substance."* I agree with this concern. In addition, if this was a "typical" TSCA section 4 review, I would reject these studies, pending receipt of additional information on purity and studies on analytical measurements of the test chemical in the test medium.

ACUTE TOXICITY TO FISH (FATHEAD MINNOW)
TEST SUBSTANCE
Identity: Perfluorooctanoic acid, tetrabutylammonium salt; may also be referred to as PFOA tetrabutylammonium salt, tetrabutylammonium perfluorooctanoate, N2803-2, or as a major component of L-13492. (Octanoic acid, pentadecafluoro-, tetrabutylammonium salt, CAS # 95658-53-0)
Remarks: The 3M production lot number was 2. The test sample is referred to by the testing laboratory as N2803-2. The T.R. Wilbury study number is 888-TH. The purity of the sample was not sufficiently characterized, although current information indicates it is a solution of 44.9% tetrabutylammonium perfluorooctanoate, 27.9% water, and 27.2% isopropanol.
The following summary applies to the test sample as a mixture of the test substance in an isopropanol/water solution with incompletely characterized concentrations of impurities. Data may not accurately relate toxicity of the test sample with that of the test substance.
METHOD
Method: U.S. EPA-TSCA Guideline 797.1400
Type: Acute static
GLP: Yes
Year completed: 1995
Species: *Pimephales promelas*
Supplier: Aquatic Biosystems, Fort Collins, CO
Analytical monitoring: DO, conductivity, pH and temperature were monitored daily.
Exposure period: 96-hours
Statistical methods: LC50 values calculated, when possible, by probit analysis, moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.
Test fish age: Not noted.
Length and weight of fish in control: Average length = 34.9 mm
Average weight = 0.36 g
Loading: 0.24 g fish/L
Pretreatment: None
Test conditions:
Dilution Water: Deionized water adjusted to a hardness of 40-48 mg/L as CaCO3/L
Dilution water chemistry: Not noted.
Lighting: Cool-white fluorescent bulbs with an intensity of 25 ft-c. Photoperiod of 16-hours light, 8 -hours dark with a 15 minute transition period.
Aeration: Initiated after the 72-hour DO measurements.
Stock and test solutions preparation: Test substance was added directly to the dilution water in the test vessels on a weight/volume basis.
Concentrations dosing rate: Once
Exposure vessels: 20 L glass aquaria containing approximately 15 L of test solution, water depth approximately 18 cm. Vessels were loosely covered during the test.

Number of replicates: 2
Number of fish per replicate: 10
Number of concentrations: five plus a negative control
Water chemistry during the study:
Dissolved oxygen range (0 – 96 hours):
6.3 – 8.6 mg/L (control exposure)
4.6 –8.4 mg/L (1,000 mg/L exposure)
Note: aeration was supplied to test vessels at 72-hours
Conductivity range (0 – 96 hours)
140 – 150 µmhos/cm (control exposure)
170 – 180 µmhos/cm (1,000 mg/L exposure)
pH range (0 – 96 hours)
7.4 – 7.7 (control exposure)
7.5 – 7.7 (1,000 mg/L exposure)
Test temperature range (0 – 96 hours)
21.6 – 21.9°C (control exposure)
21.6 – 21.9°C (1,000 mg/L exposure)
RESULTS
Nominal concentrations: Blank control, 160, 250, 400, 630, 1,000 mg/L.
Element value and 95% confidence interval:
24-hour LC50 = >1,000 mg/L (CI not calculable)
48-hour LC50 = >1,000 mg/L (CI not calculable)
72-hour LC50 = 970 (630 - >1,000) mg/L
96-hour LC50 = 960 (830 - >1,000) mg/L
96-hour NOEC = 630 mg/L
Element values were based on nominal concentrations.

