SUMMARY OF A STUDY CONDUCTED WITH POTASSIUM PERFLUOROALKYL SULFONATE (PFOS) AT DUPONT

Type:	**Biopersistence Screening Study**
Species/Strain:	Rats/Crl:CD®SD(IGS)BR
Sex/Number:	Male/5/group
Exposure Period:	5 or 10 days; 94 days of recovery
Frequency of Treatment:	*Ad libitum* for 5 or 10 days
Exposure Levels:	Ammonium perfluorooctanoate: 20 mg/kg Potassium perfluoroalkyl sulfonate (PFOS): 10 mg/kg
Method:	Six groups of rats were administered ammonium perfluorooctanoate, 1 group received the test substance for 5 consecutive days and the other 5 groups received the test substance for 10 days. Approximately 2 hours after the first dose, blood was collected from each rat from the group designated for 5 doses. These same rats were sacrificed on test day 5 approximately 2 hours post-dosing, and blood and livers were collected. The remaining groups received the test substance for 10 days. Five rats were sacrificed and had blood and livers collected on test days 10 (2 hours post-dose), 13, 24, 52, or 93/94. The total fluorine content of the blood was determined by using a Wickbold torch combustion method, followed by analysis with a fluoride ion selective electrode. Body weights and clinical signs were recorded throughout the test. Liver weights were recorded, but not reported. Six groups of rats were administered potassium perfluoroalkyl sulfonate as described above. Food and water were available *ad libitum* throughout the test. Corn oil was used as the vehicle for the ammonium perfluorooctanoate test groups. Potassium perfluoroalkyl sulfonate was dissolved in acetone before suspending it in corn oil. The ratio of acetone to corn oil was 20:80. Negative controls of corn oil and corn oil/acetone were also tested as described above.
GLP:	No
Test Substance:	Ammonium perfluorooctanoate, purity 93-97% Potassium perfluoroalkyl sulfonate, purity 82-86% CAS #2795-39-3, 3-8% CAS #3871-99-6, 3-7% CAS #29420-49-3, 1-3% CAS #60272-25-1
Results:	Rats dosed with ammonium perfluorooctanoate exhibited wet perineum and diarrhea during the dosing period. Alopecia was observed during the recovery period. The normalized µM equivalents (blood organofluoride levels) in rat blood peaked after

5 days of dosing and then decreased throughout the testing period. The Cmax was 518.12 ± 44.89 ppm with a terminal half-life of 8.3 days. The AI (Accumulation Index) was 12.5 and the BI (Bioaccumulation) was 6497.5. An area under the curve (estimated to infinity) (AUCINF/D) was calculated and normalized for fluorine content. The AUCINF/D was calculated as 70,789.6.

Rats dosed with potassium perfluoroalkyl sulfonate exhibited diarrhea, salivation, alopecia, black ocular discharge, and staining of various parts of the body during the dosing period. Alopecia was observed during the recovery period. The normalized μM equivalents (blood organofluoride levels) in rat blood continued to rise throughout the dosing period and may not have reached steady-state. The Cmax was 989.85 ± 116.90 ppm with a terminal half-life of 40.5 days. The AI was 59.0 and the BI was 58382.2. The AUCINF/D was calculated as 566,479.1.

Reference: DuPont Co. (2000). Unpublished Data, Haskell Laboratory Report No. 2922.

SUMMARY OF STUDIES CONDUCTED WITH AMMONIUM PERFLUOROOCTANOATE AT DUPONT

Ecotoxicity

Type:	**Biodegradation**
Value:	13% at Day 28
Breakdown products:	CO_2
Method:	Modified Strum Test (OECD 301 B). In this test, biodegradability was measured as CO_2 evolution. Activated sludge was used as the inoculum.
GLP:	No
Test Substance:	Ammonium perfluorooctanoate, purity assumed 100%
Results:	Ammonium perfluorooctanoate (30% solution) did not demonstrate "Ready Biodegradability." Note that failure to pass a "Ready Biodegradability Test" does not imply that the test substance will persist in the environment for an extended period of time.

The toxicity test, which included both the test substance and the reference chemical (sodium benzoate) in the same flask, yielded < 25% biodegradation within 14 days; therefore, ammonium perfluorooctanoate was considered inhibitory to microorganisms in the inoculum.

Reference: DuPont Co. (1997). Unpublished Data, AEM Laboratory Report No. 24-97.

Type: **Adsorption-Desorption Screening Studies**

Method: Ammonium perfluorooctanoate adsorption/desorption screening studies were performed on several test materials, including two clays, a washed sand, peat moss, and an agricultural soil from Delaware, USA. The adsorption/desorption screening studies were also performed using two surface soils and two sediments from the Ohio River collected in the vicinity of the DuPont Washington Works plant near Parkersburg, West Virginia.

The adsorption/desorption procedure used in this study was adapted from a method developed by the Office of Prevention, Pesticides and Toxic Substances (OPPTS), United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data (US EPA (1998). Fate, transport and transformation test guidelines: OPPTS 835.1220 sediment and soil adsorption/desorption isotherm, United State Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances, EPA712-C-98-048).

Except for the clays, all samples were screened through a 2-mm (10 mesh) stainless steel sieve in preparation for the absorption/desorption screening test. Furthermore, the sand was washed using DI (deionized) water through 2-mm and 50-µm stainless steel sieves. Material retained on the 50 µm sieve was used in the study. The sand, soils, and sediments used in the studies were air-dried prior to use. Data calculations, however, were based on oven-dry weights of the samples. Oven-dry weights were determined after drying a subsample of material in an oven at 105°C for a minimum of 24 hours.

The pH of the samples was determined in a ratio of 1:1 (test substance to water). Percent organic matter was determined using the loss on ignition method.

Screening Test: Adsorption Studies

The adsorption test was performed in duplicate on each material. A blank containing 0.01M $CaCl_2$ solution with test materials and no ammonium perfluorooctanoate and a single control at each ammonium perfluorooctanoate concentration but no material were also included. Each material was equilibrated with the aqueous phase of a solution of the test substance at 50, 500, and 5000 µg/L

prepared in 0.01M $CaCl_2$

Sterile 50 mL polypropylene centrifuge tubes were used as the test vessels. One part air-dried material (4.0 g) was weighed and 5 parts test solution (20 mL) were decanted into centrifuge tubes, except the control tube. The centrifuge tubes were secured on an end-over-end mixer and agitated at approximately 30 rpm for 24 hours. Samples were centrifuged for 20 minutes at 4000 x g, and then filtered through a 0.2 µm nylon syringe filter into a new centrifuge tube. The volume of aqueous supernatant was measured and refrigerated at approximately 5°C until analyzed for the parent compound using an LC-MS. The blank and the control tubes were subjected to the same steps as the test systems, including filtering.

Screening Test: Desorption Studies

A fresh volume of 20 mL of 0.01M $CaCl_2$ solution without the test substance was added to each solid phase (pellet). The sample was mixed, centrifuged, filtered, stored, and analyzed as was done in the adsorption studies. This desorption step was repeated a second time, resulting in two washings that were analyzed separately.

GLP: No

Test Substance: Ammonium perfluorooctanoate, purity not applicable

Results: Five of 9 materials tested for the adsorption/desorption characteristics of ammonium perfluorooctanoate (peat moss, Upstream sediment, East Wood soil, Garden Area soil, and Kaolin clay) exhibited significant adsorption (> 25% of ammonium perfluorooctanoate added) at one or more of the added concentrations of ammonium perfluorooctanoate. Sand, Montmorillonite clay, Downstream sediment, and Sassafras soil did not adsorb a significant amount of ammonium perfluorooctanoate at any concentration.

Only peat moss, however, adsorbed a significant amount at all three concentrations of added ammonium perfluorooctanoate. Kaolin clay adsorbed a significant amount of ammonium perfluorooctanoate at 500 and 5000 µg/L. The Garden Area soil collected at Washington Works also adsorbed significant amounts of ammonium perfluorooctanoate at 500 µg/L. Upstream sediments from the Ohio River and East Wood soil adsorbed a significant amount of ammonium perfluorooctanoate at 50 µg/L only.

Once adsorbed on peat moss, most of the ammonium perfluorooctanoate (> 76%) did not desorb after two washings with a solution of 0.01M $CaCl_2$. For the Kaolin clay, most of the

ammonium perfluorooctanoate adsorbed when added at 5000 µg/L desorbed with washing (> 60%). At ammonium perfluorooctanoate added at 500 µg/L, however, an average of 98% did not desorb from the Kaolin clay after two washings. The East Wood soil, which adsorbed 46% of the ammonium perfluorooctanoate added at 50 µg/L desorbed only about 2%. For the Garden Area soil 57% of the ammonium perfluorooctanoate desorbed when adsorbed from the 500 µg/L solution. No measurable desorption of ammonium perfluorooctanoate from the Upstream sediment after two washings was observed when the ammonium perfluorooctanoate was added at 50 µg/L.

Reference: DuPont Co. (2000). Unpublished Data, Report No. EMSE-053-00.

Acute Toxicity to Fish

Type: **96-hour LC_{50}**
Species: *Lepomis macrochirus* (bluegill sunfish)
Value: 634 mg/L (95% fiducial interval, 567-725 mg/L)
Method: A rangefinding study was conducted using nominal test concentrations of 0, 0.1, 1, 10, 100, and 1000 mg/L.

For the definitive study, nominal test concentrations were 0, 262, 328, 410, 512, 640, 800, and 1000 mg/L. Glass aquaria (20 L) containing 10 L of test solution were employed. Positions of test chambers in the water bath used to maintain constant temperature were assigned using random numbers. Ten fish were added to each replicate using random numbers (2 replicates per concentration; total 20 fish per concentration). Control fish ranged from 1.2-2.8 cm standard length (mean 2.1 cm) and 0.0451-0.524 g wet weight (mean 0.228 g). Control loading was 0.23 g/L at test conclusion. Fish were not fed approximately 24 hours prior to and during the test. A photoperiod of 16 hours light (312-344 Lux) versus 8 hours darkness was employed with 25 minutes of transitional light (<2.15 Lux) preceding and following the 16-hour light interval. Observations for mortality and behavioral effects were made daily.

Dissolved oxygen, pH, and temperature were measured in each replicate before addition of fish at test start, daily, at total fish mortality, and/or at test end. Total alkalinity, EDTA hardness, and conductivity of the control water were measured before fish were added at test start. A continuously recording thermometer in a water-control replicate was used to check temperature variations during the 96-hour test. Chemical analyses of the test substance concentrations in water were not performed.

The LC_{50} value and its 95% fiducial interval were calculated.

GLP: Yes

Test Substance: Ammonium perfluorooctanoate, purity 99%

Results: Mortality in the rangefinding study after 96 hours was 0, 0, 0, 0, 0, and 80% at 0, 0.1, 1, 10, 100, and 1000 mg/L, respectively.

Total mortality was 0, 5, 10, 15, 30, 40, 65, and 95% in the 0, 262, 328, 410, 512, 640, 800, and 1000 mg/L concentrations, respectively. All deaths occurred within 24 hours, with the exception of 1 death at 48 hours at 1000 mg/L. No behavioral effects were noted at ≤ 640 mg/L. Surviving fish at 800 and 1000 mg/L were lethargic.

All chemical and physical parameters were within expected ranges. Total alkalinity, EDTA hardness, and conductivity of the dilution water control were 79 mg/L $CaCO_3$, 76 mg/L $CaCO_3$, and 165 µmhos/cm, respectively. During the test, dissolved oxygen concentrations ranged from 6.7-8.5 mg/L, pH ranged from 6.9-7.4, and temperature ranged from 21.4-22.1°C.

Reference: DuPont Co. (1994). Unpublished Data, Haskell Laboratory Report No. 61-94.

Type: **96-hour LC_{50}**

Species: *Oncorhynchus mykiss* (rainbow trout)

Value: 4001 mg/L (95% confidence interval, 3327-4932 mg/L)

Method: A static rangefinding study with 5 fish per concentration was conducted using a dilution water control and nominal concentrations of 50, 100, 500, 1000, and 5000 mg/L.

Nominal concentrations of 625, 1250, 2500, 5000, and 10,000 mg/L (20% solution of ammonium perfluorooctanoate in water) were chosen for the definitive test based on the results of the preliminary rangefinding study.

Test chambers were stainless steel aquaria that held approximately 9 L of test solution. Two replicate test chambers were used per test concentration with 10 fish in each chamber (total of 20 fish per concentration). Each chamber was covered with a glass plate to prevent fish from escaping. Random numbers were used to assign test concentrations to the test chambers and position of test concentrations in the water bath.

Rainbow trout used in this study were not fed approximately 29 hours prior to and during the test, and were assigned to the test chambers using random numbers. Addition of fish to the test solutions was initiated approximately 41 minutes after mixing of

the test solutions was completed. Mortality and behavioral observations were made at test start, every 24 hours thereafter, and at test end. At test conclusion, all surviving fish were sacrificed.

A recirculating water bath was used to maintain mean temperature in the test chambers during the 96-hour test. In addition, a continuously recording thermometer was used to check for temperature variation in 1 replicate of the dilution water control. A photoperiod of 16 hours light (approximately 199-450 Lux) and 8 hours darkness was employed, which included 30 minutes of transitional light (11-157 Lux) preceding and following the 16-hour light interval.

Dissolved oxygen, pH, and temperature were measured in all replicate chambers of the control and test substance concentrations. These measurements were taken before fish were added at test start, every 24 hours thereafter, and at test end or at total mortality in a concentration. Total alkalinity, EDTA hardness, and conductivity of the water were measured before fish were added at the beginning of the test. Test solutions were not aerated during the test.

Concentrations of ammonium perfluorooctanoate were measured directly by high performance liquid chromatography/tandem mass spectrometry (LC/MS/MS).

At test conclusion, fish from the water control ranged from 2.5-3.2 cm in standard length (mean 2.8 cm) and 0.15-0.30 g in wet weight, blotted dry (mean 0.21 g). Standard length of the longest fish was not more than twice the length of the shortest fish in the control. Loading in the water control was 0.23 g/L at test conclusion.

The LC_{50} value was calculated using the moving average-angle method.

