COVANCE
THE DEVELOPMENT SERVICES COMPANY

**Sponsor**:

APME Ad-Hoc APFO Toxicology Working Group

# FINAL REPORT

**Study Title**:

4-Week Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO) in Cynomolgus Monkeys

**Author**:

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**Study Completion Date**:

December 18, 2001

**Performing Laboratory**:

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3301 Kinsman Boulevard
Madison, Wisconsin 53704-2595

**Laboratory Project Identification**:

Covance 6329-230

**Sponsor Project Identification**:

3M Study No. T-6889.2

## COMPLIANCE STATEMENT

4-Week Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO)
in Cynomolgus Monkeys

All aspects of this study were in accordance with the Environmental Protection Agency Good Laboratory Practice Standards (GLPs), 40 CFR 792, except that bile acid determinations done by the University of Dundee were not done in compliance with GLPs.

Peter J. Thomford, PhD
Study Director
Covance Laboratories Inc.

18 Dec. 2001
Date

Paul Lieder, PhD
Diplomate, ABT
Study Monitor
3M

Date

## QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Covance Laboratories Inc., in accordance with the Environmental Protection Agency (EPA) Good Laboratory Practice Standards, 40 CFR 792. The following inspections were conducted and findings reported to the study director and study director management.

| Inspection Dates | | | Date Reported to Study Director and Study Director Management |
|---|---|---|---|
| From | To | Phase | |
| 06/07/98 | 06/07/98 | Protocol Review | 06/08/98 |
| 07/16/98 | 07/16/98 | Clinical Laboratory Inspection | 07/16/98 |
| 09/22/98 | 10/01/98 | Report Review | 10/01/98 |
| 10/01/98 | 10/01/98 | Protocol Amendment Review | 10/01/98 |
| 09/15/98 | 10/02/98 | Data Review | 10/02/98 |
| 06/10/99 | 06/10/99 | Protocol Amendment Review | 06/10/99 |
| 12/07/99 | 12/07/99 | Protocol Amendment Review | 12/07/99 |
| 02/23/01 | 02/23/01 | Report Review | 02/23/01 |
| 11/27/01 | 11/27/01 | Report Review | 11/28/01 |

Caroline E Hunter
Representative
Quality Assurance Unit

18 December 2001
Date

## STUDY IDENTIFICATION

4-Week Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO)
in Cynomolgus Monkeys

| | |
|---|---|
| Test Material | Ammonium Perfluorooctanoate (APFO) |
| Sponsor | APME Ad-Hoc APFO Toxicology Working Group |
| Study Monitor | Paul Lieder, PhD, DABT<br>3M<br>Toxicology Services<br>Building 220-2E-02, 3M Center<br>St. Paul, Minnesota 55144-1000<br>612.737.2678 |
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| Study Location | Covance Laboratories Inc.<br>3301 Kinsman Boulevard<br>Madison, Wisconsin 53704-2595 |
| Study Director | Peter J. Thomford, PhD<br>Covance Laboratories Inc.<br>P.O. Box 7545<br>Madison, Wisconsin 53707-7545<br>608.241.7207 |
| Study Timetable | |
| Study Initiation Date | June 11, 1998 |
| In-Life (Experimental) Start Date | June 17, 1998 |
| In-Life Termination Date | July 16, 1998 |
| Experimental Termination Date | December 18, 2001 |

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| Anatomical Pathologist | James L. Carter, DVM, PhD<br>Diplomate, ACVP |
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## ABSTRACT

The purpose of this study was to provide data for determining an estimated maximum-tolerated dose and lower dose levels to be used in a chronic toxicity study. In addition, the effect on critical enzyme levels, hormones, and other selected biochemical parameters was determined.

Male cynomolgus monkeys were assigned to three groups (two animals in Group 1, three animals each in Groups 2 and 3). Animals in Groups 2 and 3 received gelatin capsules containing 2 and 20 mg Ammonium Perfluorooctanoate (APFO)/kg of body weight/day (mg/kg/day), respectively. Animals in Group 1 received empty gelatin capsules.