Remarks: Testing was conducted on the mixture as described in the Test Substance Remarks field. The values reported apply to that mixture and not to the fluorochemical component alone.
Cumulative percent mortality:

Nominal Test Conc., mg/L	24-hours	48-hours	72-hours	96-hours
Control	0	0	0	0
160	0	0	0	0
250	0	0	0	0
400	0	0	0	0
630	0	0	0	0
1,000	30	45	55	55

Lowest concentration causing 100% mortality: None
Mortality of controls: None
CONCLUSIONS

The test substance 96-hour LC50 for fathead minnow was determined to be 960 mg/L with a 95% confidence interval of 830 - >1,000 mg/L. The test substance 96-hour no observed effect concentration (NOEC) was 630 mg/L.

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking 2. The study lacks analytical measurement of test substance concentrations in thetest solutions and sample purity is not sufficiently characterized.

REFERENCES

This study was conducted at T.R. Wilbury Laboratories, Inc., Marblehead, MA, at the request of the 3M Company, Lab Request number N2803-2, 1995.

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Purity of the test material also is a major concern and was not sufficiently characterized in this test. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. In fact, 3M in their summary of each test state: *"Data may not accurately relate toxicity of the test sample with that of the test substance."* I agree with this concern. In addition, if this was a "typical" TSCA section 4 review, I would reject these studies, pending receipt of additional information on purity and studies on analytical measurements of the test chemical in the test medium.

TOXICITY TO MICROORGANISMS

Title: Microtox Toxicity Test

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; also referred to as PFOA ammonium salt, ammonium perfluorooctanoate, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS# 3825-26-1)

Remarks: The 3M production lot number was 390. The test sample was FC-126, a white powdery solid. Its purity was not sufficiently characterized, although information indicated it was a mixture of 78-93 percent test substance and 7-22 percent C5, C6, and C7 perfluoro analogue compounds.

METHOD

Method/guideline followed: Beckman's Microtox "BASIC" Procedure

Test type: Static

GLP (Y/N): No

Year study performed: 1987

Species/strain: *Photobacterium phosphoreum*

Supplier: Not noted

Concentrations tested: 0, 420, 560, 750, and 1000 mg/L as nominal concentrations.

Exposure period: 30 minutes

Analytical monitoring: No measurements of the test substance were taken throughout the test.

Statistical methods: Not noted

Test conditions:
-Diluent was 2% NaCl Microbic's Reagent
-Deionized water pH was 6.5
- A 2000 mg/L stock solution was prepared by dissolving 200 mg test solution in 100 mL diluent.
- Test solutions were prepared using aliquots of the stock solution.
- Media was not renewed
-Stability of the test chemical solutions was not noted.
-Exposure vessels were cuvettes.
-Two replicates were taken.

Remarks: none

RESULTS

Dose of each endpoint (as mg/L): 5 minute EC20 = 810 mg/L
5 minute EC50 = >1000 mg/L
5 minute EC80 = > 1000 mg/L
15 min EC20 = 520 mg/L
15 min EC50 = >1000 mg/L
15 min EC80 = >1000 mg/L
30 min EC20 = 420 mg/L
30 min EC50 = 870 (810-930) mg/L
30 min EC80 = > 1000 mg/L

Was control response satisfactory (yes/no/unknown): unknown

Statistical results, as appropriate: Not noted

Remarks: none

CONCLUSIONS

The 30-minute EC50 was determined to be 870 mg/L with 95% confidence intervals of 810 to 930 mg/L

Submitters' remarks: A Klimisch data quality ranking of 2 was given. Testing meets all criteria for quality testing, but lacks analytical confirmation of test substance concentrations. Characterization of the test sample is also lacking.

Reviewers' remarks: none

REFERENCE

3M Company. [No title given.] Lab Request Number E1282. St. Paul, MN.

OTHER

General remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

TOXICITY TO MICROORGANISMS DATA

Title: Microbics Microtox® Toxicity Test

TEST SUBSTANCE

Identity: PFOA ammonium salt also referred to as 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), as stated in the submitter's summary.

Remarks: The 3M production lot number was 2327; the test substance was FX-1003. The purity of the test substance was not sufficiently characterized, though current information indicated it was a solution of < 45% ammonium perfluorooctanoate, 50% water, < 3% inert perfluorinated compound, and 1 – 2% C_5 and C_7 perfluoro-analogue compounds.