GLP: Yes

Test Substance: Ammonium perfluorooctanoate, purity 99.4%

Results: At the end of 96 hours in the rangefinding study, the mortality was 0, 0, 0, 0, 0, and 60% at 0, 50, 100, 500, 1000, and 5000 mg/L, respectively. Test substance solutions were clear and colorless with no insoluble test substance present during the study.

In the definitive study, mean measured concentrations of ammonium perfluorooctanoate were 554, 1090, 2280, 4560, and 9360 for the 625, 1250, 2500, 5000, and 10,000 mg/L dose levels, respectively. Control solutions showed no detectable

concentrations of ammonium perfluorooctanoate. All test substance solutions were clear and colorless with no insoluble test substance present during the test.

All chemical and physical parameters for the definitive test were within expected ranges. Total alkalinity, EDTA hardness, and conductivity of the dilution water control were 49 mg/L $CaCO_3$, 122 mg/L $CaCO_3$, and 240 µmhos/cm, respectively. During the test, dissolved oxygen concentrations ranged from 7.5-11.2 mg/L, pH ranged from 7.1-7.2, and mean temperature was 11.8°C (range 11.6-12.1°C).

No mortality or sublethal effects were seen in the dilution water control fish. No mortality or sublethal effects were observed at concentrations ≤ 2500 mg/L. At 5000 mg/L, mortality was 8/20, 11/20, 14/20, and 15/20 at 24, 48, 72, and 96 hours, respectively. At 10,000 mg/L all fish were dead by 24 hours.

Surviving fish exposed to 5000 mg/L exhibited sublethal effects such as swimming at the surface, labored respiration, dark coloration, lethargy, partial loss of equilibrium, rapid respiration, and erratic swimming.

Reference: DuPont Co. (1999). Unpublished Data, Haskell Laboratory Report No. Dupont-3381.

Mammalian Toxicity

Acute Toxicity

Type: **Oral LD_{50}**

Species/strain: Male rats/Crl:CD®

Value: Male rats receiving ammonium perfluorooctanoate alone: 478 mg/kg (95% confidence limits, lower 361 mg/kg, upper 571 mg/kg, slope 7.9)

Male rats receiving ammonium perfluorooctanoate and pre-treatment of phenobarbital sodium: 547 mg/kg (95% confidence limits, lower 517 mg/kg, upper 582 mg/kg, slope 22.1)

Male rats receiving ammonium perfluorooctanoate and pre-treatment of proadifen hydrochloride: 520 mg/kg (95% confidence limits, lower 450 mg/kg, upper 618 mg/kg, slope 9.8)

Method: Ammonium perfluorooctanoate was administered by intragastric intubation as a suspension in corn oil in single doses to 10 rats/group at concentrations of 400, 500, or 650 mg/kg. Another 30 rats, 10/group, were treated by single intraperitoneal injections

with aqueous solutions of phenobarbital sodium at 80 mg/kg/day for 3 days. One day after the final treatment, the rats were administered ammonium perfluorooctanoate by intragastric intubation using the same treatment as described above. An additional group of 30 rats, 10/group, were treated with an intraperitoneal injection of aqueous solutions of proadifen hydrochloride at 50 mg/kg. One hour after this treatment, ammonium perfluorooctanoate was administered by intragastric intubation, using the same treatment as described above. The surviving rats from all treatments were weighed and observed during a 14-day recovery period and then sacrificed. The LD_{50} values were calculated from the mortality data.

The phenobarbital sodium phase of the study was repeated. The rats from both phases were combined and the LD_{50} value was based on all 60 rats.

GLP: No

Test Substance: Ammonium perfluorooctanoate, purity approximately 100%

Results: There were no significant differences in the LD_{50} values of ammonium perfluorooctanoate, either when tested alone or following pre-treatment with either phenobarbital sodium or proadifen hydrochloride.

Mortality ratios of 2/10, 7/10, and 8/10 were observed for the rats treated with 400, 500, and 650 mg/kg ammonium perfluorooctanoate, respectively. Wet and/or stained perineal area and weight loss were observed at all levels tested. Other clinical signs observed included stained face (500 and 650 mg/kg), weakness (500 and 650 mg/kg), and chromodacryorrhea (500 mg/kg). All deaths occurred within 6 days after dosing.

Mortality of 0/20, 4/20, and 19/20 was observed for the rats pretreated with phenobarbital sodium and then treated with 400, 500, and 650 mg/kg ammonium perfluorooctanoate, respectively. Wet and/or stained perineal area, stained face, diarrhea, and weight loss were observed at all levels tested. Other clinical signs observed included weakness (500 and 650 mg/kg) and lethargy (650 mg/kg). All deaths occurred within 4 days after dosing.

Mortality of 1/10, 5/10, and 8/10 was observed for the rats pretreated with proadifen hydrochloride and then treated with 400, 500, and 650 mg/kg ammonium perfluorooctanoate, respectively. Stained face, wet and/or stained perineal area, weight loss, and weakness were observed at all levels tested. Other clinical signs observed included tremors (400 mg/kg), chromodacryorrhea (400 mg/kg), diarrhea (500 and 650 mg/kg), and lacrimation

(650 mg/kg). All deaths occurred within 5 days after dosing.

Reference: DuPont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 567-81.

Type: **Oral LD_{50}**

Species/strain: Male and female rats/Crl:CD®

Value: Male rats: 470 mg/kg (95% confidence limits, lower 403 mg/kg, upper 536 mg/kg, slope 8.6)

Female rats: 482 mg/kg (95% confidence limits, lower 438 mg/kg, upper 541 mg/kg, slope 12.3)

Method: The test substance, as a suspension in corn oil, was administered by intragastric intubation in single doses to 6 groups of male and 6 groups of female young adult rats. Ten male and female rats/group were administered 200, 400, 450, 500, 670, or 1000 mg/kg of the test substance. The surviving rats were weighed and observed during a 14-day recovery period and then sacrificed. The LD_{50} values were calculated from the mortality data.

GLP: No

Test Substance: Ammonium perfluorooctanoate, purity approximately 100%

Results: Mortality ratios of 2/10, 3/10, 4/10, 6/10, 9/10, and 10/10 were found in the 200, 400, 450, 500, 670, and 1000 mg/kg male groups, respectively. Mortality ratios of 0/10, 0/10, 5/10, 7/10, 9/10, and 10/10 were found in the 200, 400, 450, 500, 670, and 1000 mg/kg female groups, respectively.

Clinical signs observed in male rats included stained and/or wet perineal area, weakness, and weight loss at all levels tested. Other clinical signs observed in the male rats included stained face (450 mg/kg and above), chromodacryorrhea (500 and 1000 mg/kg), hunched posture (500 mg/kg), morbundity (670 mg/kg), eyes half closed (670 mg/kg), and gasping (670 mg/kg). All deaths occurred within 4 days of dosing.

Clinical signs observed in female rats included stained and/or wet perineal area and weight loss at all levels tested. Stained face was observed at all levels except 200 mg/kg. Other clinical signs noted in female rats included alopecia (400 mg/kg), weakness (400, 450, 670, and 1000 mg/kg), hunched posture (450 and 670 mg/kg), chromodacryorrhea (670 mg/kg), eyes half closed (670 mg/kg), and ataxia (670 mg/kg). All deaths occurred within 3 days after dosing.

Reference: DuPont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 295-81.

Type:	**Oral LD_{50}**
Species/strain:	Male and female rats/Crl:CD®
Value:	Male newborn rats: 243 mg/kg (95% confidence limits, undefined, slope 55.7) Female newborn rats: 258 mg/kg (95% confidence limits, lower 244 mg/kg, upper 276 mg/kg, slope 19.9) Male weanling rats: 573 mg/kg (95% confidence limits, lower 514 mg/kg, upper 664 mg/kg, slope 8.0) Female weanling rats: 580 mg/kg (95% confidence limits, lower 470 mg/kg, upper 2258 mg/kg, slope 4.7) Male young adult rats: 470 mg/kg (95% confidence limits, lower 403 mg/kg, upper 536 mg/kg, slope 8.6) Female young adult rats: 453 mg/kg (95% confidence limits, lower 413 mg/kg, upper 503 mg/kg, slope 15.0) Male older rats: 336 mg/kg (95% confidence limits, undefined) Female older rats: 343 mg/kg (95% confidence limits, lower 285 mg/kg, upper 390 mg/kg, slope 8.0)
Method:	The test substance, as a suspension in corn oil, was administered by intragastric intubation in single doses to groups of rats. The groups (10 rats/group) were male and female newborn rats (< 2 days old), male and female weanling rats (21 days old), male and female young adults (~8-10 weeks old), and male and female older rats (age undefined). Male newborn rats were administered 130, 200, 240, 280, 330, or 370 mg/kg. Female newborn rats were administered 130, 160, 200, 220, 240, 280, or 320 mg/kg. Male weanling rats were administered 350, 400, 450, 525, 670, or 710 mg/kg. Female weanling rats were administered 350, 400, 450, or 670 mg/kg. Young adult rats were administered 350, 425, 500, or 670 mg/kg. Young adult male rat data are reported in DuPont Report No. 295-81. Older male rats were administered 200, 240, 300, 350, 400, 500, or 720 mg/kg. Older female rats were administered 225, 350, 400, 450, or 670 mg/kg. The surviving rats were weighed and observed during a 14-day recovery period and then sacrificed. The LD_{50} values were calculated from the mortality data.
GLP:	No
Test Substance:	Ammonium perfluorooctanoate, purity approximately 100%
Results:	Mortality ratios for male newborn rats were 0/10, 0/10, 0/10, 10/10,

10/10, and 10/10 for the 130, 200, 240, 280, 330, and 370 mg/kg groups, respectively. No clinical signs were reported. Deaths occurred up to 6 days after dosing.

Mortality ratios for female newborn rats were 0/10, 0/10, 0/10, 0/10, 5/10, 6/10, and 10/10 for the 130, 160, 200, 220, 240, 280, and 320 mg/kg groups, respectively. No clinical signs were reported. Deaths occurred up to 4 days after dosing.

Mortality ratios for male weanling rats were 0/10, 2/10, 2/10, 3/10, 7/10, and 8/10 for the 350, 400, 450, 525, 670, and 710 mg/kg groups, respectively. Stained and wet perineal area, weakness, and weight loss were observed. Deaths occurred up to 3 days after dosing.

Mortality ratios for female weanling rats were 1/10, 3/10, 3/10, and 6/10 for the 350, 400, 450, and 670 mg/kg groups, respectively. Stained and wet perineal area, stained face, weakness, and weight loss were observed. Deaths occurred up to 3 days after dosing.

Mortality ratio data for the young adult males are covered in DuPont Report No. 295-81.

Mortality ratios for female young adult rats were 1/10, 2/10, 8/10, and 10/10 for the 350, 425, 500, and 670 mg/kg groups, respectively. Stained and wet perineal area, stained face, nasal discharge, diarrhea, and weight loss were observed. Deaths occurred up to 4 days after dosing.

Mortality ratios for the older male rats were 0/10, 0/10, 0/10, 9/10, 10/10, 10/10, and 10/10 for the 200, 240, 300, 350, 400, 500, and 720 mg/kg groups, respectively. Stained face, stained and wet perineal area, weakness, tremors, lethargy, chromodacryorrhea, diarrhea, and weight loss were observed. Deaths occurred up to 9 days after dosing.

Mortality ratios for the older female rats were 1/10, 5/10, 6/10, 9/10, and 10/10 for the 225, 350, 400, 450, and 670 mg/kg groups, respectively. Stained face, stained and wet perineal area, weakness, tremors, lethargy, and weight loss were observed. Deaths occurred up to 5 days after dosing.

References:

DuPont Co. (1982). Unpublished Data, Haskell Laboratory Report No. 788-82.

DuPont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 295-81.

Type:	**Oral LD_{50} Rangefinder and Liver Weight Comparison Test**
Species/strain:	Male and female rats/Crl:CD®
Value:	Male rats: 439 mg/kg (confidence limits, lower 294 mg/kg, upper 554 mg/kg, slope 6.1) Castrated male rats: 491 mg/kg (confidence limits, lower 276 mg/kg, upper 619 mg/kg, slope 6.7) Female rats: 459 mg/kg (confidence limits, lower 315 mg/kg, upper 607 mg/kg, slope 5.2) Overiectomized female rats: 400 mg/kg (confidence limits, lower 259 mg/kg, upper 494 mg/kg, slope 7.2)
Method:	In an LD_{50} rangefinder test, the test substance, as a suspension in corn oil, was administered by intragastric intubation in single doses to normal male rats, castrated male rats, normal female rats, and ovariectomized female rats. Dose levels of 200, 480, and 670 mg/kg were tested in each of the test systems mentioned above (10 rats/group). In the liver weight comparison test, 5 rats/group were administered the test substance, as a suspension in corn oil, as a single dose. Male groups were defined as male control, male 100 mg/kg, male 200 mg/kg, male castrated control, and male castrated 200 mg/kg. Female groups were defined as female control, female 100 mg/kg, female 200 mg/kg, female ovariectomized control, and female ovariectomized 200 mg/kg. All rats were weighed and observed during a 14-day recovery period. The rats were then sacrificed and the livers were weighed.
GLP:	No
Test Substance:	Ammonium perfluorooctanoate, purity approximately 100%
Results:	In the LD_{50} study, mortality in the male rats occurred in 0/10, 7/10, and 8/10 rats in the 200, 480, and 670 mg/kg groups, respectively. Mortality in the castrated male rats occurred in 0/10, 5/10, and 8/10 rats in the 200, 480, and 670 mg/kg groups, respectively. Mortality of the female rats occurred in 0/10, 7/10, and 7/10 rats in the 200, 480, and 670 mg/kg groups, respectively. Mortality in the ovariectomized female rats occurred in 0/10, 8/10, and 9/10 mg/kg at 200, 480, and 670 mg/kg, respectively. In the liver weight comparison study tested at dose levels of 0, 100, and 200 mg/kg, no changes in liver weight or in liver to body weight ratios were seen in the female rats given single doses of up to 200 mg/kg (ovariectomized or normal). A single oral dose of either 100 or 200 mg/kg produced an increase in liver weight of

male rats. Castration reduced the magnitude of the liver weight increase, but rats castrated and given 200 mg/kg had heavier livers than did the controls. Clinical signs seen in the ammonium perfluorooctanoate-treated rats included stained face and perineal area, and sporadic weight loss. No deaths occurred in this phase of the experiment.