Food was provided once or twice daily. Water was provided *ad libitum*. The animals were observed twice daily (a.m. and p.m.) for mortality and moribundity. At least once daily, animals were examined for abnormalities and signs of toxicity, and food consumption was assessed qualitatively. In addition, animals were observed approximately 30, 60, and 90 minutes postdose for signs of poor health or abnormal behavior. Body weight data were collected before initiation of treatment, on the first day of treatment, and weekly thereafter. Blood samples for hormone analyses [estradiol, estrone, estriol, thyroid stimulating hormone, total and free triiodothyronine (T3), and total and free thyroxin (T4)] were collected before initiation of treatment and on Day 30. Serum was harvested, and the samples were sent to Ani Lytics Inc., for analyses. Blood samples for cholecystokinin analyses were collected before initiation of treatment and on Day 30. Plasma was harvested, and the samples were sent to the DuPont Haskell Company for possible analyses. Blood samples for serum APFO concentration analyses were collected before initiation of treatment and on Day 30. Serum was harvested, and samples were sent to 3M for analyses. Blood samples were collected for hematology and clinical chemistry tests twice before initiation of treatment and on Day 30. In addition, blood for clinical chemistry tests was collected on Day 2. Additional blood was collected at the time of exsanguination, and samples were shipped to 3M for possible future analysis. On Day 30, the animals were anesthetized, weighed, exsanguinated, and necropsied. At necropsy, macroscopic observations were recorded, and selected tissues were collected and preserved. In addition, a sample of liver was collected from each animal for palmitoyl CoA oxidase activity analyses. Representative samples of liver, testes, and pancreas were collected from each animal and sent to Pathology Associates, A Charles River Company for proliferation cell nuclear antigen evaluation. A sample from

the liver and pancreas was collected from each animal and sent to the DuPont Haskell Company for possible receptor level determinations. Bile was collected from each animal and sent to the University of Dundee for bile acid determination. A sample of liver was collected from each animal and sent to 3M for APFO concentration analyses. Microscopic examinations were done on the adrenals, liver, pancreas, spleen, and testes from each animal.

All animals survived to the scheduled sacrifice. There were no apparent test material-related effects on estradiol, estriol, thyroid stimulating hormone, total and free triiodothyronine, and total and free thyroxin. Estrone levels were notably lower for males given 2 and 20 mg/kg/day.

There were no apparent test material-related effects on clinical observations, body weights, food consumption, clinical pathology parameters, or macroscopic or microscopic findings.

There did not appear to be a cell proliferative response to the test material in the liver, testes or pancreas, as determined by proliferating indices which were similar in all animals.

Based on the results of this study, daily capsule administration of Ammonium Perfluorooctanoate to cynomolgus monkeys for 29 days at dose levels of 2 and 20 mg/kg/day was well tolerated and produced no apparent evidence of toxicity.

## PURPOSE

The purpose of this study was to provide data for determining an estimated maximum-tolerated dose and lower dose levels to be used in a chronic toxicity study. In addition, the effect on critical enzyme levels, hormones, and other selected biochemical parameters was determined.

## REGULATORY COMPLIANCE

All aspects of this study were done in accordance with the Environmental Protection Agency Good Laboratory Practice Standards (GLPs), 40 CFR 792, except that bile acid determinations done by the University of Dundee were not done in compliance with GLPs.

## TEST MATERIAL

### Test Material

The test material, Ammonium Perfluorooctanoate (APFO), Lot No. 332 (expiration date: December 15, 2001), is a white powder and is 95.2% pure. It was received at Covance on June 10, 1998. The test material was stored at room temperature.

Information on synthesis methods, composition, or other characteristics that define the test material is on file with the Sponsor. The Certificate of Analysis is in Appendix 1.

### Reserve (Archive) Samples

A reserve sample (1 g) of the test material was taken before initiation of treatment and stored at room temperature. This sample was transferred to the Sponsor on May 2, 2001.

### Disposition

The remaining test material was returned on May 2, 2001.

## TEST SYSTEM

### Test Animal

Young adult to adult cynomolgus monkeys were obtained from Covance Research Products Inc. (Denver, Pennsylvania and Alice, Texas), on March 10, 1998. The animals weighed 2.1 to 3.6 kg at initiation of treatment.