METHOD

Method/guideline followed: Microbics Microtox "BASIC" Procedure

Test type: Static

GLP (Y/N): No

Year study performed: 1990

Species/strain/supplier: *Photobacterium phosphoreum*/3M

Doses (concentrations) tested: 0 (blank control), 125, 250, 500, and 1000 mg/L

Exposure period: 30 minutes

Analytical monitoring: Concentrations of the test substance were not measured during the study.

Test conditions: Two replicates of each of the four test concentrations and blank control were prepared using aliquots of the stock solution, a 2000 mg/L solution prepared in Millipore Milli-Q® water. 2% NaCl Microbic's Reagent was used as the diluent. The initial pH of 6.8 was adjusted to pH 6.7 with dilute H_2SO_4; for osmotic adjustments, 200 mg NaCl was added to 10 ml of sample. The final solutions were clear and colorless. Cuvettes were used as the exposure vessels. Percent of light-loss by the dosed organisms, in reference to the mean light generated by the control organisms, was used to assess the toxicity of the test substance.

Statistical methods: EC_{10} and EC_{50} values were calculated using a statistical linear-regression program provided for Microtox®.

Remarks: The test conditions and procedures were not further described.

RESULTS

Dose of each endpoint (calculated by statistical linear-regression program):

5-min EC_{50} >1000 mg/L
15-min EC_{50} >1000 mg/L
30-min EC_{50} >1000 mg/L

Was control response satisfactory (yes/no/unknown): Unknown

Statistical results, as appropriate: Not specified

Submitter's remarks: **Testing met all criteria for quality testing, but lacked analytical confirmation of test-substance concentrations. Also, there was a lack of characterization of the test sample.**

Reviewer's remarks: **Unable to determine % mortality of the controls.**

CONCLUSIONS

The authors conclude the FX-1003 30-minute EC_{50} for *Photobacterium phoshphoreum* to be >1,000 mg/L (nominal concentration).

Submitter's remarks: Klimisch-ranking 2. The study summary applies to the test sample as a mixture of the test substance in water solution with incompletely characterized concentrations of impurities. Data may not accurately relate toxicity of the test sample with that of the test substance.

Reviewer's remarks: None

REFERENCE

3M Environmental Laboratory. 1990. Microbics Microtox® Toxicity Test. St. Paul, Minnesota. Lab request number G2882.

OTHER

Remarks: This summary was based on a summary report and limited raw data. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

TOXICITY TO MICROORGANISMS

Title: Microbics Microtox® Toxicity Test of FC-143

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)

Remarks: The test sample is FC-143. Its purity was not sufficiently characterized, though current information indicates it is a mixture of 96.5 – 100% test substance and 0 – 3.5% C_6, C_7, and C_9 perfluoro analog compounds. The 3M product lot number was 427.

METHOD

Method/guideline followed: Microbics Microtox® "BASIC" Procedure

Type (test type): static

GLP (Y/N): N

Year study performed: 1996

Species/strain: *Photobacterium phosphoreum*

Supplier: Microbics

Concentrations tested: 0, 125, 250, 500, and 1000 mg/L. The concentrations were nominal. Two replicates of each were tested at each concentration.

Exposure period: 30 minutes

Analytical monitoring: none

Statistical methods: EC50 values were calculated by a statistical linear regression program provided for Microtox®.

Test conditions: A primary 2000 mg/L stock solution was prepared in Millipore Milli-Q™ water. The pH of the stock solution was then adjusted from 5.8 to 7.6 using 1.0 N NaOH. An osmotic adjustment was made using 200 mg NaCl dissolved in 10 mL stock solution. The water hardness was not reported. Appearance of test solutions was noted as "clear and colorless." All test solutions were made by proportional dilutions. The test temperature was not noted. Cuvettes (3 mL) were used as the exposure vessels. Two replicates at each of four concentrations were tested, along with two controls.

Remarks: The number of microbes per replicate and the growth phase of the microbes were not indicated.