Reference: DuPont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 600-81.

Type: **Acute Oral Toxicity**
Species/strain: Male rats/Crl:CD®
Method: The test substance, ammonium perfluorooctanoate (3M) and ammonium perfluorooctanoate (Rimar Co.), as suspensions in corn oil, were administered by intragastric intubation in single doses to young adult male rats. Rats (10/group) were administered 200, 480, or 670 mg/kg of the test substance. The surviving rats were weighed and observed during a 14-day recovery period and then sacrificed.
GLP: No
Test Substance: Ammonium perfluorooctanoate (3M), purity approximately 100%

Ammonium perfluorooctanoate (Rimar Co.), purity 95% minimum
Results: Mortality ratios of 0/10, 6/10, and 10/10 were found in the 200, 480, and 670 mg/kg ammonium perfluorooctanoate (3M) groups, respectively. Mortality ratios of 0/10, 10/10, and 10/10 were found in the 200, 480, and 670 mg/kg ammonium perfluorooctanoate (Rimar Co.) groups, respectively.

Clinical signs observed in ammonium perfluorooctanoate (3M) rats included stained and/or wet perineal area, stained face, and weight loss at all levels tested. Other clinical signs observed included weakness (480 and 670 mg/kg), diarrhea (480 mg/kg), and alopecia (200 mg/kg). All deaths occurred within 4 days after dosing.

Clinical signs observed in ammonium perfluorooctanoate (Rimar Co.) rats included stained and/or wet perineal area, stained face, and weight loss at all levels tested. Other clinical signs noted included weakness (480 and 670 mg/kg) and lethargy (670 mg/kg). All deaths occurred within 4 days after dosing.

Reference: DuPont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 565-81.

Type: **Oral ALD (Approximate Lethal Dose)**
Species/strain: Male rats/ChR-CD
Value: 670 mg/kg
Method: The test substance, as an aqueous solution (pH ~6), was

	administered by intragastric intubation to young adult male rats (1 rat/group) in single doses. Concentrations tested were 1, 1.5, 2.3, 3.4, 5.1, 26, 40, 60, 77, 90, 120, 130, 170, 200, 300, 450, 670, and 2250 mg/kg. Survivors were sacrificed 14 days later, and body weights and liver weights were recorded.
GLP:	No
Test Substance:	Ammonium perfluorooctanoate, purity not specified
Results:	Deaths did not occur at dose levels of 1-450 mg/kg. The rat dosed with 670 mg/kg died 2 days after dosing, and the rat dosed with 2250 mg/kg died 1 day after dosing. Slight initial weight losses occurred at 26 mg/kg and above for 1-2 days. At 90 mg/kg and above, the feces were small and irregularly-shaped for 1-6 days after dosing, and the rats were uncomfortable for 1-5 days after dosing. The compound caused liver enlargement in rats with single doses as low as 60-90 mg/kg. Based on clinical signs, it also acted as a gastrointestinal irritant. Additional toxic signs at the lethal doses (670 and 2250 mg/kg) included chewing motions, polyuria, increased respiration rate, and face-pawing on the day of dosing. Shovel-nosing was observed at 2250 mg/kg and slight salivation after dosing occurred at 670 mg/kg.
Reference:	DuPont Co. (1968). Unpublished Data, Haskell Laboratory Report No. 128-68.

Type:	**Oral ALD**
Species/strain:	Male rats/ChR-CD
Value:	670 mg/kg
Method:	The test substance was administered by intragastric intubation to male rats (1 rat/group) in single doses. Concentrations tested were 1.5, 12, 40, 120, 200, 300, 450, 670, 1000, 1500, and 2250 mg/kg. Survivors were sacrificed 14-15 days later, and body weights and liver weights were recorded.
GLP:	No
Test Substance:	Ammonium perfluorooctanoate, purity 99%
Results:	Mortality occurred at 670 mg/kg and above. Clinical signs of toxicity included inactivity (120, 1000, 1500, and 2250 mg/kg), red discharge around the nose (670, 1500, and 2250 mg/kg), perineal discoloration (300 and 450 mg/kg), ruffled fur (300 and 450 mg/kg), irritability (670 mg/kg), and weight loss (40, 200, 300, 450, 670, and 1000 mg/kg). Animals sacrificed 14-15 days after having received doses down to and including 200 mg/kg had enlarged livers when compared to control rats weighing between 500-550 g with liver weights of 22.6, 20.7, 19.1, and 21.7 g and liver weight/body weight percentages of 4.2, 3.8, 3.6, and 3.8, respectively. Liver weights at 200, 300, and 450 mg/kg were 30.6,

25.2, and 28.2 g, with liver weight/body weight percentages of 5.6, 5.3, and 6.2, respectively. Liver enlargement was also possible in rats dosed below 200 mg/kg, but additional test and control rats would have to be compared in order to establish this.

Reference: DuPont Co. (1961). Unpublished Data, Haskell Laboratory Report No. 55-61.

Type: **Acute Oral Effects, Does pretreatment with ethanol modify the toxicity of ammonium perfluorooctanoate?**

Species/strain: Male rats/Crl:CD®

Method: Young adult male rats (6/group) were administered ammonium perfluorooctanoate as a 60% aqueous solution at dose levels of 200, 480, and 670 mg/kg. Additional groups of rats were pre-treated with ethanol (15% v/v) in drinking water for 14 days and then dosed with ammonium perfluorooctanoate at the same concentrations as listed above on day 15. Three other groups of rats were pretreated with a single 6000 mg/kg dose of ethanol via intragastric intubation followed 24 hours later with the ammonium perfluorooctanoate treatment at the levels listed above. An additional group of untreated rats served as controls. Groups of rats were also treated with ethanol alone at either 6000 mg/kg or 15% v/v in drinking water for 14 days. Clinical signs and body weights were recorded. Liver weights were recorded at the 14-day sacrifice.

GLP: No

Test Substance: Ammonium perfluorooctanoate, purity approximately 100%

Results: All rats died within 8 days after dosing with ammonium perfluorooctanoate at 670 mg/kg. Liver weight/body weight ratios showed an increase in all rats that received ammonium perfluorooctanoate when compared to the untreated controls and the animals that received only ethanol. There were no significant differences between the untreated controls and the animals that received only ethanol. There were no significant differences between rats that received ethanol and ammonium perfluorooctanoate and the rats that received only ammonium perfluorooctanoate.

Reference: DuPont Co. (1984). Unpublished Data, Haskell Laboratory Report No. 79-84.

Type: **Oral LD_{50}**

Species/strain: Male and female mice/CD-1

Value: Male and female mice: 457 mg/kg (95% confidence limits, lower 344 mg/kg, upper 560 mg/kg, slope 6.6)

Method: The test substance, as a suspension in corn oil, was administered by intragastric intubation in single doses to 6 groups of male and 6 groups of female young adult mice. Ten male and female

mice/group were administered 250, 500, 750, 1000, 2000, or 4000 mg/kg of the test substance. The surviving rats were weighed and observed during a 14-day recovery period and then sacrificed. The LD_{50} values were calculated from the mortality data. The male and female 4000 and 2000 mg/kg dose levels were not calculated in the LD_{50} data due to limitations of the computer.

GLP: No

Test Substance: Ammonium perfluorooctanoate, purity approximately 100%

Results: Mortality ratios of 1/10, 4/10, 10/10, 10/10, 10/10, and 10/10 were found in the 250, 500, 750, 1000, 2000, and 4000 mg/kg male and female groups, respectively.

Clinical signs observed in male mice included stained and/or wet perineal area, weakness, and weight loss in all surviving mice. Other signs included eyes half closed (250 and 1000 mg/kg), ruffled fur (250 and 500 mg/kg), ataxia (250, 500, and 1000 mg/kg), and tremors (500 and 750 mg/kg). All deaths in male mice occurred within 6 days after dosing.

Clinical signs observed in female mice included stained and/or wet perineal area, weakness, and weight loss in all surviving mice. Other signs included eyes half closed (750 mg/kg), stained face (750 mg/kg), tremors (750 mg/kg), ataxia (500 mg/kg), and ruffled fur (250 and 750 mg/kg). All deaths in female mice occurred within 7 days after dosing.

Reference: DuPont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 329-81.

Type: **Oral LD_{50}**

Species/strain: Male and female guinea pigs/Duncan Hartley

Value: Male guinea pigs: 178 mg/kg (95% confidence limits, lower 144 mg/kg, upper 202 mg/kg, slope 8.9)

Female guinea pigs: 217 mg/kg (95% confidence limits, lower 187 mg/kg, upper 246 mg/kg, slope 8.6)

Method: The test substance, as a suspension in corn oil, was administered by intragastric intubation in single doses to 6 groups of male and 6 groups of female young adult guinea pigs. Ten male and female guinea pigs/group were administered 150, 200, 250, 300, 400, or 670 mg/kg of the test substance. The surviving guinea pigs were weighed and observed during a 14-day recovery period and then sacrificed. The LD_{50} values were calculated from the mortality data.

GLP: No

Test Substance: Ammonium perfluorooctanoate, purity approximately 100%

Results: Mortality ratios of 3/10, 6/10, 9/10, 10/10, 10/10, and 10/10 were

found in the 150, 200, 250, 300, 400, and 670 mg/kg male groups, respectively. Mortality ratios of 2/10, 1/10, 8/10, 9/10, 10/10, and 10/10 were found in the 150, 200, 250, 300, 400, and 670 mg/kg female groups, respectively.

Clinical signs observed in male guinea pigs included stained and/or wet perineal area and weakness at all levels tested. Other clinical signs observed in the male guinea pigs included stained face (150, 200, and 670 mg/kg), eyes half closed (200 and 300 mg/kg), lacrimation (150 and 200 mg/kg), ataxia (670 mg/kg), tremors (150 mg/kg), and hunched posture (200 mg/kg). Weight loss occurred at all levels tested except 400 and 670 mg/kg. All deaths occurred within 4 days after dosing.

Clinical signs observed in female guinea pigs included stained and/or wet perineal area and weakness at all levels tested except 400 and 670 mg/kg. Other clinical signs noted in female guinea pigs included convulsions (670 mg/kg), ataxia (670 mg/kg), gasping (670 mg/kg), eyes half closed (150 and 250 mg/kg), tremors (400 mg/kg), lacrimation (250 mg/kg), stained face (200 mg/kg), and lethargy (150 mg/kg). Weight loss occurred at all levels tested except for 670 mg/kg. All deaths occurred within 6 days after dosing.

Reference: DuPont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 291-81.

Type: **Acute Toxicity**
Species/strain: Male dogs/beagle
Method: Dogs (2/group) received either 200 or 450 mg/kg of the test substance. The following biochemical measurements were made on the blood: sugar, urea nitrogen, total cholesterol, and alkaline phosphatase. When the 450 mg/kg dose was administered, the level of activity of lactic dehydrogenase (LDH), isocitric dehydrogenase (ICDH), aldolase, glutamic oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were also measured. A routine hematological examination and an analysis of a 24-hour urine specimen was made at intervals on these animals. The level of various components of the blood following dosing was compared with an average value observed prior to exposure and with a similar measurement made at the same time on specimens from stock colony dogs. For the enzyme activities, a normal range was established from measurements made on stock dogs. The activity was also measured at least once prior to treatment.
GLP: No
Test Substance: Ammonium perfluorooctanoate, purity 99%
Results: The GPT and GOT levels were elevated within 48 hours in both

dogs that received 200 mg/kg. One week later, they were in the normal range. When 450 mg/kg was administered, all of the enzymes measured were markedly elevated 24 to 48 hours later. The greatest change occurred in the GPT and ICDH. Both animals that received 450 mg/kg died within 48 hours after dosing.

Reference: DuPont Co. (1965). Unpublished Data, Haskell Laboratory Report No. 123-65.

Type: **Inhalation ALC (Acute Lethal Concentration)**
Species/strain: Rats/ChR-CD
Exposure Time: 4 hours
Value: 0.8 mg/L
Method: Male rats (6/exposure level), weighing 250-270 grams were exposed to dusts of ammonium perfluorooctanoate at exposure levels of 0.38, 0.81, 0.83, 2.2, 4.8, or 5.7 mg/L. Exposures were head-only, except for the 2.2 mg/L exposure level where rats had whole-body exposure. Two different methods of dust generation were used to generate ammonium perfluorooctanoate aerosols. In the first method, a heavy cloud was generated by blowing approximately 30 L/minute of air through a high velocity Cu-tubing jet submerged in a mechanically stirred reservoir of the test substance. The air carried the dust particles into an 8 L exposure chamber. Suitable diluting air was introduced between the generator and the chamber to achieve lower concentrations. The generator did not fractionate the dust, and all particle sizes, large and small, were delivered into the exposure chamber. In the second method, a generator was used for low atmospheric dust concentrations. A falling stream of dust particles from a stirred reservoir impinged on a pneumatic jet. The air stream from this jet carried the particles to a cyclone head where the larger ones were returned to the reservoir. This generator achieved some particle size fractionation. The generator was run under constant conditions and dust concentrations were lowered by diluting the stream with air between the generator and the exposure chamber. The airborne concentrations were determined 5 times during each 4-hour exposure. At the mid-point of each exposure, a particle size distribution measurement was made.

Clinical signs were recorded. The eyes of the rats were stained with fluoroscein immediately after exposure. Rats were sacrificed for histopathologic examination at 1, 7, 14, 27, or 42 days post-exposure. Tissues from two rats dying during exposure were also examined microscopically.

GLP: No
Test Substance: Ammonium perfluorooctanoate, purity approximately 100%
Results: The mortality ratios for the 0.38, 0.81, 0.83, 2.2, 4.8, and 5.7 mg/L

exposure groups were 1/6 (not test substance-related), 2/6, 1/6, 6/6, 6/6, and 6/6, respectively. All deaths occurred either during exposure or within 48 hours after exposure.