### Identification

Each animal was assigned a permanent number upon arrival and identified with collar tag before initiation of treatment. All data for an animal are recorded under this number.

### Acclimation

Nine males were received on March 10, 1998, transferred from the Covance in-house stock colony on June 3, 1998. All animals were acclimated in Animal Room 2015; animals were acclimated at least 30 days before initiation of treatment (duration includes days animals were in the stock colony). In general, animals in this shipment appeared healthy. During acclimation, the animals were examined for abnormalities indicative of health problems. In addition, tuberculosis tests were done on each animal.

Results of the tuberculosis tests were negative for all animals. One male was eliminated from study consideration because it had the highest body weight. There were eight males in the randomization for assignment to groups (two males in Group 1 and three males each in Groups 2 and 3). The animal not selected for the study was returned to the Covance stock colony.

### Housing and Maintenance

Animal Room 2015 was used for this study. Environmental controls for the animal room were set to maintain 18 to 29$^{o}$C, a relative humidity of 30 to 70%, and a 12-hour light/12-hour dark cycle.

The animals were housed individually in stainless steel cages.

Certified primate diet (#8726C, Harlan Teklad) was provided once or twice daily. The lot numbers are recorded in the data. The diet is routinely analyzed by the manufacturer

for nutritional components and environmental contaminants. Results of specified nutrient and contaminant analyses are on file with Covance-Madison. Fruit or additional supplements were provided, but did not require analysis.

Water was provided *ad libitum*. Samples of the water are analyzed for specified microorganisms and environmental contaminants. The results are on file with Covance-Madison.

There were no known contaminants in the diet or water at levels that would have interfered with this study.

**Justification**

APFO is a known hepatic peroxisome proliferator (PP) in the rat. When exposed to PP, nonhuman primates (such as the cynomolgus monkey) respond similarly to humans (i.e., low to no hepatic response) and therefore are an appropriate human surrogate species.

## PROCEDURES

This study was conducted in accordance with the Protocol dated June 11, 1998, and Protocol Amendment No. 1 through 3. The protocol and protocol amendments are in Appendix 1.

**Group Designations and Dose Levels**

Selection of animals for the study was based on clinical observations, body weights, and other data as appropriate. Animals were assigned to treatment groups using a computerized blocking procedure designed to achieve body weight balance with respect to treatment group.

| Group | Dose Level (mg/kg/day) | Total Material Dose Level (mg/kg/day) | Number of Males |
|---|---|---|---|
| 1 (Control) | 0[a] | 0[a] | 2 |
| 2 (Low-dose) | 2 | 2 | 3 |
| 3 (High-dose) | 20 | 20 | 3 |

a The control group (Group 1) received the same size and number of empty gelatin capsules as used for Group 3.

**Dose Preparation**

Gelatin capsules (Torpac, Inc., Fairfield, New Jersey), Size No. 2, Lot Nos. 122630 (expiration date June 26, 2002) and 122932 (expiration date June 12, 2003), were used for dose administration.

The test material was dispensed into capsules weekly. The dose levels were based on the test material as supplied. For Groups 2 and 3, the specified amount of test material was weighed and transferred into the gelatin capsules. The top and bottom halves of each capsule were joined, and the capsules were placed into the appropriate container labeled with study number, group number, animal number, and dose level. The prepared capsules were stored at room temperature.

**Dose Analyses**

Because the test material was not being mixed with a vehicle, dose analyses were not required.

**Method of Administration**

Gelatin capsules were used for test material administration to compare with previously conducted toxicology studies using the oral route.

The dose preparations were administered orally in gelatin capsules once daily (7 days/week) for 29 days. The dosing tubes were flushed with 5 mL of reverse osmosis water. Individual doses were calculated based on the most recently recorded body weights, with the exception of body weight collection days when the previous body weight was used. Animals were dosed at approximately the same time each day.

**Clinical Observations**

The animals were observed twice daily (a.m. and p.m.) for mortality and moribundity. Each animal was observed daily, and food consumption was assessed qualitatively; abnormal findings were recorded. Animals were observed approximately 30, 60, and 90 minutes postdose for signs of poor health or abnormal behavior. Effects were recorded as they were observed. Animals were observed once weekly; abnormal findings or an indication of normal was recorded.