RESULTS

JC0324

Dose of each endpoint (as mg/L): 5 minute EC50 ≥ 1000 mg/L
15 minute EC50 = 800 (790 – 820) mg/L
30 minute EC50 = 730 (630 – 850) mg/L

Was control response satisfactory (yes/no/unknown): unknown

Statistical results, as appropriate: none

Remarks: none

CONCLUSIONS

The FC-143 30 minute EC50 for *Photobacterium phosphoreum* was determined to be 730 mg/L with a 95% confidence interval of 630 to 850 mg/L.

Submitters' remarks: For data reliability, the study was assigned a Klimisch ranking of 2. Testing meets all criteria for quality testing, but lacks analytical confirmation of test substance concentrations. There is also a lack of characterization of the test sample.

Reviewers' remarks: none

REFERENCE

3M Environmental Laboratory. 1996. Microbics Microtox® Toxicity Test of FC-143. Lab Request number P1626. St. Paul, Minnesota.

OTHER

General Remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

TOXICITY TO MICROORGANISMS

Title: Microbics Microtox® Toxicity Test of FC-118

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, FC-143, or as the major component of FC-118. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)

Remarks: The test sample is FC-118. Its purity was not sufficiently characterized, though current information indicates it is 20% FC-143 in 80% water. The 3M product lot number was not noted. Data may not accurately relate toxicity of the test sample with that of the test substance.

METHOD

Method/guideline followed: Microbics Microtox® "BASIC" Procedure

Type (test type): static

GLP (Y/N): N

Year study performed: 1996

Species/strain: *Photobacterium phosphoreum*

Supplier: Microbics

Doses (concentrations) used: 0, 625, 1250, 2500, and 5000 mg/L. The concentrations were nominal. Two replicates were tested at each concentration.

Exposure period: 30 minutes

Analytical monitoring: none

Statistical methods: EC50 values calculated by statistical linear regression program provided for Microtox®.

Test Conditions: A primary 10 g/L stock solution was prepared in Millipore Milli-Q™ water. The pH of the stock solution was 6.0 (no adjustment) and an osmotic adjustment was made using 200 mg NaCl dissolved in 10 mL stock solution. Appearance of test solutions was noted as "clear and colorless." All test solutions were made by proportional dilutions. The test temperature was not noted. Cuvettes (3 mL) were used as the exposure vessels. Two replicates were tested at each concentration, as well as the control.

Remarks: The number of microbes per replicate and the growth phase of the microbes were not indicated.

RESULTS

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Dose of each endpoint (as mg/L): 5 minute EC50 = 4460 (4020 – 4950) mg/L
15 minute EC50 = 3360 (3090 – 3641) mg/L
30 minute EC50 = 3150 (2910 – 3420) mg/L

Was control response satisfactory (yes/no/unknown): unknown

Statistical results, as appropriate: none

Remarks: none

CONCLUSIONS

The FC-118 30 minute EC50 for *Photobacterium phosphoreum* was determined to be 3150 mg/L with a 95% confidence interval of 2910-3420 mg/L.

Submitters' remarks: For data reliability, the study was assigned a Klimisch rating of 2. Testing meets all criteria for quality testing, but lacks analytical confirmation of test substance concentrations. There is also a lack of characterization of the test sample.

Reviewers' remarks: none

REFERENCE

3M Environmental Laboratory. 1996. Microbics Microtox® Toxicity Test of FC-118. Lab Request number P1626. St. Paul, Minnesota.

OTHER

General Remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

TOXICITY TO MICROORGANISMS

Title: Microbics Microtox® Toxicity Test of FC-1015-X

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, FC-143, or as the major component of FC-1015 or FC-1015-X. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)

Remarks: The test sample is FC-1015-X. Its purity was not sufficiently characterized, though current information indicates it is a 30% straight carbon chain version of FC-143 in 80% water. The 3M product lot number was not noted. Data may not accurately relate toxicity of the test sample with that of the test substance. Data were used to compare toxicity of the branched/ straight chain ammonium perfulorooctanoate homolog mixture in FC-143 vs. FC-1015-X.