Clinical signs observed during exposure included gasping (all exposure levels), irregular breathing (all exposure levels), red discharge around the eyes and nose (all exposure levels), and salivation (2.2, 4.8, and 5.7 mg/L). Clinical signs observed post-exposure included some rupture of eyes, all other eyes opaque (2.2 and 4.8 mg/L), and > 80% rats having opaque and corroded-appearing eyes (0.81 and 0.83 mg/L). External damage of the eyes was confirmed with fluorescein stain (0.81, 0.83, and 2.2 mg/L), with the eyes appearing normal after 20 days (0.81 mg/L).

Inhalation of the test substance caused liver enlargement which reached a maximum (2 times normal) 7-14 days after exposure. Liver weights returned to the normal range 42 days after exposure. No changes in liver cell morphology were observed. Microscopic examination indicated that acute pulmonary edema developed promptly, but disappeared in approximately 1 week, leaving no residual injury. No dust particles were seen in the lungs microscopically. There was irritation of the stomach that cleared in 2 weeks. Ammonium perfluorooctanoate also caused corneal opacity and ulceration that were still microscopically evident 42 days after exposure.

References:

DuPont Co. (1969). Unpublished Data, Haskell Laboratory Report No. 160-69.

Kennedy, G. L., Jr. et al. (1983). The Toxicologist, 3:22.

Kennedy, G. L., Jr. et al. (1986). Food Chem. Toxicol., 24:1325-1329.

Type: **Dermal LD_{50}**
Species/strain: Male rats/ChR-CD
Exposure Time: 24 hours
Value: 6959 mg/kg (95% confidence limits undefined)
Method: Rats were clipped free of hair over the back and trunk area. Five male rats/dose level were dosed with 3000, 5000, and 7500 mg/kg ammonium perfluorooctanoate. The test substance, as a 50/50 aqueous suspension, was applied to the back of each rat under a square of aluminum foil and held in place with elastic bandages. After 24 hours of exposure, the rats were unwrapped, sponged off with a mild detergent, rinsed, dried, and returned to their cages for 13 days observation or until death. During the

	observation period, the rats were weighed and observed for clinical signs. Gross pathology was done on all survivors and 1 animal that was found dead at 7500 mg/kg. Liver weights were recorded in all rats except 1. The LD_{50} value was calculated from the mortality data.
GLP:	No
Test Substance:	Ammonium perfluorooctanoate, purity approximately 100%
Results:	Mortality ratios were 0/5, 0/5, and 4/5 at 3000, 5000, and 7500 mg/kg, respectively. Death occurred within 2 days of dosing. All rats dosed at 3000, 5000, and 7500 mg/kg had initial weight loss. Clinical signs observed included lethargy (5000 and 7500 mg/kg), wet perineal area (5000 and 7000 mg/kg), stained perineal area (7500 mg/kg), stained nose (7500 mg/kg), and chromodacryorrhea (7500 mg/kg). Gross pathology showed an increase in liver weights in all surviving rats examined at 14 days post-treatment.
References:	DuPont Co. (1979). Unpublished Data, Haskell Laboratory Report No. 659-79.
	Kennedy, G. L., Jr. (1985). Toxicol. Appl. Pharmacol., 81:348-355.

Type:	**Dermal LD_{50}**
Species/strain:	Female rats/ChR-CD
Exposure Time:	24 hours
Value:	> 7500 mg/kg (the maximum feasible dose)
Method:	Rats were clipped free of hair over the back and trunk area. Five female rats/dose level were dosed with 5000 or 7500 mg/kg ammonium perfluorooctanoate. The test substance, as a 50/50 aqueous suspension, was applied to the back of each rat under a square of aluminum foil and held in place with elastic bandages. After 24 hours of exposure, the rats were unwrapped, sponged off with a mild detergent, rinsed, dried, and returned to their cages for 13 days observation or until death. During the observation period, the rats were weighed and observed for clinical signs. Surviving rats were sacrificed.
GLP:	No
Test Substance:	Ammonium perfluorooctanoate, purity approximately 100%
Results:	The mortality was 0/5 and 1/5 at 5000 and 7500 mg/kg, respectively. Death occurred 3 days after treatment. Ammonium perfluorooctanoate caused mild skin irritation and weight loss at both dose levels. In addition, stained face and wet and stained perineal area were observed at 7500 mg/kg.
References:	DuPont Co. (1980). Unpublished Data, Haskell Laboratory Report No. 682-80.

Kennedy, G. L., Jr. (1985). Toxicol. Appl. Pharmacol., 81:348-355.

Type:	**Dermal LD_{50}**
Species/strain:	Male rabbits/New Zealand White
Exposure Time:	24 hours
Value:	4278 mg/kg (95% confidence limits, lower 2369 mg/kg, upper 9814 mg/kg, slope 6.2504)
Method:	Rabbits were clipped free of hair over the back and trunk area, and were fitted with plastic collars. Five, 5, 5, and 2 male rabbits were dosed with 1500, 3000, 5000, and 7500 mg/kg ammonium perfluorooctanoate, respectively. The test substance was made into a slurry with water, was applied to the back of each rabbit, and the test site was occluded (wrapped with plastic wrap, gauze, and elastic bandages). After 24 hours of exposure, the animals were unwrapped, washed with water, dried, and returned to their cages for 14 or 15 days observation or until death. During the observation period, the animals were weighed and observed for clinical signs. Gross pathology was done on all survivors and 1 animal that was found dead at 3000 mg/kg. The LD_{50} value was calculated from the mortality data.
GLP:	No
Test Substance:	Ammonium perfluorooctanoate, purity approximately 100%
Results:	Mortality ratios of rabbits were 0/5, 1/5, 3/5, and 2/2 at 1500, 3000, 5000, and 7500 mg/kg, respectively. Death occurred within 4 days of dosing. Gross pathological examination showed no compound-related changes; however, there was skin irritation at all dose levels. Clinical signs included weight loss and labored breathing at all dose levels. In addition, lethargy (3000 mg/kg), nasal discharge (5000 mg/kg), shallow breathing (5000 mg/kg), pallor (5000 mg/kg), diarrhea (5000 mg/kg), weakness (5000 mg/kg), wet underneath the body (5000 and 7500 mg/kg), and cyanosis (7500 mg/kg) were observed.
References:	DuPont Co. (1979). Unpublished Data, Haskell Laboratory Report No. 659-79.

Kennedy, G. L., Jr. (1985). Toxicol. Appl. Pharmacol., 81:348-355.

Type:	**Dermal Irritation**
Species/strain:	Male rabbits/New Zealand White
Method:	Six male rabbits were clipped free of hair on the trunk and lateral areas, and placed in FDA-type stocks. Doses of 0.5 g solid ammonium perfluorooctanoate were applied as an aqueous paste to the intact skin under gauze squares, and the test site was semi-occluded (rubber sheeting was loosely wrapped around the

	trunk and secured with adhesive tape). After 24 hours, the rabbits were removed from the stocks, the patches removed, and the reactions observed. Observations were also made at 48 hours.
GLP:	No
Test Substance:	Ammonium perfluorooctanoate, purity approximately 100%
Results:	Ammonium perfluorooctanoate caused mild to moderate skin irritation in 24 hours, and slight to moderate irritation in 48 hours when tested on the shaved intact skin of rabbits under semi-occluded conditions.
Reference:	DuPont Co. (1979). Unpublished Data, Haskell Laboratory Report No. 636-79.
Type:	**Dermal Irritation**
Species/strain:	Male rabbits/New Zealand White
Method:	Twenty-one male rabbits were clipped free of hair over the back and trunk areas, and fitted with plastic collars. Ammonium perfluorooctanoate impregnated Teflon® strips or ammonium perfluorooctanoate impregnated Kevlar® strips were applied to the back of each rabbit. Three rabbits served as controls and were wrapped in non-ammonium perfluorooctanoate-treated material. The trunk of each rabbit was then occluded (wrapped with a layer of plastic wrap, gauze stretch bandage, and adhesive stretch tape). After 4-, 8-, or 24-hour exposure periods, the wrappings were removed and the exposed areas were wiped with gauze pads that were soaked in a 50:50 ethanol:water solution. The animals were observed after unwrapping for skin irritation.
GLP:	No
Test Substance:	Ammonium perfluorooctanoate impregnated Teflon® (48% Teflon® fibers, 38% Teflon® resin that included 2.9% Triton® X-100 and 0.061% ammonium perfluorooctanoate, and 14% dimethylsilicone oil) Ammonium perfluorooctanoate impregnated Kevlar® (40.5% Kevlar® fibers, 42% Kevlar® resin that included 3.2% Triton® X-100 and 0.067% ammonium perfluorooctanoate, and 17.5% dimethylsilicone oil)
Results:	No skin irritation was observed during the study.
Reference:	DuPont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 736-81.
Type:	**Eye Irritation**
Species/strain:	Rabbits/New Zealand White
Method:	One-tenth mL (38.3 mg) of solid test substance was placed into the right conjunctival sac of each of 2 rabbits (sex not specified). After 20 seconds, 1 treated eye was washed with tap water for 1 minute. The treated eye of the other rabbit was not washed. Observations

	of the cornea, iris, and conjunctiva were made with a hand-slit lamp at 1 and 4 hours, and at 1, 2, 3, 7, 14, 21, and 28 days. Fluor-I-strip® stain and a biomicroscope were used at examinations after the day of treatment.
GLP:	No
Test Substance:	Ammonium perfluorooctanoate, purity 100%
Results:	Ammonium perfluorooctanoate caused generalized moderate corneal opacity with a small area of severe opacity, intermittent moderate iritis, and moderate conjunctivitis when tested in a rabbit eye that was unwashed after treatment. The ocular effects gradually receded; however, the small area of corneal opacity persisted, and at 21-28 days was mild with vascularization (sign of healing). An eye dosed with the test substance and promptly washed had a small area of slight to moderate corneal opacity and moderate to slight conjunctivitis with no iritic effect. The eye was normal within 7 days, except for mild conjunctival redness, which was normal within 14 days.
Reference:	DuPont Co. (1979). Unpublished Data, Haskell Laboratory Report No. 635-79.

Repeat Dose Toxicity

Type:	**Repeated Dose Oral Toxicity Study**
Species/Strain:	Mice/CD-1 Rats/Crl:CD®
Sex/Number:	Male and female/5/group
Exposure Period:	9 doses (3/week)
Frequency of Treatment:	3 weeks
Exposure Levels:	0, 0.1, 1.0, 10 mg/kg
Method:	The test substance, as aqueous solutions, was administered by intragastric intubation to rats and mice. Control rats and mice received water only. Body weights and clinical signs were recorded. Animals were sacrificed 3 days after the final dose and the livers were removed and weighed.
GLP:	No
Test Substance:	Ammonium perfluorooctanoate, purity approximately 100%
Results:	A significant liver weight increase was noted in both male and female mice at the 1 and 10 mg/kg levels. Significantly increased liver weights were noted in male rats, but only at 10 mg/kg. There were no significant changes in the liver weights of female rats at any dose.

	Mortality occurred in 2/5 female mice at 10 mg/kg. No mortality occurred in male mice, male rats, or female rats at any dose level tested. Sporadic weight loss occurred in the 0.1, 1.0, and 10 mg/kg female rats and in the 0.1, 1.0, and 10 mg/kg male and female mice. Weakness was observed in the 10 mg/kg female mice.
Reference:	DuPont Co. (1983). Unpublished Data, Haskell Laboratory Report No. 138-83.
Type:	**Repeated Dose Oral Toxicity Study**
Species/Strain:	Rats/Crl:CD
Sex/Number:	Male/12
Exposure Period:	12 days; 14 days of recovery
Frequency of Treatment:	10 daily doses (5 doses, 2 non-dose days, 5 doses)
Exposure Levels:	0, 6.7 mg/kg
Method:	Each of 6 rats received 10 daily doses of ammonium perfluorooctanoate by intragastric intubation as an aqueous solution. Body weights were recorded. Three rats were killed 4 hours after receiving the 10^{th} dose, and the remainder were killed 14 days later. Organ weights were recorded. Six undosed rats served as controls.
GLP:	No
Test Substance:	Ammonium perfluorooctanoate, purity 99%
Results:	The most prominent effect of the test substance was enlargement of the liver, which, at the end of the dosage regimen, was about 45% heavier than that of the control rats. This change persisted after cessation of dosage, but the weight discrepancy was somewhat less since 14 days later the livers were only 20% heavier than those of control rats. The renal weights were 20% heavier than those of corresponding control rats, and this increase persisted after cessation of dosage, being 22% above that of the control rats 14 days later. These weight changes were not accompanied by morphological changes. The pancreatic weights were slightly depressed, being 8% and 12% lower than those of controls at the end of the test and recovery phases, respectively. The adrenals and testes were slightly increased in weight after the last dose, +14% and +11%, respectively, but returned to normal 14 days later.
Reference:	DuPont Co. (n.d.). Unpublished Data.