**Body Weights**

Individual body weight data were recorded before initiation of treatment, on the first day of treatment, and weekly thereafter. An additional body weight was recorded on Day -1 for the Day 1 dose calculations.

**Blood Hormone Determination**

Blood was collected from a femoral vein of each animal before initiation of treatment (Day -8) and on Day 30 (after 29 days of treatment). Blood was collected between 07:00 and 09:00. Animals were fasted overnight before collections. Approximately 3 mL (Day -8) or 6 mL (Day 30) of blood for plasma samples were collected into tubes with potassium EDTA at the anticoagulant. Approximately 5 mL (Day -8) or 6 mL (Day 30) of blood for serum samples were collected without anticoagulant and allowed to clot for serum samples. Blood samples for plasma (Day 30) were maintained chilled until plasma was harvested. Blood samples collected on Day -8 for serum and plasma and blood samples collected on Day 30 for serum were maintained at room temperature until serum and plasma were harvested. Samples for serum were centrifuged within 1 hour after collection, and serum was harvested. Serum was divided into two approximately equal aliquots and stored in a freezer set to maintain -60 to -80°C until packed on dry ice and shipped to Ani Lytics Inc., for analyses. The serum samples were analyzed for estradiol (E2), estrone (E1), estriol (E3), thyroid stimulating hormone (TSH), total and free triiodothyronine (T3 and FT3, respectively), and total and free thyroxin (T4 and FT4, respectively). Results of these analyses provided by Ani Lytics Inc., are in Appendix 6.

Samples for plasma were centrifuged within 1 hour after collection. Plasma was harvested and stored in a freezer set to maintain -60 to -80°C until packed on dry ice and

shipped to DuPont Haskell Company for possible future analyses. Results of the analyses for cholecystokinin, if any, will be reported separately.

**Serum APFO Level Determination**

Approximately 2 mL of whole blood were collected from a femoral vein of each animal before initiation of treatment (Day -8) and on Day 30 (after 29 days of treatment). Animals were fasted overnight before collections. All samples were collected without anticoagulant, maintained at room temperature, and allowed to clot. Samples were centrifuged within 1 hour after collection, serum was harvested and stored in a freezer set to maintain -10 to -30°C until packed on dry ice and shipped to 3M for analyses. The samples were analyzed for APFO. Results of analyses will be reported separately by 3M.

**Clinical Pathology**

Blood samples were collected from each animal twice before initiation of treatment (Days -8 and -5) and on Day 30 (after 29 days of treatment) for hematology and clinical chemistry tests. In addition, blood for clinical chemistry tests was collected from each animal on Day 2 (approximately 24 hours after the first dose). Animals were fasted overnight; water was provided *ad libitum*. Blood was collected from a femoral vein. The following were evaluated:

**Hematology**

- red blood cell (erythrocyte) count
- hemoglobin
- hematocrit
- mean corpuscular volume
- mean corpuscular hemoglobin
- mean corpuscular hemoglobin concentration
- platelet count
- white blood cell (leukocyte) count
- differential blood cell count
  - segmented neutrophil count
  - lymphocyte count
  - monocyte count
  - eosinophil count
  - basophil count
- blood cell morphology
- reticulocyte count

**Clinical Chemistry**

| | |
|---|---|
| glucose | gamma glutamyl transferase |
| urea nitrogen | sorbitol dehydrogenase |
| creatinine | creatine kinase |
| total protein | calcium |
| albumin | inorganic phosphorus |
| globulin | sodium |
| total bilirubin | potassium |
| cholesterol | chloride |
| triglycerides | bile acids |
| aspartate aminotransferase | amylase |
| alanine aminotransferase | lipase |
| alkaline phosphatase | pancreatic-specific amylase |

**Additional Blood Collection**

Whole blood (approximately 15 mL) was collected from the vena cava of each animal at the time of exsanguination. Approximately equally sized samples (approximately 5 mL each) of serum (collected without anticoagulant) and whole blood and plasma using potassium EDTA as the anticoagulant were transferred into containers and stored in a freezer set to maintain -60 to -80°C until packed on dry ice and shipped to 3M for possible future analysis.