METHOD

Method/guideline followed: Microbics Microtox® "BASIC" Procedure

Type (test type): static

GLP (Y/N): N

Year study performed: 1996

Species/strain: *Photobacterium phosphoreum*

Supplier: Microbics

Doses (concentrations) used: 0, 416, 832, 1665, and 3330 mg/L. The concentrations were nominal. Two replicates were tested at each concentration.

Exposure period: 30 minutes

Analytical monitoring: none

Statistical methods: EC50 values calculated by statistical linear regression program provided for Microtox®.

Test Conditions: A primary 6.660 g/L stock solution was prepared in Millipore Milli-Q™ water. The pH of the stock solution was 6.9 and an osmotic adjustment was made using 200 mg NaCl dissolved in 10 mL stock solution. Appearance of test solutions was noted as "clear and colorless." All test solutions were made by proportional dilutions. The test temperature was not noted. Cuvettes (3 mL) were used as the exposure vessels. Two replicates were tested at each concentration, as well as two controls.

Remarks: The number of microbes per replicate and the growth phase of the microbes were not indicated.

RESULTS

Doses of each endpoint (as mg/L): 5 minute EC50 = 2300 (2070 - 2560) mg/L
15 minute EC50 = 1960 (1730 - 2210) mg/L
30 minute EC50 = 1950 (1760 - 2160) mg/L

Was control response satisfactory (yes/no/unknown): unknown

Statistical results, as appropriate: none

Remarks: none

CONCLUSIONS

The FC-1015-X 30 minute EC50 for *Photobacterium phosphoreum* was determined to be 1950 mg/L with a 95% confidence interval of 1760-2160 mg/L.

Submitters' Remarks: For data reliability, the study was assigned a Klimisch rating of 2. Testing meets all criteria for quality testing, but lacks analytical confirmation of test substance concentrations. There is also a lack of characterization of the test sample.

Reviewers' Remarks: none

REFERENCE

3M Environmental Laboratory. 1996. Microbics Microtox® Toxicity Test of FC-1015-X. Lab Request number P1626. St. Paul, Minnesota.

OTHER

General Remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of this study, are limited.

TOXICITY TO BACTERIA

Title: Activated Sludge Respiration Inhibition

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)

Remarks: The 3M production lot number was 37. The test sample was FC-143. The purity of the substance was not sufficiently characterized, though current information indicates it was a mixture of 96.5 – 100% test substance and 0 – 3.5% C_6, C_7, and C_9 perfluoro analogue compounds.

METHODS

Method/guideline followed: Protocol reference EAR, 11/8/79

Test type: Not noted

GLP (Y/N): No

Year study performed: 1980

Test organism: Activated sludge mixed liquor

Source: Metro Wastewater Treatment Plant, St. Paul, MN.

Concentrations tested: Blank control, 1000 mg/L

Exposure period: Seven minutes

Analytical monitoring: None

Statistical methods: Graphed dissolved oxygen versus time in minutes.

Test conditions: Not noted other than the stock solution was prepared with 0.6 g FC-143 diluted to 100 mL with D.I. water.

Remarks: No further details on testing methods were provided by the submitter.

RESULTS

Dose of each endpoint (as mg/L): No acute inhibitory effect on activated sludge respiration rate at 1000 mg/L with a contact time of 7 minutes.

Remarks: Results based on nominal concentrations.

Was control response satisfactory (yes/no/unknown): unknown

Statistical results, as appropriate: Not noted

CONCLUSIONS

Limited contact time estimates that ammonium perfluorooctanoate is not expected to inhibit the activity of activated sludge.

Submitters' remarks: Klimisch ranking 3. Testing lacks record of methodology used. The method was not an agency approved method. There is lack of characterization of the test sample and test solutions not analyzed for test substance concentrations.

Reviewers' remarks: No EC50 or other effect level was given.

REFERENCE

3M Company. [No title given]. Lab request number 5625S. St. Paul, MN.

OTHER

General remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

TOXICITY TO BACTERIA

Title: Activated Sludge Respiration Inhibition Test

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; also referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS# 3825-26-1.)