Type:	**14-Day Feeding Study**
Species/Strain:	Rat/Crl:CD®BR
Sex/Number:	Male/20/group
Exposure Period:	14 days; 56 days of recovery
Frequency of Treatment:	*ad libitum* for 14 days
Exposure Levels:	0, 30, 300 ppm
Method:	Rats were approximately 6 weeks old at arrival. At study start, the body weights ranged from 196 to 240 g. Rats were housed singly and were fed food and water *ad libitum*. During the test period each group were fed diet that contained 0, 30, or 300 ppm of the test substance. Diets were prepared once for the 2-week feeding period and were stored refrigerated until used. All rats were weighed and observed for clinical signs of toxicity. Rats not sacrificed at the end of the feeding period were weighed and observed during the recovery period. Cageside examinations to detect moribund or dead rats were conducted twice daily. Five rats/group were sacrificed at the end of the 14-day feeding period, and on recovery days 7, 28, and 56. Livers were removed and weighed at each sacrifice period. Blood samples were taken for organofluoride concentration analysis from rats sacrificed at the end of the feeding period and on recovery day 7. Appropriate statistical methods were used to analyze body weights, body weight gains, and organ weight data.
GLP:	No
Test Substance:	Ammonium perfluorooctanoate, purity approximately 100%
Results:	Diets were not analyzed. No mortalities were observed during the study. Clinical signs of toxicity noted in the 30 and 300 ppm groups during the feeding period included irregular respiration, rapid breathing, red nasal discharge, and hunched posture. Statistically significant decreases in mean body weights were observed in rats from the 300 ppm dose group on days 7 and 14 of the feeding period and on recovery day 7. Mean body weight gains from this same group were significantly depressed during the first week of feeding. Significant increases in mean absolute liver weights were observed in rats from the 30 and 300 ppm dose groups sacrificed at the end of the feeding period and on recovery days 7 and 28. Significantly increased mean relative liver weights were observed in rats from the 30 and 300 ppm dose groups sacrificed at the end of the feeding

	period and on recovery day 7, and in the 300 ppm dose group rats sacrificed on recovery day 28. Mean blood organofluoride concentrations of rats sacrificed on recovery day 0 were 0.3 ppm in control rats, 33.2 ppm in the 30 ppm rats, and 71.5 ppm in the 300 ppm rats. On recovery day 7, mean blood organofluoride concentrations were 0.9 ppm for controls, 19.3 ppm for the 30 ppm group, and 22.2 ppm for the 300 ppm group.
Reference:	DuPont Co. (1995). Unpublished Data, Haskell Laboratory Report No. 326-95.
Type:	**14-Day Feeding Study**
Species/Strain:	Mice/Crl®:CD-1
Sex/Number:	Male and female/5/group
Exposure Period:	14 days
Frequency of Treatment:	*ad libitum* for 14 days
Exposure Levels:	0, 30, 300, 3000 ppm
Method:	Male and female mice (age 43 and 44 days, weighing 21 to 38 g) were fed diets containing ammonium perfluorooctanoate for 14 consecutive days. Male and female controls were observed concurrently and fed only ground chow. Individual body weights, food consumption, and clinical signs were recorded. Liver weights were recorded at the 14-day sacrifice.
GLP:	No
Test Substance:	Ammonium perfluorooctanoate, purity approximately 100%
Results:	All male and female mice died after dosing with 3000 ppm. One female mouse died at 300 ppm. Body weight loss occurred at 300 and 3000 ppm. Liver weight/body weight ratios showed a dose-response increase at ≥ 30 ppm in male and female mice. Clinical signs observed included unkempt head area (300 and 3000 mg/kg) and weakness (300 mg/kg).
References:	DuPont Co. (1982). Unpublished Data, Haskell Laboratory Report No. 12-82. Kennedy, G. L., Jr. (1987). Toxicol. Lett., 39:295-300.
Type:	**14-Day Feeding Study**
Species/Strain:	Mice/Crl®:CD-1
Sex/Number:	Male and female/5/group/gender
Exposure Period:	14 days

Frequency of Treatment:	*ad libitum* for 14 days
Exposure Levels:	0, 10, 30, 100, 300, 1000, 3000, 10,000 ppm
Method:	Male and female mice (age 41 and 42 days, weighing 19 to 30 g) were fed diets containing ammonium perfluorooctanoate for 14 consecutive days. Male and female controls were observed concurrently and fed only ground chow. Individual body weights, food consumption, and clinical signs were recorded. Liver weights were recorded at the 14-day sacrifice.
GLP:	No
Test Substance:	Ammonium perfluorooctanoate, purity approximately 100%
Results:	Death occurred at 1000, 3000, and 10,000 ppm. All female mice at 3000 ppm and all male mice at 10,000 ppm were dead at the 2-week time point. All 10,000 ppm female mice and 3000 ppm male mice were dead at the 1-week time point. Although death occurred in the 1000 ppm mice, details on the number of deaths was not reported. Body weight loss occurred at the end of each week at 300, 1000, 3000, and 10,000 ppm male and female mice. Liver weight/body weight ratios showed a dose-response increase at $\geq$ 10 ppm in male and female mice. Clinical signs observed included weakness (100 – 3000 ppm), tremors (300 and 1000 ppm), piloerection (100, 1000, and 3000 ppm), pallor (10,000 ppm), stained perineal area (100 – 1000 ppm), weight loss (100, 1000, 3000, and 10,000 ppm), and unkempt appearances (300 – 10,000 ppm).
Reference:	DuPont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 560-81.

Type:	**14-Day Feeding Study**
Species/Strain:	Mice/Crl®:CD-1
Sex/Number:	Male and female/5/group
Exposure Period:	14 days
Frequency of Treatment:	*ad libitum* for 14 days
Exposure Levels:	Ammonium perfluorooctanoate: 0, 30 ppm Nonadecafluorodecanoic acid: 3, 10, 30, 300, 3000 ppm Mixtures of nonadecafluorodecanoic acid/ammonium perfluorooctanoate: 15/15, 5/25, 25/5 ppm
Method:	Male and female mice (age 44 days, weighing 24 to 35 g) were fed diets containing either ammonium perfluorooctanoate, nonadecafluorodecanoic acid, or mixtures of the two test substances for 14 consecutive days. Male and female controls were observed concurrently and fed only ground chow. Individual body

	weights and clinical signs were recorded. Liver weights were recorded at the 14-day sacrifice.
GLP:	No
Test Substance:	Ammonium perfluorooctanoate, purity approximately 100%
Results:	Liver weight/body weight ratio showed an increase at all levels tested. Mixing the 2 test substances did not alter the effects. Liver enlargement appeared to be dose-related with nonadecafluorodecanoic acid appearing the more potent of the two substances.
Reference:	DuPont Co. (1982). Unpublished Data, Haskell Laboratory Report No. 537-82.

Type:	**21-Day Feeding Study**
Species/Strain:	Mice/Crl®:CD-1
Sex/Number:	Male and female/5/group
Exposure Period:	21 days
Frequency of Treatment:	*ad libitum* for 21 days (Mice were mistakenly fed ground chow, without compound, for 2 days)
Exposure Levels:	0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30 ppm
Method:	Male and female mice (age 41 and 42 days, weighing 21 to 33 g) were fed diets containing ammonium perfluorooctanoate (suspended in 1% corn oil before being mixed in ground chow) for 21 consecutive days. Male and female controls were observed concurrently and fed only ground chow. Individual body weights and clinical signs were recorded. Liver weights were recorded at the 21-day sacrifice.
GLP:	No
Test Substance:	Ammonium perfluorooctanoate, purity approximately 100%
Results:	Livers were significantly heavier at 30 ppm, slightly heavier at 3 ppm, and appeared normal at 10 ppm in male and female mice. No changes in liver weight were seen at feeding levels of 1 ppm or lower, and the significance of the change observed at 3 ppm, in light of no differences at 10 ppm, is questionable. The only clinical sign observed was sporadic weight loss.
References:	DuPont Co. (1982). Unpublished Data, Haskell Laboratory Report No. 323-82.
	Kennedy, G. L., Jr. (1987). Toxicol. Lett., 39:295-300.

Type:	**2-Year Feeding Study**
Species/Strain:	Rat/Crl:CD®BR
Sex/Number:	Male/156/group
Exposure Period:	2 years

Frequency of Treatment:	*ad libitum* for 2 years
Exposure Levels:	0, 0 (pair-fed), 300 ppm
Method:	Male rats were administered either 0 (*ad libitum* control group (control)), 0 (control pair-fed to the ammonium perfluorooctanoate group (CP-C8)), or 300 ppm ammonium perfluorooctanoate in the diet for approximately 2 years. After assignment to treatment groups each rat was designated for either hormonal evaluation (10/group/time point), cell proliferation evaluation (6/group/time point), or evaluation of peroxisome proliferation (6/group/time point). All rats were provided food and tap water *ad libitum*. Stability of ammonium perfluorooctanoate was confirmed by analyses at the beginning, middle, and end of the study. Throughout the study, concentration of the test compound in the diet and the homogeneity of the test diets were determined. All rats were approximately 49 days of age on the day of study start. Body weights, food consumption, and clinical signs were monitored throughout the study. Blood was collected for hormonal analyses at approximately 1, 3, 6, 9, 12, 15, 18, and 21 months after initiation of the study. Serum was analyzed for testosterone, estradiol, luteinizing hormone (LH), follicle stimulating hormone (FSH), and prolactin concentrations. Rats were sacrificed at the following interim time points: 1, 3, 6, 9, 12, 15, 18, and 21 months for cell proliferation and peroxisome proliferation evaluations. The following organs were weighed: testes, epididymides, accessory sex gland (ASG) unit with fluid, coagulating gland/seminal vesicle with fluid removed, prostate, and liver. Immediately after weighing, the liver and testes from animals selected for peroxisome proliferation evaluation were placed in ice-cold homogenization buffer for peroxisomal preparation. The following tissues were collected from the rats selected for cell proliferation evaluation: right and left testes, epididymides, ASG, liver, duodenum, pituitary, and all organs with gross lesions. All rats surviving the 24-month test period were sacrificed and necropsied. Brain, heart, liver, spleen, kidneys, ASG unit, coagulating gland/seminal vesicles with fluid removed, prostate, epididymides, and testes were weighed at necropsy. The liver, testes, epididymides, pancreas, and organs with gross lesions were examined microscopically. Data were analyzed by appropriate statistical methods.
GLP:	No

Test Substance:	Ammonium perfluorooctanoate, purity 98-100%
Results:	Increased relative liver weights were observed in the ammonium perfluorooctanoate-treated rats. Hepatic ß-oxidation activity was also increased in the ammonium perfluorooctanoate-treated rats at all time points. Ammonium perfluorooctanoate did not significantly alter the rate of Leydig cell ß-oxidation or Leydig cell proliferation. Moreover, the rate of ß-oxidation in Leydig cells was approximately 20-times less than the rate of hepatic ß-oxidation. Serum testosterone, FSH, prolactin, and LH levels were unchanged. There were, however, significant increases in serum estradiol levels in the ammonium perfluorooctanoate-treated rats at 1, 3, 6, 9, 15, 18, and 21 months. At 12 months, the ammonium perfluorooctanoate-treated rats had elevated serum estradiol levels when compared to the pair-fed control. Histopathological evaluation revealed compound-related increases in liver, Leydig cell, and pancreatic acinar cell tumors. Based on these data, the Leydig cell tumors appear to be due to the combination of elevated estradiol levels and reduced prolactin levels.
Reference:	DuPont Co. (2000). Unpublished Data (Draft Manuscript).

Type:	**Repeated Exposure Inhalation Study**
Species/Strain:	Rats/ChR-CD
Sex/Number:	Male/20/group
Exposure Period:	2 weeks; 42 days of recovery
Frequency of Treatment:	6 hours/day, 5 days/week
Exposure Levels:	0, 8, 80 mg/m^3
Method:	Houseline air (approximately 20 L/min) was passed through a cyclone-head dust generator connected to a particle agitator-reservoir that contained ammonium perfluorooctanoate. The resulting airborne particulate was passed into a 30 L battery jar exposure chamber. The atmospheric concentration of ammonium perfluorooctanoate in the exposure chamber was monitored at 30-minute intervals. Rats were exposed, head only, for 5 consecutive days, 6 hours per day. After 5 days, the rats were given a 2-day recovery (weekend), which was followed by 5 daily, consecutive 6-hour exposures. All rats were weighed and observed daily (except weekends) during the exposure and recovery periods. Food and water were available *ad libitum* at all times other than during the actual exposure. Clinical laboratory examinations were performed on 10 rats from each group at 0, 14, and 28 days post-exposure. After a total of

10 exposures, 5 rats from each group were pathologically evaluated at 0, 14, 32, and 42 days post-exposure. Organs were weighed, and absolute and relative organ weights were calculated.

On the 5th and 9th days of exposure, 10 rats were selected from each group for eye examinations.

GLP: No

Test Substance: Ammonium perfluorooctanoate, purity approximately 100%

Results: Overall mean concentrations were 0, 11 ± 5, 83 ± 17 for the 0, 8, and 80 mg/m^3 exposure levels, respectively.

Rats exposed to ammonium perfluorooctanoate showed a suppression of body weight throughout the test. The 83 mg/m^3 group was more severely affected during exposure and for 14 days of recovery. During each exposure, sporadic cases of blinking, pawing, chewing, and red eye and nasal discharge were seen in all groups.

Some rats exposed to ammonium perfluorooctanoate dust displayed elevated alkaline phosphatase activity. Effects were seen in glutamic-pyruvic transaminase and glutamic-oxalacetic transaminase after 0, 14, and 28 recovery days. The incidence was related to dose; higher activities were found at the higher exposure level. However, only the latter activity at 83 mg/m^3 was statistically different from controls.

Pathologic evaluations showed cloudy swelling or granular degeneration of hepatocytes in the livers of rats exposed to ammonium perfluorooctanoate for 10 days with no recovery. This effect was not seen after a 14-day or longer recovery period. No other compound-related histologic changes were noted. Exposure-related increase in liver weights was observed.

No corneal, iritic, or conjunctival effects were seen in any of the rats examined after 5 or 9 exposure days.

References: DuPont Co. (1979). Unpublished Data, Haskell Laboratory Report No. 253-79.

Kennedy, G. L., Jr. et al. (1983). The Toxicologist, 3:22.