**Necropsy**

On Day 30, animals that were fasted overnight were anesthetized with ketamine and xylazine, weighed, bled for the additional blood collection, exsanguinated, and necropsied. Animals were necropsied in random order.

The necropsy included a macroscopic examination of the external surface of the body; all orifices; the cranial cavity; the brain and spinal cord; the nasal cavity and paranasal sinuses; cervical tissues and organs; and the thoracic, abdominal, and pelvic cavities and viscera.

**Palmitoyl CoA Oxidase Determinations**

Representative samples of the right lateral lobe of liver were collected from each animal at the scheduled sacrifice. The sample was weighed, flash-frozen in liquid nitrogen, and

stored in a freezer set to maintain -60 to -80$^{o}$C until analyzed for palmitoyl CoA oxidase activity.

**Cell Proliferation Evaluation**

Representative samples of the left lateral lobe of the liver, left and right testes, and pancreas were collected and preserved in zinc formalin. After fixation, samples for proliferation cell nuclear antigen (PCNA) evaluation were embedded in paraffin and maintained at ambient temperature (with slides stained with hematoxylin and eosin) until shipped to Pathology Associates, A Charles River Company for PCNA analyses. Results of the evaluation provided by Pathology Associates, A Charles River Company are in Appendix 7.

**Receptor Level Determination**

Samples (approximately 2 g) of liver and pancreas was collected from each animal at the scheduled sacrifice, flash-frozen in liquid nitrogen, and stored in a freezer set to maintain -60 to -80°C until packed on dry ice and shipped to the DuPont Haskell Company for possible analysis. Results of the receptor level determination, if any, will be reported separately.

**Bile Acid Determination**

Approximately 1.0 to 2.0 mL of bile were collected from each animal at the scheduled sacrifice, flash-frozen in liquid nitrogen, and stored in a freezer set to maintain -60 to -80°C until packed on dry ice and shipped to the University of Dundee for bile acid determination. Results of the bile acid determination will be reported separately by the University of Dundee.

**Liver APFO Determination**

A section of liver (approximately 20 g) was collected from each animal at the scheduled sacrifice, weighed, flash-frozen in liquid nitrogen, and stored in a freezer set to maintain -60 to -80°C until packed on dry ice and shipped with plasma samples to 3M for APFO analysis. Results of these analysis will be reported separately by 3M.

**Tissue Preservation**

The following tissues (when present) or representative samples were collected and preserved in 10% neutral-buffered formalin, unless otherwise specified, for possible future microscopic examination:

adrenal (2)
aorta
brain
cecum
colon
duodenum
epididymis (2)
esophagus
eyes [preserved in Davidson's fixative(2)]
femur with bone marrow (articular surface of the distal end)
gallbladder
heart
ileum
jejunum
kidney (2)
lesions
liver
lung
lymph node (mesenteric)
mammary gland
muscle (thigh)
pancreas
pituitary
prostate
rectum
salivary gland [mandibular (2)]
sciatic nerve
seminal vesicle (2)
skin
spinal cord (cervical, thoracic, and lumbar)
spleen
sternum with bone marrow
stomach
testis (2)
thymus
thyroid (2) with parathyroid
trachea
urinary bladder

**Histopathology**

The adrenals, liver, pancreas, spleen, and testes were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically from each animal.

**Statistical Analyses**

Only data collected on or after the first day of treatment were analyzed statistically.

One-way analysis of variance [ANOVA (Winer, 1971a)] was used to analyze initial body weights; palmitoyl CoA oxidase activities, and continuous clinical pathology values.

Levene's test (Levene, 1960) was done to test for variance homogeneity. In the case of heterogeneity of variance at $p \leq 0.05$, transformations were used to stabilize the variance.

ANOVA was done on the homogeneous or transformed data. If the ANOVA was significant, Dunnett's t-test (Dunnett, 1964) was used for control versus treated group comparisons.

One-way analysis of covariance [ANCOVA (Winer, 1971b)] was used to analyze body weights, with initial body weights as the covariate. Although Levene's test for variance homogeneity was done (see above), no transformations were used because covariance adjustment removed extraneous heterogeneity. If the ANCOVA was significant, least squares means t-test was used for control versus treated group comparisons.