Remarks: The 3M production lot number is 390. The test sample was FC-126, a white powdery solid. The purity was not completely characterized, although information suggested it was a mixture of 78-93 percent test substance and 7-22 percent C5, C6, and C7 perfluoro analogue compounds.

METHODS

Method/guideline followed: OECD 209

Test type: Static

GLP (Y/N): No

Year study performed: 1987

Test Organism: Activated sludge mixed liquor was used the same day it was collected.

Source: Metro Wastewater Treatment Plant, St. Paul, MN.

Concentrations tested: 0, 100, 180, 320, 560, and 10000 mg/L test material solution. (Two blank controls - 0 mg/L - were used, as well as a reference substance.)

Exposure period: 30 minutes; 3 hours

Analytical monitoring: It was not stated whether the concentrations were monitored throughout the test.

Statistical methods: Not stated.

Test conditions:

- Dilution water was aerated, distilled, deionized water with a pH of 6.7
- Synthetic sewage was prepared according to OECD Guideline # 209
- Suspended solids were 2.7 g/L
    - Test solutions were prepared by adding individual weights to solutions containing 284 mL distilled water, 16 mL synthetic sewage feed, and 200 mL inoculum. The pH of the solutions was adjusted to 7.0 using 1 N NaOH. The reference solutions were created using 500 mg/L stock solution of 3,5-diochlorophenol.

- pH of the activated sludge mixed liquor was 7.8 (initial) and 8.0 (final); pH of the 1000 mg/L test concentration was 7.4 (initial) and 8.1(final).
- Temperature of the test was 20-21 C (for both 30 min and 3 hr).
- Exposure vessel type was not described.
- Number of replicates not stated, except that two blank controls were used.
- Respiration inhibition was determined by measuring dissolved oxygen consumption.

Remarks: Hardness of the dilution water was not presented.

RESULTS

Dose of each endpoint (as mg/L):

At 30 min: EC50 (for respiration inhibition) = > 1000 mg/L
At 3 hrs: EC50 (for respiration inhibition) = > 1000 mg/L

Remarks:
- A reference substance was used, but no results were presented.
- None of the doses resulted in 100 percent inhibition.

Was control response satisfactory (yes/no/unknown): Control response appeared adequate; less than 1 percent respiration rate inhibition occurred. Also, the difference between the two controls was 2.2 percent at 30 minutes and 0.8 percent at 3 hours; this meets the guideline criterion of being within 15 percent of each other.

Statistical results, as appropriate: none

CONCLUSIONS

The authors sate that the test material induced 38 percent inhibition in respiration rate at 1000 mg/L after 3 hours of exposure.

Submitters' remarks: The authors state that the Klimisch data quality ranking was 2, and that the study meets the criteria for quality testing. However, the study lacked characterization of the test substance purity and analytical confirmation of concentrations. Also, no results were presented for the reference compound.

Reviewers' remarks:

REFERENCE

3M Company. Activated Sludge Respiration Inhibition Test. Environmental Laboratory; Lab Request Number E1282. St. Paul, MN.

OTHER

General remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

TOXICITY TO BACTERIA

Title: Activated Sludge Respiration Inhibition

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be 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 3M production lot number was 2327. The test sample was FX-1003. The purity of the test substance was not sufficiently characterized, though available information indicated it was a solution of <45% ammonium perfluorooctanoate, 50% water, <3% inert perfluorinated compound and 1-2% C_5 and C_7 perfluoro- analogue compounds. The test substance was a clear liquid. The reference substance used for this study, 3,5-dichlorophenol, was Aldrich red label, lot number D7-060-0.

METHODS

Method/guideline followed: OECD 209

Test type: Static

GLP (Y/N): No

Year study performed: 1990

Test organism: Mixed liquor activated sludge was collected from Metro Wastewater Treatment Plant. The test organisms were used on the day obtained. The condition of the organisms was not specified.

Supplier: Metro Wastewater Treatment Plant

Concentrations tested: 0 (blank control) and 1000 mg/L FX-1003 were used for each exposure period (30 minutes and 3 hours). Two replicates of the blank control and one replicate of 1000 mg/L FX-1003 were used for each exposure period. A reference control, 3,5-dichlorophenol, was included at a concentration of 10 mg/L. One replicate of the reference control was used for each exposure period. Nominal concentrations of the test substance and reference control were used during this study.