Type: **Repeated Exposure Inhalation Study**

Species/Strain: Rats/Crl:CD®

Sex/Number: Male/24/group

Exposure Period: 2 weeks; 84 days of recovery

Frequency of Treatment:	6 hours/day, 5 days/week
Exposure Levels:	0, 1, 8, 80 mg/m^3
Method:	Dust atmospheres of ammonium perfluorooctanoate were generated by passing air through a glass generator. For the high concentration (80 mg/m^3), chamber atmosphere concentrations were primarily determined by gravimetric analysis. For the intermediate and low concentrations (8 and 1 mg/m^3, respectively), chamber atmospheres were determined by a chemical analyses. Male rats (age 7-8 weeks, weighing 240-279 g) were exposed head-only to dust atmospheres for 6 hours/day, 5 days/week for 2 weeks (weekends excluded). During exposure, rats were observed and clinical signs were noted. Post-exposure rats were weighed and observed daily for 14 recovery days, then weighed and observed 2 times/week through 84 days of recovery. Five rats/group were sacrificed at 0, 14, 28, 42, and 84 days of recovery, for a total of 96 test days. Clinical laboratory examinations were performed on 5 rats from each group at 0, 14, 28, 42, and 84 days post-exposure. After a total of 10 exposures, 5 rats from each group were pathologically evaluated at 0, 14, 28, 42, and 84 days post-exposure. The rats were examined grossly and tissues and organs were saved for microscopic evaluation. In addition, lungs, heart, thymus, spleen, liver, testes, and kidneys were weighed. At necropsy, blood was collected for analysis of organofluoride levels in rat blood. Blood samples from the 0 and 80 mg/m^3 groups were analyzed at each recovery period. Blood samples from the 1 and 8 mg/m^3 groups were analyzed only at 0 and 28 recovery days.
GLP:	No
Test Substance:	Ammonium perfluorooctanoate, purity approximately 100%
Results:	Overall mean concentrations were 1.0 ± 0.5, 7.6 ± 2.5, and 83.9 ± 12.8 mg/m^3 for the 1, 8, and 80 mg/m^3 groups, respectively. Body weight analysis demonstrated no significant differences between controls and 1 mg/m^3. Body weights from animals exposed to 8 mg/m^3 were significantly higher than controls from test days 17-33. Body weights from animals exposed to 80 mg/m^3 were significantly lower than controls from test days 2-16. Observations of clinical signs during exposures showed only slight signs of nasal and ocular discharge. However, at the high concentration, after 3-4 days on test 1 rat died during exposure and 1 rat was sacrificed

in extremis. Both of these rats had severe weight loss. Mortality was probably exposure-related, although pathologic evaluation could not determine the cause of death. Three of 24 rats in the 80 mg/m^3 exposure group exhibited lung noise during the 12-day exposure period.

Organ to body weight ratios demonstrated a significant, dose-related increase in lung, liver, and testes weights after 0 recovery days. The liver/body weight ratios were significantly higher in animals exposed to 80 mg/m^3 through 28 days of recovery. Mean absolute liver weights were significantly higher in the 8 mg/m^3 animals through 28 days of recovery, but this may be an artifact caused by an unexplainable increase in body weight accompanied by a normal increase in liver weights at 8 mg/m^3.

Clinical laboratory measurements demonstrated an increase in alkaline phosphatase in all groups exposed to ammonium perfluorooctanoate after 10 exposures, but this finding was significant only at 8 and 80 mg/m^3. This increase persisted in the 80 mg/m^3 animals through 14 days of recovery. After 28, 42, and 84 days of recovery no differences were found.

Compound-related pathologic findings included heavy livers, panlobular hepatocellular hypertrophy, centrolobular hepatocellular hypertrophy, and hepatocellular necrosis in animals exposed to 8 and 80 mg/m^3. These findings showed an exposure-response relationship, but were reversible by 28 days of recovery (8 mg/m^3) or 42 days of recovery (80 mg/m^3).

Blood organofluoride analysis clearly demonstrated an exposure-related presence in all groups (including the controls, this finding remains unexplained). Blood organofluoride levels decreased with time, but was detectable after 84 days of recovery in both the control and 80 mg/m^3 exposure levels.

References:

DuPont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 205-81.

Kennedy, G. L., Jr. et al. (1983). The Toxicologist, 3:22.

Kennedy, G. L., Jr. et al. (1986). Food Chem. Toxicol., 24:1325-1329.

Type: **Repeated Dose Dermal Study**
Species/Strain: Rats/ChR-CD
Sex/Number: Male/15/group

Exposure Period:	2 weeks; 42 days of recovery
Frequency of Treatment:	6 hours/day, 5 days/week
Exposure Levels:	0, 20, 200, 2000 mg/kg
Method:	Male rats (age 8 weeks, weighing 210-245 g) were collared to prevent ingestion of the compound when preening and grooming during the 2-week exposure period. Ammonium perfluorooctanoate, as an aqueous paste, was applied to the backs of each rat that had been shaved free of hair. Daily 6-hour exposures ended when the compound was wiped from the rats' backs with a gauze pad. Collars were removed after exposure day 10. Throughout the test period, food and water were available *ad libitum*. Body weights and clinical signs were recorded. After exposure day 10 and on recovery days 14 and 42, blood was taken from 5 rats from each group for hematology measurements. Gross necropsy and histopathological examinations were performed on 5 rats/group after exposure 10, and on recovery days 14 and 42. Mean absolute and relative organ to body weight analyses were performed. After exposure 10 and on recovery days 14 and 42, blood was collected from 5 rats/group for organofluorine determinations. Eye examinations were performed on each rat after exposure 9, and on recovery days 13 and 41. The procedure included gross observation of the eyes using a bright light, and semimicroscopic observation using a hang magnifying lamp and a slit-lamp biomicroscope. Data were analyzed by appropriate statistical methods.
GLP:	No
Test Substance:	Ammonium perfluorooctanoate, purity approximately 100%
Results:	Rats treated with 20 mg/kg ammonium perfluorooctanoate showed normal body weights and no unusual clinical signs during the experiment. During the 10-day exposure period, rats treated with either 200 or 2000 mg/kg lost weight, followed by a normal growth after the exposure period. Slight redness of the skin was observed in these 2 groups, along with salivation in the 2000 mg/kg group only. Clinical enzyme determinations monitoring liver function (alkaline phosphatase, GPT, and GOT) showed dose-related increases in all treated groups after exposure 10. These values returned to normal at recovery days 14 and 42. Liver damage characterized by coagulative necrosis was observed in all treated groups following the 10^{th} dose. The incidence and

severity of liver damage was dose-related. Recovery was complete in the 20 mg/kg group 14 days following the 10th dose, and was essentially complete in the 200 mg/kg group at the same time. On recovery day 42, reversal of liver damage was essentially complete in the 2000 mg/kg group. Two rats exposed to 2000 mg/kg had coagulative necrosis of the epidermis at the dose site following 10 exposures. Liver weights, both on an absolute and relative to body weight basis, showed a dose-related increase on exposure day 10, with a return to normal weight seen in the 20 mg/kg group at 14 days and in the 200 mg/kg group at 42 days of recovery. The increased liver weight persisted for 42 days in the 2000 mg/kg group, although a trend toward normal was observed. On exposure day 10, the mean kidney weights of the 20 mg/kg group were significantly greater than controls, and the mean absolute spleen and kidney weights from the 2000 mg/kg group were significantly less than controls. The mean relative testicular weights from the 2000 mg/kg group showed a statistically significant increase on exposure day 10. On recovery day 14, there was a statistically significant increase in the mean relative kidney and testes weights of the 200 and 2000 mg/kg groups.

Blood organofluoride levels showed dose-related elevation on exposure day 10, followed by a decrease in the levels at recovery days 14 and 42. These values after the 10th exposure ranged from 52, 81, and 118 ppm (20, 200, and 2000 mg/kg, respectively) to 1, 4, and 8 ppm (20, 200, and 2000 mg/kg, respectively) at recovery day 42. At a blood organofluoride concentration of approximately 10 ppm, there were normal liver weight to body weight ratios and serum enzyme activity, and no clinical signs or body weight differences from controls. One rat showed liver changes at this level. At a blood organofluoride level of approximately 50 ppm, there were marked liver changes and significant increases in the mean absolute and relative liver weights. No other toxic effects were evident at this blood organofluoride concentration.

References: DuPont Co. (1980). Unpublished Data, Haskell Laboratory Report No. 589-80.

Kennedy, G. L., Jr. (1985). Toxicol. Appl. Pharmacol., 81:348-355.

Developmental Toxicity

Type:	**Developmental Toxicity in Rats**
Species/Strain:	Rats/Crl:CD®(SD)BR
Sex/Number:	Females/6-15/group
Route of Administration:	Inhalation

Exposure Period:	Days 6-15 of Gestation
Frequency of Treatment:	6 hours/day
Exposure Levels:	0, 0.1, 1.0, 10.0, 25.0 mg/m^3
Method:	Female rats were mated to males, and mating was verified by detection of spermatozoa in the vaginal lavage each morning following overnight cohabitation. The day that spermatozoa were detected was designated as Day 1 of gestation (Day 1G).

For Experiment I (females sacrificed before parturition), a total of 24 mated females were to have been assigned to each group (12 females/group/Run). However, due to the degree of maternal toxicity in evidence in the 25.0 mg/m^3 group in Run I, this concentration was reduced to 10.0 mg/m^3 for Run II, and 15 mated females were assigned to this new group. Furthermore, two more control groups (6 mated females/group) were added to Run II; one was pair-fed to the 25.0 mg/m^3 group, and the other was pair fed to the 10 mg/m^3 group.

For Experiment II (females allowed to give birth), in Run I 12 mated female rats were distributed to each group. With the addition of the 10.0 mg/m^3 group in Run II, 6 mated females were added to both the control and the 10.0 mg/m^3 groups.

Rats were exposed whole-body to atmospheres of ammonium perfluorooctanoate in 150 L glass and stainless steel Rochester-type chambers within which the rats were housed individually in wire-mesh modules. Chamber concentrations were determined by gravimetric analysis either each ½ hour (1.0 mg/m^3, and 10 or 25 mg/m^3) or each hour (0.1 mg/m^3), and by a spectrophotometric technique (on each 0.1 mg/m^3 sample, and on 5-6 samples per exposure day for the other levels tested).

Dams were weighed and observed for clinical signs. Feed consumption was measured during gestation (2 females/cage). The dams were coded from before sacrifice until all maternal and fetal data were collected, and until all structural alterations noted among the fetuses were classified. After sacrifice, the dams were examined for gross pathologic changes, liver weight was recorded, and reproductive status was determined. The number of corpora lutea and implantation sites were counted, and the number and position of all live, dead, and resorbed fetuses were recorded.

All live and dead fetuses were weighed and sexed externally and

internally, and the live fetuses were examined for external alterations. Approximately ½ of the fetuses of each litter that were alive when removed from the dam were examined for visceral alterations, and all stunted or malformed fetuses were examined similarly. The fetuses that were examined for visceral alterations were also examined for head alterations. Sections, containing the eyes of 3 fetuses from each litter of the 25.0 mg/m^3 group and of 2 fetuses from each litter of the control group, were processed histologically for examination. In Run II, one fetal head from each of 4 litters from the 10.0 mg/m^3 group and the control group were examined. In addition, the heads from all fetuses in the group pair fed to the 25 mg/m^3 group were processed for examination. All fetuses were examined for skeletal alterations.

For Experiment II, the procedures used until Day 21G were the same as for Experiment I, except that the dams were weighed less frequently during gestation, feed consumption was not measured, and the identity of each offspring within litters was not retained. Before expected parturition, each dam was housed in a polycarbonate cage that contained bedding. The date of parturition was noted and was termed Day 1 PP. The dams were weighed and examined for clinical signs. For each test group fertility index and gestation index were calculated, and for each litter viability index and lactation index were calculated. All dams were sacrificed on Day 22 PP.

The pups from each dam were counted, sexed, weighed, and examined for external alterations toward the end of Day 1 PP. Thereafter, each pup was weighed and inspected for adverse clinical signs on Day 4, 7, 14, and 22 PP. The eyes of the pups in all groups of Run I (Experiment II) were examined by an ophthalmologist on Days 15, 16, or 17 PP, shortly after the eyes opened. This examination was conducted with the exposure levels coded. On Day 35 PP, each pup was sacrificed and the eyes were fixed for possible future evaluation.

Data were analyzed by appropriate statistical methods.

GLP: Yes

Test Substance: Ammonium perfluorooctanoate, purity > 95%

Results: The actual mean concentrations achieved were 0 and approximately 0.14, 1.2, 9.9, and 21.0 mg/m^3 for the 0, 0.1, 1.0, 10.0, and 25.0 mg/m^3 exposure groups.

No effects were observed at 0.1 or 1.0 mg/m^3. None of the 21 dams exposed to ammonium perfluorooctanoate at 10.0 mg/m^3 died, but they showed similar clinical signs to a lesser degree than

that seen at the 25.0 mg/m^3 level. At 25 mg/m^3, ammonium perfluorooctanoate was overtly toxic to rats in that 5/24 did not survive to term, most of the survivors had wet abdomens; reddish-brown discoloration around the eyes, nose, and mouth; lethargy; decreased feed consumption and body weight gain during the exposure period; and an unkempt appearance.

Maternal liver weight changes among groups on a relative weight basis indicated that exposure to ammonium perfluorooctanoate at 10.0 mg/m^3 or greater resulted in significantly larger livers. This increase in liver weight occurred despite the significant decrease in body weight gain in these groups, which significantly reduced the relative liver weights of the pair-fed control groups.

No differences from control were observed in the mean number of implants, mean number of corpora lutea, or fetal death in any dose level tested.

Developmental toxicity was not demonstrated upon sacrifice of the dams on Day 21 of gestation at any concentration of ammonium perfluorooctanoate tested. Concentration-related embryo-fetal toxicity, expressed as decreased fetal weight, occurred only at 25.0 mg/m^3, which was overtly toxic to the dams. This decreased body weight persisted to Day 1 PP, but not to Day 4 PP.

On Days 15, 16, or 17 PP, coded examination of the pups' eyes *in vivo* from Run I did not reveal concentration-related malformations. In view of these negative results, similar *in vivo* examination of the eyes of the pups from Run II was not conducted.

Ammonium perfluorooctanoate did not demonstrate a unique hazard to the conceptus.

Reference: DuPont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 881-81.

Staples, R. E. et al. (1984). Fundam. Appl. Toxicol., 4:429-440.

Type: **Developmental Toxicity in Rats**
Species/Strain: Rats/Crl:CD®(SD)BR
Sex/Number: Female/25/group (Experiment I), 12/group (Experiment II)
Route of Administration: Gavage
Exposure Period: Days 6-15 of Gestation

40

Exposure Levels:	0, 100 mg/kg
Method:	In a pretest, 2 non-pregnant female rats were administered ammonium perfluorooctanoate by gavage at 150 mg/kg/day or 100 mg/kg/day to determine the maximum dose that the dams could tolerate for the planned exposure period of 10 days.

For the definitive study, female rats were mated to males, and the day that spermatozoa were detected in vaginal lavage was designated as Day 1 of gestation (Day 1G).

For Experiment I, body weights, clinical signs, and feed consumption during gestation were recorded. The dams were coded from just before sacrifice until all maternal and fetal data were collected, and until all structural alterations noted among the fetuses were classified.