Groups 2 and 3 were compared with Group 1 (control). Group comparisons were evaluated at the 5.0%, two-tailed probability level.

## RECORD RETENTION

All raw data, documentation, records, protocol, and specimens generated as a result of this study will be archived in the storage facilities of Covance-Madison for a period of 1 year. One year after the submission of the final report, the Sponsor will determine the final disposition of the materials. All raw data stored on magnetic media and an original copy of the final report will be retained by Covance-Madison.

Within 1 year after submission of the final report, all the aforementioned materials from the Sponsor's designees (Ani Lytics Inc., DuPont Haskell Company, Pathology Associates International, and the University of Dundee) will be sent to the Sponsor (Paul Lieder, PhD, DABT, 3M). Pathology Associates, A Charles River Company (PAI) is responsible for maintenance of any raw data or specimens produced by PAI.

## RESULTS

### Clinical Observations

Clinical observations are summarized in Tables 1 through 4; individual data are in Appendix 2. Individual animal fate data are also in Appendix 2.

All animals survived to the scheduled sacrifice. There were no test material-related clinical observations.

On Day 23, Animal No. I05419 (Group 2) was observed with a 4-cm laceration on the inner left thigh. The animal was anesthetized with ketamine, and the wound was cleaned and sutured. This condition was not considered to be test material-related.

**Body Weights**

Body weight data are summarized in Table 5; individual data are in Appendix 3.

There were no test material-related effects on body weights.

**Food Consumption**

Food consumption data are summarized in Table 1 (Summary of Clinical Observation - A.M./Weekly); individual data are included in the individual clinical observations in Appendix 2.

Low or no food consumption was noted for one animal (Animal No. I05235) given 20 mg/kg/day. For this animal, no food consumption was noted on Day 12, and low food consumption was noted on Days 5, 7, 11, 14, 16, 17, and 24. The decrease in food consumption for this animal may be test material-related**.**

**Blood Hormone Analyses**

Summary and individual hormone analyses data are in Appendix 6.

There were no apparent test material-related effects on estradiol, estriol, thyroid stimulating hormone, total and free triiodothyronine, and total and free thyroxin. Estrone levels were notably lower for males given 2 and 20 mg/kg/day.

**Clinical Pathology**

Hematology and clinical chemistry data are summarized in Tables 6 through 12; individual data are in Appendix 4. The Pathology Report contains a discussion of the data.

Administration of APFO had no effects on clinical pathology test results.

**Cell Proliferation Evaluation**

Results of cell proliferation evaluation provided by Pathology Associates, A Charles River Company are in Appendix 7.

Cell proliferation, as determined by measuring the proliferating index, was not increased in the liver, testes or pancreas of monkeys. Thus, there did not appear to be a cell proliferative response to the test material in the liver, testes or pancreas, as determined by proliferating indices which were similar in all animals.

**Anatomical Pathology**

Incidences of macroscopic and microscopic observations are summarized in Tables 13 and 14. Individual data are in Appendix 5. The Pathology Report contains a discussion of the data.

Administration of APFO had no effects on anatomical pathology test results.

## CONCLUSIONS

Based on the results of this study, daily capsule administration of Ammonium Perfluorooctanoate to cynomolgus monkeys for 29 days at dose levels of 2 and 20 mg/kg/day was well tolerated and produced no apparent evidence of toxicity.

## SIGNATURES

Patricia K. McKee Pesik, BS, LAT
Study Coordinator
Covance Laboratories Inc.

18 Dec 2001
Date

Peter J. Thomford, PhD
Study Director
Covance Laboratories Inc.

18 Dec. 2001
Date

## REFERENCES

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Levene, H., "Robust Tests for Equality of Variances," Contributions to Probability and Statistics, (eds.) I. Olkin et al., Ch. 25, pp. 278-292, Stanford University Press: Stanford, California (1960).

Winer, B. J., "Design and Analysis of Single-Factor Experiments," Statistical Principles in Experimental Design, Second Ed., Ch. 3, pp. 149-260, McGraw-Hill: New York, New York (1971a).