Exposure period: 30-minutes and 3-hours

Analytical monitoring: Dissolved oxygen concentrations were monitored in order to determine respiration inhibition, as determined by oxygen consumption.

Statistical methods: Not specified

Test conditions: The inoculum contained 3.2 g/L of mixed liquor suspended solids. The test substance was created by a mass addition to a solution containing 284 mL Millipore Milli-Q® water, 16 mL

synthetic sewage feed, and 200 mL inoculum. The test substance was apparently miscible with water. A reference solution was created using a 500 mg/L stock solution of 3,5-dichlorophenol. The initial and final pH of the activated sludge mixed liquor was 6.7 and 7.1, respectively. The initial pH of the 1000 mg/L test solution was 7.3. The initial pH of the reference solution, 10 mg/L 3,5-dichlorophenol, was 7.5. The temperature range was 20 – 21°C during the study. Two replicates of the blank control, and one replicate of each concentration of FX-1003 (1000 mg/L) and 3,5-dichlorophenol (10 mg/L), were used for each exposure period (30 minutes and 3 hours).

Remarks: No additional comments

RESULTS

Dose of each endpoint (as mg/L): 30-minute EC50 >1000 mg/L
3-hour EC50 >1000 mg/L

Remarks: Testing was conducted on the mixture of the described test substance. The values reported apply to that mixture and not the test substance.

Was control response satisfactory (yes/no/unknown): Yes

Statistical results, as appropriate: No additional comments

CONCLUSIONS

The FX-1003 3-hour EC50 for activated sludge respiration inhibition was determined to be >1000 mg/L.

Submitters' remarks: Klimisch ranking 2. Testing meets all criteria for quality testing, but lacks analytical confirmation of test substance concentrations. There is a lack of characterization of the test sample. The testing procedure was not fully documented.

Reviewers' remarks: The conclusions appear to be supported by the data, however, limited data were available to adequately assess the study.

REFERENCE

3M Environmental Laboratory. 1990. Activated Sludge Respiration Inhibition. St. Paul, Minnesota. Lab Request number G2882.

OTHER

General remarks: This summary was based on a summary report and limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

TOXICITY TO BACTERIA

Title: Activated Sludge Respiration Inhibition Test

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA tetrabutylammonium salt, Ammonium perfluorooctanoate PFO, FC-116, FC-126, FC-169, FC-143, or as a major component of GC-1015 or FC-1015-X. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)

Remarks: The test sample was FC-1015-X. It's purity was not sufficiently characterized, though current information indicates it is a 30% straight chain version of FC-143 in 80% water. The 3M product lot number was "HOGE 205." Data were used to compare the toxicity of the branched/ straight chain ammonium perfluorooctanoate homologue mixture in FC-143 with what is supposed to be the 100% straight carbon chain ammonium perfluorooctanoate in FC-1015-X.

METHODS

Method/guideline followed: OECD Test #209

Test type: static

GLP (Y/N): no

Year study performed: 1996

Test Organism: activated sludge

Supplier: The activated sludge mixed liquor was collected from the Metro Wastewater Treatment Plant in St. Paul, MN.

Concentrations tested: 420, 840, 1660, and 3320 mg/L. Two blank controls and a reference substance were also tested. The concentrations were nominal.

Exposure period: 30 minutes and 3 hours

Analytical monitoring: none

Statistical methods: none

Test conditions: The dilution water used was Millipore Milli-Q™ water. A stock solution of the reference substance, 3,5-dichlorophenol, was prepared by dissolving 500 mg in 10 mL 1N NaOH, diluted to 30 mL, then brought to the point of incipient precipitation with 1N H_2SO_4 and diluted to 1L. The pH of the reference solution was measured to be 7.2. The test substance was added directly to the test vessels.

Synthetic sewage per OECD guidelines (Test #209) was used.