After sacrifice of the dams on Day 21G, gross pathologic changes were examined, liver weight was recorded, and reproductive status was determined. The number of corpora lutea and implantation sites were counted, and the number and position of all live, dead, and resorbed fetuses were recorded. All live and dead fetuses were weighed and sexed externally and internally, and the live fetuses were examined for external alterations. Approximately ½ of the fetuses of each litter that were alive when removed from the dam were examined for visceral alterations. In addition, all stunted or malformed fetuses were similarly examined. The heads of all fetuses examined for visceral alterations and a sufficient number of the remainder to total 2/3 of each litter were fixed and examined for head alterations. All fetuses, except the heads of those that were fixed, were examined for skeletal alterations.

For Experiment II, the procedures used until Day 21G were the same as for Experiment I, except that body weights were collected on different gestation days, feed consumption was not measured, and the identity of each offspring within litters was not retained. At least two days before expected parturition, each dam was housed in a polycarbonate cage with bedding. The date of parturition was noted, and was termed Day 1 PP. The dams were weighed and examined for clinical signs. For each test group fertility index and gestation index were calculated, and for each litter viability index and lactation index were calculated. All dams were sacrificed on Day 23 PP, without pathological examination.

The pups from each dam were counted, sexed, weighed, and examined for external alterations toward the end of Day 1 PP.

Thereafter, each pup was weighed and inspected for adverse clinical signs on Day 4, 7, 14, and 22 PP. The eyes of the pups in both groups were examined by an ophthalmologist between Days 27 and 31 PP. This examination was conducted with the exposure levels coded. On Day 35 PP, each pup was sacrificed and the eyes were fixed for histologic evaluation.

Data were analyzed by appropriate statistical methods.

GLP: Yes

Test Substance: Ammonium perfluorooctanoate, purity ≥ 95%

Results: In the pretest, one rat (weighing 278 g) dosed at 150 mg/kg showed severe clinical signs of toxicity by the 4th day and was found dead on the morning of the 5th day, by which time it had lost approximately 40 g body weight. Another rat (weighing 260 g) dosed at 150 mg/kg lost approximately 11 g by the 3rd day. After 5 days of dosing at 100 mg/kg, adverse clinical signs were not noted in 1 rat that lost approximately 6 g, and were minimal in the other rat that lost approximately 14 g. On this basis, the 100 mg/kg/day dosage level was judged to be the maximum that the dams could tolerate for the planned exposure period of 10 days.

In Experiments I and II, 5 of the 37 dams given ammonium perfluorooctanoate were found dead and 1 was sacrificed in a moribund state, as compared to 0 of the 37 control animals. During the dosing period, all but 1 of the dams that subsequently died had wet perineal areas and were lethargic. Two also had chromodacryorrhea and chromorhinorrhea. Among the remaining dams, 4 developed alopecia, 1 had lung noise, and 1 had diarrhea. In the control group, the only clinical sign noted was focal alopecia that developed in 1 dam.

From Days 6-15G, the treated group in Experiment I gained approximately 1/3 less than the control group, and during the post-treatment period (Days 16-21G), the body weight gain of the ammonium perfluorooctanoate-treated group significantly exceeded that of the control group. In Experiment II, the Day 16G body weight was not taken, therefore, Days 6-15 body weight gains were not calculated.

Feed consumption was measured only for Experiment I. During the dosing period, the treated group consumed significantly less feed than the control group. Feed consumption was similar to the control value in the post-exposure period.

Mean maternal liver weight for the treated group was increased, but the difference was not statistically significant. At sacrifice, 1 of the

dams given ammonium perfluorooctanoate was observed to have several red areas on the visceral surface of the median lobe of the liver.

In Experiment I, the maintenance of pregnancy, the incidence of resorptions, and fetal body weight were not adversely affected by ammonium perfluorooctanoate administration. Similarly, in Experiment II, no adverse effect on reproductive performance or on pup viability or growth was demonstrated.

The only embryo-fetal toxicity finding noted that could be compound-related was an increased incidence of fetuses with ossification sites on the first lumbar vertebrae versus the incidence in the control group. This difference in incidence was statistically significant only if analyzed by a one-tailed test. Its presence was probably a response to generalized stress evoked by the toxic state of the dams. The postpartum viability, growth rate, and development of the offspring from additional dams given ammonium perfluorooctanoate were not affected.

In vivo examination of pups' eyes between Days 27 and 31 PP revealed no compound-related alterations in the pups.

Ammonium perfluorooctanoate did not demonstrate a unique hazard to the conceptus.

Reference: DuPont Co. (1982). Unpublished Data, Haskell Laboratory Report No. 1-82.

Miscellaneous

Type: **Metabolism**

Species/strain: Female and male rats/Crl:CD®

Method: Female albino rats (7-8 weeks old and weighing approximately 200 g); pregnant primigravida females (weighing approximately 200 g); and male rats (approximately 8 weeks old and weighing approximately 250 g) were used. The rats were housed 2/cage and allowed food and water *ad libitum*.

Groups A-E rats were tested to measure blood organofluoride levels in female rats as a function of post-exposure time following oral administration and to investigate multiple versus single doses. Group A (21 female rats) was dosed with 25 mg/kg orally and 3 rats were sacrificed at ¼, ½, 1, 2, 4, 8, and 24 hours after dosing. Groups B and C (12 female rats/group) were orally dosed with 2.5 or 150 mg/kg, respectively, and 3 rats were sacrificed at ½, 2, 8, and 24 hours after dosing. Group D (21 female rats) was dosed

11 days with 25 mg/kg orally each day and 3 rats were sacrificed at ¼, ½, 2, 4, 8, 24, and 168 hours after the 11th dose. Group E (12 male rats) was dosed at 25 mg/kg orally and 3 rats were sacrificed at ½, 8, 24, and 168 hours after dosing.

Groups F-I rats were tested to measure blood organofluoride levels in female rats following inhalation exposure. Group F (24 female rats) was exposed to a single 6-hour exposure of 10 mg/m^3 and 3 rats were sacrificed at ¼, ½, 1, 2, 4, 8, 24, and 168 hours after exposure. Groups G and H (12 female rats/group) were exposed to a single 6-hour exposure of 1 mg/m^3 or 0.1 mg/m^3, respectively, and 3 rats were sacrificed at ½, 2, 8, and 24 hours after exposure. Group I (12 male rats) was exposed to a single 6-hour exposure of 10 mg/m^3 and 3 rats were sacrificed at ½, 2, 8, and 24 hours after exposure.

Groups J-N were tested to compare blood organofluoride levels in pregnant versus non-pregnant rats and to compare oral exposure versus inhalation exposure. Group J (12 pregnant female rats) was administered 25 mg/kg orally on gestation day 15 and 3 rats were sacrificed at ½, 2, 8, and 24 hours after dosing. Group K (6 pregnant female rats) was administered 25 mg/kg orally on gestation days 6-11 and 3 rats were sacrificed at ½ and 2 hours after the 6th dose. Group L (12 pregnant female rats) was administered 25 mg/kg orally on gestation days 6-15 and 3 rats were sacrificed at ½, 2, 8, and 24 hours after the 10th dose. Group M (6 pregnant female rats) was exposed via a single 6-hour inhalation exposure to 10 mg/m^3 on gestation day 15 and 3 rats were sacrificed at ½ and 2 hours after exposure. Group N (3 pregnant female rats) was exposed via inhalation, 6 hours/day, to 10 mg/m^3 on gestation days 6-15 and 3 rats were sacrificed at ½ hour after the 10th exposure.

Blood samples were obtained from each rat.

GLP: No

Test Substance: Ammonium perfluorooctanoate, purity approximately 100%

Results: The uptake and clearance of ammonium perfluorooctanoate from the blood of female rats following a single oral dose was rapid with the peak reached 1-2 hours post-treatment and with virtual total clearance by 24 hours. A dose-response was demonstrated with no apparent changes in blood organofluoride levels following multiple oral dosing. A slower clearance rate in male rats was demonstrated following a single oral dose.

A single 6-hour inhalation exposure resulted in peak blood levels within 1 hour after cessation of exposure. The test substance

rapidly cleared from the blood, and the number of exposures did not affect blood levels. Male rats cleared the compound much more slowly.

Pregnant and non-pregnant rats showed similar organofluoride blood levels following either oral or inhalation exposures.

The amount of ammonium perfluorooctanoate present as the straight chain isomer increases relative to the non-straight chain isomers as the time following ammonium perfluorooctanoate administration increases. This suggested that the non-straight chain isomers are cleared from the blood more rapidly than the straight chain isomer, or that a metabolite is present.

Reference: DuPont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 593-81.

Type: **Metabolism**

Species/strain: Male rats/Crl:CD®
Male mice

Method: ^{14}C-Ammonium perfluorooctanoate (0.5 μCi/mg) as an aqueous solution was administered by intragastric intubation to 8 young adult male rats and 6 adult male mice at a dose of 10 mg/kg. One control group (2 rats and 2 mice) was sacrificed at 24 hours and 1 control group (3 rats and 2 mice) was sacrificed at 96 hours. The treated group (3 rats and 2 mice) was dosed with cholestyramine (1000 mg/kg) 24 hours after dosing with the test substance and then sacrificed at 96 hours. After dosing, rats and mice were placed in individual glass metabolism chambers. Exhaled ^{14}C was sampled at 24, 48, 72, and 96 hours. Feces and urine were collected. At sacrifice time, 1-2 mL of blood was removed from the mice and approximately 8 mL of blood was removed from the rats. Livers were removed, homogenized, weighed, and 0.5 g samples were oxidized and counted.

GLP: No

Test Substance: Ammonium perfluorooctanoate, purity approximately 100%

Results: Although a previous study (DuPont Report No. 828-81 – described below) indicated that cholestyramine resin could reduce the acute lethal effects of ammonium perfluorooctanoate in this study, there was no sign of enhanced elimination of ^{14}C-ammonium perfluorooctanoate via feces, urine, or exhaled air. The differences between the 2 studies are most likely because of differences in absorption of the test substance. In the previous study, much of the dose was probably still in the gastrointestinal tract when cholestyramine was administered. The non-absorbed ammonium perfluorooctanoate then associated with cholestyramine, was removed before absorption could occur, and thus prevented the

acute lethal effects of ammonium perfluorooctanoate.

For both rats and mice, the primary route of excretion was urinary, followed by fecal and expired air.

After 96 hours, fecal elimination was nearly the same for both species; however, the mouse expired significantly more ^{14}C in the air than the rat. Most notably, the mouse excreted less in the urine than the rat. This difference in urinary excretion was reflected as a greater concentration in mouse liver.

Reference: DuPont Co. (1982). Unpublished Data, Haskell Laboratory Report No. 405-82.

Pastoor, T. P. et al. (1983). The Toxicologist, 3:82.

Type: **Metabolism**

Species/strain: Male and female rats
Male and female mice
Male and female hamsters
Male and female rabbits

Method: A male and female of each species received a single 10 mg/kg dose of ^{14}C-ammonium perfluorooctanoate via intragastric intubation. The rats, mice, and hamsters were housed individually in glass metabolism units immediately after dosing. Expired CO_2, urine, and feces were collected at 12, 24, 48, 72, 96, and 120 hours after dosing. The male and female rabbits were individually housed in stainless steel metabolism cages immediately after dosing. Urine and feces were collected 24, 48, 72, 96, 120, 144, and 168 hours after dosing. Expired CO_2 was not collected. Blood was drawn from the rabbits 168 hours after dosing.

The rats, mice, and hamsters were all sacrificed 120 hours after dosing and blood was drawn. All animals were dissected with following tissues excised, weighed, and frozen: heart, lungs, liver, kidneys, spleen, testes or ovaries, brain, G. I. tract, and muscle, skin, and fat samples. The carcasses were then weighed and frozen. Metabolism units were washed and the cage washes were collected and refrigerated.

Urine, CO_2 samples, and cage washes were analyzed directly for radioactivity using a liquid scintillation counter. Blood, feces, tissues, organs, and homogenized carcass samples were analyzed for ^{14}C content by tissue oxidation using a tissue oxidizer and liquid scintillation counter. Rabbit carcasses were not analyzed for ^{14}C content.

GLP: No

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Test Substance: Ammonium perfluorooctanoate, purity 70%

Results: Substantial sex differences in rats and hamsters were observed in the excretion of ^{14}C activity following a single oral dose. The female rat and the male hamster excreted over 99% of the original ^{14}C activity by 120 hours after dosing, conversely the male rat and the female hamster excreted 39 and 60% of the original ^{14}C activity, respectively, by 120 hours post-dosing. Both sexes of rabbits excreted the ^{14}C activity as rapidly and completely as the female rat and the male hamster. The male and female mice excreted only 21% of the original ^{14}C activity by 120 hours post-dosing. The rapid excretors (female rat, male hamster, and male and female rabbits) contained negligible amounts of ^{14}C in organs and tissues at sacrifice. The slow excretors exhibited the highest ^{14}C concentrations in the blood and liver with substantial levels in the kidneys, lungs, and skin.

Reference: DuPont Co. (1982). Unpublished Data, Haskell Laboratory Report No. 62-82.

Type: **Placental Transfer**

Species/strain: Pregnant rats/strain not specified

Sex/Number: Females/6

Method: ^{14}C-labeled ammonium perfluorooctanoate was labeled on the carbonyl position and had a specific activity of 0.5 µCi/mg. Water was used as the dosing vehicle. The pregnant female rats received a single 10 mg/kg dose of ^{14}C-labeled ammonium perfluorooctanoate by gavage on the 19th day of gestation. The rats were individually placed in glass metabolism units immediately after dosing.

Two rats were sacrificed at each time interval of 2, 4, and 8 hours after dosing. Blood was drawn at sacrifice and refrigerated. The placentas, umbilical cords, and fetuses were then removed, dissected from each other, and weighed. The umbilical cords and placentas were individually placed into paper combustion cones and fetuses were frozen. Organs and tissues were also excised, weighed, and frozen. The carcasses were also weighed and frozen. Urine and fecal samples excreted between dosing and sacrifice were collected and frozen. The metabolism units were washed successively with dilute detergent, water, and acetone. The washes were collected and stored.

The placentas, umbilical cords, and fetuses were oxidized in their entirety. Samples of the maternal tissues, carcass, and feces were also oxidized and analyzed for ^{14}C activity by liquid scintillation counting. The urine and cage washes were analyzed directly by liquid scintillation counting.