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# PATHOLOGY REPORT

## SUMMARY

The purpose of this study was to provide data for determining an estimated maximum-tolerated dose and lower dose levels to be used in a chronic toxicity study and to assess the effect of the test material, Ammonium Perfluorooctanoate (APFO), on critical enzyme levels, hormones, and other selected biochemical parameters.

Administration of APFO had no effects on clinical or anatomical pathology test results.

## METHODS

Three groups of male cynomolgus monkeys were administered the test material orally via capsule at a dose level of 0 (control group; received empty gelatin capsules), 2, or 20 mg/kg of body weight/day (mg/kg/day) for 29 days. There were two animals in the control group and three animals in each of the groups receiving APFO. All animals survived to the scheduled sacrifice.

Blood was collected for hematology and clinical chemistry tests twice before initiation of treatment (Days -8 and -5) and on Day 30. In addition, blood for clinical chemistry tests was collected on Day 2. The animals were sacrificed and necropsied on Day 30; macroscopic observations were recorded, and tissues were preserved in fixative as specified by the protocol. Microscopic examinations were done on adrenals, liver, pancreas, spleen, and testes from all animals. Samples of liver were collected and frozen for determination of hepatic palmitoyl CoA oxidase activity and for determination of APFO content (done by 3M). Samples of liver, testes, and pancreas were collected and preserved in zinc formalin for evaluation of proliferation cell nuclear antigen (PCNA; done by Pathology Associates, a Charles River Company). Samples of liver and pancreas were collected and frozen for possible receptor level determination (done by the DuPont Haskell Company). Samples of bile were collected and frozen for bile acid determination (done by the University of Dundee).

Statistically significant differences cited in the Results and Discussion section are based on comparisons between the control and treated groups.

## RESULTS AND DISCUSSION

### Mortality

All animals survived to the scheduled sacrifice.

### Clinical Pathology

Individual values for hematology and clinical chemistry tests are in Appendix 4. Mean values and the results of statistical comparisons are in Tables 6 through 12.

**Days -8 and -5.** Clinical pathology test results indicated no obvious group or individual health abnormalities.

**Day 2.** Administration of the test material had no effects on clinical chemistry test results at this collection interval.

There were only two statistically significant differences for clinical chemistry results between the control and treated groups; lower glucose for the low-dose group and lower total bilirubin for the high-dose group. These differences were considered incidental because similar differences were present before treatment was initiated. There were no other notable differences for clinical chemistry results between the control and treated groups.

**Day 30.** Administration of the test material had no effects on hematology or clinical chemistry test results at Day 30.

The only statistically significant differences between the control and treated groups was lower glucose for the high-dose males. This difference was considered incidental because a similar difference was present before treatment was initiated. There were no other notable differences for clinical pathology results between the control and treated groups.

### Anatomical Pathology

Individual animal pathology data are in Appendix 5. Incidence summaries of the macroscopic and microscopic observations are in Tables 13 and 14, respectively.

**Macroscopic Findings.** There were no test material-related gross findings noted at necropsy. A few commonly occurring macroscopic findings frequently observed as spontaneous lesion in monkeys were present.

**Microscopic Findings.** There were no test material-related microscopic findings. The only microscopic findings were minimal mineralization of the adrenals for one male given 2 mg/kg/day and immature testes for one male given 2 mg/kg/day and one male given 20 mg/kg/day. There were no differences in the appearances of the testes of the other two males given 2 mg/kg/day or the other two males given 20 mg/kg/day.

Robert L. Hall, DVM, PhD
Diplomate, ACVP
(Clinical Pathology)

18 December 2001
Date

James L. Carter, DVM, PhD
Diplomate, ACVP

18 December 2001
Date

## COMMENTS ON THE DATA

Various models of calculators, computers, and computer programs were used to analyze data in this study. Because different models round off or truncate numbers differently, values in some tables (e.g., means, standard deviations, or individual values) may differ slightly from those in other tables, from individually calculated data, or from statistical analysis data. Neither the integrity nor the interpretation of the data was affected by these differences.

Some tabular data were compiled using Excel® Version 7.0 software.