The temperature ranged from 19.1 to 22.1 °C. Initially, total suspended solids were measured at 3.22 g/L. At the end of the test, TSS = 1.3 g/L. The initial pH = 7.8, while the final pH = 7.9. The water hardness was not indicated.

The element basis was respiration inhibition as determined by oxygen consumption.

Remarks: Authors reported that values were corrected to 20C for calculations.

RESULTS

Dose of each endpoint (as mg/L): 30 min EC50 > 3320 mg/L
3 h EC50 > 3320 mg/L

Remarks: Testing was conducted on the mixture of the test substance as described in the test substance remarks field. The values reported apply to that mixture and not the test substance. A reference substance of 3,5-dichlorophenol was used, but EC50 values were not reported.

Was control response satisfactory (yes/no/unknown): unknown

Statistical results, as appropriate: none

CONCLUSIONS

The FC-1015-X 3 hour EC50 for activated sludge respiration inhibition was determined to be greater than 3320 mg/L.

Submitters' remarks: The study was assigned a Klimisch ranking of 2. Testing meets all criteria for quality testing, but lacks analytical confirmation of test substance concentrations. There is a lack of characterization of the test sample.

Reviewers' remarks: none

REFERENCE

3M Company Environmental Laboratory. 1996. Lab Request N2169. St. Paul, MN.

OTHER

General remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

MICROBICS' MICROTOX® TOXICITY TEST
TEST SUBSTANCE
Identity: Perfluorooctanoic acid, tetrabutylammonium salt; may also be referred to as PFOA tetrabutylammonium salt, tetrabutylammonium perfluorooctanoate, N2803-2, or as a major component of L-13492. (Octanoic acid, pentadecafluoro-, tetrabutylammonium salt, CAS # 95658-53-0)
Remarks: The 3M production lot number was 2 . The test sample is referred to by the testing laboratory as L-13492. The purity of the sample was not sufficiently characterized, although current information indicates it is a solution of 44.9% tetrabutylammonium perfluorooctanoate, 27.9% water, and 27.2% isopropanol.
The following summary applies to the test sample as a mixture of the test substance in an isopropanol/water solution with incompletely characterized concentrations of impurities. Data may not accurately relate toxicity of the test sample with that of the test substance.
METHOD:
Method: Microbics' Microtox® "BASIC" Procedure
GLP: No
Year Completed: 1995
Species: *Photobacterium phosphoreum*
Analytical monitoring: pH, light output
Replicates: 2
Statistical methods: EC50 values calculated by statistical linear regression program provided for Microtox®
Test organism source: Microbics Corporation, Carlsbad, CA
Test Conditions:
Dilution water: Millipore Milli-Q TM water.
Stock and test solution preparation: A primary 2000 mg/L stock solution was prepared in Millipore Milli-Q™ water. The pH of the stock solution was then adjusted from 4.7 to 6.7 using 0.1 N NaOH and an osmotic adjustment was made using 200 NaCl dissolved in 10 mL stock solution. Appearance of test solutions was noted as "clear and colorless". A ll test solutions were made by proportional dilutions.
Exposure vessels: 4 mL glass cuvettes.
Number of replicates: Two
Number of concentrations: Four plus blank control.
Element Basis: Percent light loss.
RESULTS
Nominal concentrations: Blank control, 125, 250, 500, 1000 mg/L
Element value and 95% confidence interval:
5-minute EC50 = 630 (590 - 665) mg/L
15-minute EC50 = 300 (270 - 330) mg/L
30-minute EC50 = 260 (220 - 300) mg/L
Element values based on nominal concentrations.
Remarks: Testing was conducted on a mixture as described in the Test Substance Remarks field. The values reported apply to that mixture and not the fluorochemical component alone.
CONCLUSIONS

The test sample 30-minute EC50 for *Photobacterium phosphoreum* was determined to be 260 mg/L with a 95% Confidence Interval of 220-300 mg/L.

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking 2. Testing meets all criteria for quality testing, but lacks analytical confirmation of test substance concentrations in the test solutions and sample purity is not sufficiently characterized.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, St Paul, MN, Lab Request number N2169, 1995

OTHER