GLP:	No
Test Substance:	Ammonium perfluorooctanoate, purity approximately 70%
Results:	Placental transfer of ^{14}C-labeled ammonium perfluorooctanoate was shown to occur after administration of a single oral dose of ^{14}C-labeled ammonium perfluorooctanoate. The comparison of fetal levels of ammonium perfluorooctanoate at 2 and 4 hours relative to the concentrations observed in the maternal blood, placenta, and other organs revealed evidence of resistance to placental transfer of the test compound. However, by 4 hours, the fetal concentrations of ammonium perfluorooctanoate increased substantially more than all other organs and tissues examined. In contrast to other tissues examined, ammonium perfluorooctanoate in the fetuses did not decrease between 4-8 hours. The peak fetal ammonium perfluorooctanoate concentrations were similar in magnitude to the levels observed in the spleen, heart, lungs, and fat. Significant quantities of ammonium perfluorooctanoate can, therefore, be transferred from the placenta to the fetus with the placental barrier offering minimal resistance to transfer.
Reference:	DuPont Co. (1982). Unpublished Data, Haskell Laboratory Report No. 61-82.

Type:	**Effects of Dowex® Ion Exchange on the Toxicity of Ammonium Perfluorooctanoate in Rats**
Species/strain:	Male rats/Crl:CD®
Method:	A range-finding study was conducted prior to the test to find the tolerated dose of Dowex® 1-X2-C1 (cholestyramine). In that study, rats were dosed from 200-1000 mg/kg, 1 rat/dose level, and 6 rats dosed at 1000 mg/kg. No clinical signs of toxicity were noted in this study. Ammonium perfluorooctanoate, as a suspension in corn oil, was administered by intragastric intubation to young adult male rats. Four groups, 6 rats/group, were used in the study. Group I was dosed with 500 mg/kg of ammonium perfluorooctanoate, Group II was dosed with 500 mg/kg of ammonium perfluorooctanoate and immediately dosed with 1000 mg/kg of Dowex® 1-X2-C1, Group III was dosed with 1000 mg/kg Dowex® 1-X2-C1 and 2 hours later dosed with 500 mg/kg of ammonium perfluorooctanoate, and Group IV was dosed with 500 mg/kg of ammonium perfluorooctanoate and 2 hours later dosed with 1000 mg/kg of Dowex® 1-X2-C1. All rats were weighed and observed over a 14 day recovery period and then sacrificed.
GLP:	No
Test Substance:	Ammonium perfluorooctanoate, purity approximately 100%
Results:	Pre-dosing or post-dosing with Dowex® 1-X2-C1 Ion Exchange Resin at 1000 mg/kg changes the toxic effects of ammonium

perfluorooctanoate in rats. All rats dosed with the Dowex® 1-X2-C1, either before or after the ammomium perfluooctanoate had reduced mortalities compared to the rats dosed with ammomium perfluooctanoate alone. Mortality ratios were 5/6, 0/6, 1/6, and 0/6 for Groups I, II, III, and IV, respectively.

A follow-up study (DuPont Report No. 405-82 – described above) was performed to determine whether cholestyramine would enhance the elimination of absorbed ammonium perfluorooctanoate from the body.

Reference: DuPont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 828-81.

Type: **Respirator Evaluation**

Method: The purpose was to evaluate M.S.A. Combination Type GMA-H respirator cartridges against ammonium perfluorooctanoate dust for breakthrough. The cartridge pairs were tested against an average concentration of 0.56 mg/m^3 ammonium perfluorooctanoate generated in a dust generator, and introduced into an air blow of 60 L/min maintained at 50% relative humidity and ambient room temperature. Both upstream and downstream airborne concentrations were monitored for ammonium perfluorooctanoate dust by filter paper cassettes backed up with an impinger sampling train to absorb possible ammonium perfluorooctanoate vapors. Aliquots of the aqueous extracts of the filter paper and aliquots of the aqueous impinger samples were analyzed colorimetrically for ammonium perfluorooctanoate.

GLP: No

Test Substance: Ammonium perfluorooctanoate, purity not specified

Results: After 40 hours of continuous exposure, no detectable amount of ammonium perfluorooctanoate dust or vapor was found downstream on the breathing side of the cartridge pairs tested. The minimum detectable limit was 1.5 µg of ammonium perfluorooctanoate. Based on a 420 L air sample, this would calculate to be 0.004 mg/m^3. The mean upstream airborne concentrations were 0.67 ± 0.33 and 0.48 ± 0.15 mg/m^3 at 40 and 54 hours, respectively. The results demonstrated that the M.S.S. combination type GMA-H cartridges effectively filter ammonium perfluorooctanoate dust and vapor concentrations at the test conditions for a minimum of 40 hours.

Reference: DuPont Co. (1980). Unpublished Data, Haskell Laboratory Report No. 664-80.

Type:	**Glove Permeation Testing**
Method:	Five types of gloves (neoprene; neo-synthetic latex rubber, floc lined; latex; natural latex; neoprene rubber) were evaluated for their permeation resistance to a 30% aqueous solution of ammonium perfluorooctanoate and ammonium perfluorooctanoate dry powder. Samples of the gloves were tested in duplicate in 10 mL glass permeation cells with water used as a collection medium on the inside surface of the gloves. A 10 mL aliquot of the collecting medium was analyzed for ammonium perfluorooctanoate by spectrophotometry after an 8-hour exposure. Breakthrough was determined when a detectable amount of ammonium perfluorooctanoate was found in the collection medium. The minimum detection limit for ammonium perfluorooctanoate was 1 µg/mL in the collection medium.
GLP:	No
Test Substance:	Ammonium perfluorooctanoate, purity not specified
Results:	Results of the evaluation show that 3 of the glove samples (neoprene; neo-synthetic later rubber, floc lined; and natural latex) do have a measurable breakthrough time and permeation rate after 8 hours of continuous exposure to a 30% solution of ammonium perfluorooctanoate. However, the permeation rates are very low indicating that the materials do offer some resistance to the test substance. Results of the tests performed with ammonium perfluorooctanoate powder show that in 4 of the samples (neoprene; neo-synthetic latex rubber, floc lined; latex; and neoprene rubber) no breakthrough was observed after 8 hours of continuous exposure, and only a very small amount permeated the latex sample.
Reference:	DuPont Co. (1981). Unpublished Data, Haskell Laboratory Report No. 612-81.

Type:	**Biopersistence Screening Study** Summarized in the PFOS section, see DuPont (2000). Haskell Laboratory Report No. 2922.

SUMMARY OF STUDIES CONDUCTED WITH AMMONIUM PERFLUORONONANOATE AT DUPONT

Mammalian Toxicity

Acute Toxicity

Type:	**Oral ALD**
Species/strain:	Male rats/ChR-CD
Value:	187 mg/kg
Method:	The test substance, as a solution in water, was administered by intragastric intubation to young adult male rats (1/group) in single doses. Concentrations tested were 1.0, 1.5, 2.3, 3.4, 5.1, 7.7, 12, 17, 26, 40, 60, 90, 130, 187, 300, 450, 670, 1000, 1500, and 2250 mg/kg. Survivors were sacrificed 14 days later, and body weights and liver weights were recorded.
GLP:	No
Test Substance:	Ammonium perfluorononanoate, purity not specified
Results:	Mortality was observed at concentrations of 187 mg/kg and above. Deaths occurred within 6 days after dosing. Slight initial weight losses occurred at 26 and 40 mg/kg. Weight loss occurred for 9, 12, and 15 days at 60, 90, and 130 mg/kg, respectively. No clinical signs were observed below 26 mg/kg. Clinical signs observed at 26 mg/kg and above included pallor, salivation, polyuria, and chewing-motions. Additional clinical signs observed at 187 mg/kg and above included belly-crawling, half-closed eyes, incoordination, ruffled fur, diarrhea, and emanciation. Increased liver weights and increased liver/body weight ratios occurred at 3.4 mg/kg and above.
Reference:	DuPont Co. (1968). Unpublished Data, Haskell Laboratory Report No. 129-68.

Type:	**Inhalation ALC**
Species/strain:	Male rats/Crl:CD®(SD)BR
Value:	590 mg/m^3
Method:	Male rats (6/group), 8 weeks old and weighing between 234 and 298 g, were exposed via nose-only inhalation for a single, 4-hour period to a dust atmosphere of ammonium perfluorononanoate in air. Concentrations tested were 620, 910, 1600, and 4600 mg/m^3. Dust atmospheres were generated with a bin feeder regulated with a volumetric feed controller. The bin feeder metered test substance into a glass transfer tube. Air introduced at the tube swept the test substance through a size-reducing cyclone and into the exposure chamber. For the lowest exposure concentration, the atmosphere was generated by passing pressurized air through a glass generator. A flask at the bottom of the generator served as a dust reservoir. A

cyclone elutriator was inserted above the reservoir. A motorized stirring rod agitated dust in the generator. Air introduced at the bottom of the reservoir and at the cyclone elutriator swept dust particles into the exposure chamber. The atmospheric concentrations was determined at approximately 30 minute intervals by drawing calibrated volumes of chamber atmosphere through filters. The atmospheric concentration of particulate was determined from the filter weight differential before and after sampling. Chamber temperature, relative humidity, and chamber oxygen content were measured. Except during exposure, food and water were available *ad libitum*. Body weights and clinical signs were recorded. Survivors were sacrificed 14 days later.

To monitor the effects of ammonium perfluorononanoate on the liver, 2 groups of 10 rats, 8 weeks old and weighing between 237 and 277 g, were exposed to 67 and 590 mg/m^3, respectively. Two groups of 10 rats, 8 weeks old and weighing 231 and 267 g, were exposed to air only. Each control group was exposed concurrently with one of the test groups. Five rats/group were sacrificed 5 or 12 days after exposure for pathologic examination of the liver.

GLP: Yes

Test Substance: Ammonium perfluorononanoate, purity >99%

Results: Chamber temperatures ranged between 23-27°C, relative humidities ranged from 19-45%, and chamber oxygen contents were 21%.

One of the rats in the 590 mg/m^3 died on the 12th day of exposure. Mortality ratios of 0/6, 4/6, 6/6, and 6/6 were observed in the 620, 910, 1600, and 4600 mg/m^3. Clinical signs observed during or immediately post-exposure included red or brown facial discharge (67-1600 mg/m^3), test substance on the head (620, 1600, and 4600 mg/m^3), labored breathing (4600 mg/m^3), profuse clear nasal and oral discharges (4600 mg/m^3), and diminished startle response (4600 mg/m^3). No adverse clinical signs were observed in rats exposed to 67 mg/m^3 throughout the recovery period. Common clinical signs at higher concentrations included hunched posture, ruffled or discolored fur, red or brown facial discharges, wet or stained perineum, pallor, lung noise or labored breathing, lethargy, limpness, and hair loss.

Rats exposed to 67 mg/m^3 lost 1-9% of initial body weight 1 day post-exposure, followed by normal weight gain. At concentrations greater than 67 mg/m^3, rats lost approximately 6-15% of initial body weight 1 day post-exposure, and continued to lose weight either throughout the recovery period or until they died. Most

surviving rats exposed to 590, 620, or 910 mg/m^3 weighed only 54-71% of initial body weight when they were sacrificed 12 days post-exposure or at the end of the recovery period.

Rats exposed to 67 mg/m^3 had significantly elevated mean liver weights and liver-to-body weight ratios on the 5th and 12th days after exposure. Rats exposed to 590 mg/m^3 had significantly elevated liver-to-body weight ratios on the 12th day after exposure. Liver weights for these rats were not significantly different from the controls on the 5th day of recovery, and mean liver weights were significantly depressed on the 12th day of recovery. However, these seemingly inconsistent changes were due to severe body weight loss in rats exposed to 590 mg/m^3. Gross pathologic examination of rats exposed to 590 mg/m^3 revealed discolored livers with prominent lobular patterns in 4/5 rats sacrificed on the 5th day of recovery, and similar gross liver lesions in 2/5 rats sacrificed on the 12th day of recovery.

References: DuPont Co. (1985). Unpublished Data, Haskell Laboratory Report No. 293-85.

Kinney, L. A. et al. (1989). Food Chem. Toxicol., 27:465-468.

Repeat Dose Toxicity

Type:	**Repeated Dose Oral Toxicity Study**
Species/Strain:	Mice/Crl:CD®-1(ICR)BR
Sex/Number:	Male and female/5/group
Exposure Period:	14 days
Frequency of Treatment:	*Ad libitum* for 14 days
Exposure Levels:	0, 1, 3, 10, 30, 100, 300, 1000, 3000, 10,000 mg/kg
Method:	Male and female mice (5-6 weeks old) were fed diets containing ammonium perfluorononanoate for 14 consecutive days. Verification of the concentration of the test substance in the prepared diets were performed. Body weights and clinical signs were recorded throughout the test. After sacrifice, liver weights were recorded. Body weights and liver weights were analyzed with appropriate statistical methods. Sacrifices occurred on test day 6 at concentrations of 1000 ppm and greater, on test day 8 at concentrations of 100 and 300 ppm, and on test day 14 for the remaining test groups.
GLP:	No
Test Substance:	Ammonium perfluorononanoate, purity 99%

Results: Mortality ratios of 0/5, 0/5, 0/5, 3/5, and 1/5 were observed for the male mice fed 100, 300, 1000, 3000, and 10,000 ppm, respectively. Mortality ratios of 0/5, 3/5, 4/5, 5/5, and 3/5 were observed for the female mice fed 100, 300, 1000, 3000, and 10,000 ppm, respectively. Mice fed concentrations of 100 ppm or less exhibited slight to severe sporadic weight loss. Mice fed concentrations of 300 ppm and higher exhibited severe weight loss, ruffled fur, lethargy, low posture, and limpness.

Male and female mice fed diets of 3 ppm or higher had significantly increased mean absolute and mean relative liver weights. The mean relative liver weights of male mice fed the 1 ppm diet were also significantly heavier than the controls. Comparisons of mean body and liver weights of mice fed diets above 30 ppm were not possible because there were no concurrent control groups.

Reference: DuPont Co. (1985). Unpublished Data, Haskell Laboratory Report No. 401-85.