The summary tables for clinical observations indicate the number of animals for which a condition was observed without regard to the specific nature, severity, reversibility, number of incidences/animal, or the length of time the condition persisted.

Only observations other than normal are indicated on the summary clinical observations tables.

The day of initiation of treatment is “Day 1.”

Animal No. I05419 (Group 2) was observed and treated by the laboratory animal veterinary staff, and the data were recorded on the day the examination was done. These data are referenced in the Request for Veterinary Services and are archived with the raw data but do not appear on the summary or individual clinical observations tables.

## CODES, ABBREVIATIONS, AND UNITS

General Codes and Abbreviations
Codes for Clinical Pathology
Abbreviations and Units for Clinical Hematology
Abbreviations and Units for Clinical Chemistry
Codes for Anatomical Pathology

Note: The following lists of codes, abbreviations, and units are used by Covance. Some, but not necessarily all, of this information may be needed for this report.

## General Codes and Abbreviations

| | |
|---|---|
| WK | Week. |
| N | Number of measurements in a group. |
| Mean; MEAN | Arithmetic mean. |
| CAM | Covariate-adjusted mean. |
| SD; S.D.; STAND DEV; STANDARD DEV; sd | Standard deviation. |
| * | Group mean is significantly different from the mean of the control group (Group 1) at $p \leq 0.05$. |
| -; NA | No value; not applicable; not present. |
| P | Present. |
| UNSCHED | Unscheduled. |
| DISPATCH | Observations transferred from the in-life module of the data collection system to the necropsy module for reference during necropsy. Observations are duplicates of the last in-life observations. |
| MIN | Minute. |
| OBS | A.M. daily/weekly observation. |

**Animal Death Codes:**

| | |
|---|---|
| T | Terminal sacrifice. |

## Codes for Clinical Pathology

### GENERAL CODES

| | |
|---|---|
| NS | No sample |
| QS/QNS | Quantity not sufficient |
| NR | No repeat (sample volume not sufficient for repeat analysis) |
| FS | Fibrin strands |
| SC | Sample clotted |
| SH | Slightly hemolyzed |
| H | Hemolyzed |
| SL | Slightly lipemic |
| L | Lipemic |
| SI | Slightly icteric |
| I | Icteric |
| NF | Animal not fasted |
| U | Unscheduled/moribund bleed |
| DT/DOT | Animal died on test |
| DB | Died during bleeding |
| TJ | Technician judgment to repeat test |
| TE | Technical error (instrument or technician error that results in unacceptable data, e.g., unacceptable instrument output, sample spilled, entry of invalid data) |
| RE | Recording error (recorded incorrect data, e.g., wrong number, spelling error, incorrect date) |
| EE | Entry error (incorrect keyboard entry) |
| SE | Sampling error |
| PC | Platelets clumped |
| PD | Platelets decreased |
| PI | Platelets increased |
| PL | Platelets large |
| PA | Platelets appear adequate |
| CO | Color interferes with test |
| HB | Heinz bodies observed |
| PLASMO | Plasmodium |
| NO AGG | No aggregation |
| FR | Fractious |
| UTD | Unable to determine |
| NO COAG | No coagulation |

## Codes for Clinical Pathology (Continued)

## RESULTS NOT INCLUDED IN STATISTICAL ANALYSES

Hemolyzed clinical chemistry or coagulation samples
Samples from animals at unscheduled intervals
Prothrombin times (PT) greater than 50 seconds
Activated partial thromboplastin times (PTT) greater than 110 seconds
Bleed times (BLETIME) greater than 30 minutes

## CODES FOR BLOOD CELL MORPHOLOGY

The following scale was used to measure the degree of anisocytosis (ANISO), poikilocytosis (POIK), polychromasia (POLY), hypochromasia (HYPO), and toxic neutrophils (TOXNEUT):

| Scale | Degree | Presence |
|---|---|---|
| - | Normal for the species | Not present |
| 1 | Slight | Rare |
| 2 | Moderate | Few |
| 3 | Marked | Moderate |
| 4 | Not applicable | Many |

## Abbreviations and Units for Clinical Hematology

| Test | Abbreviation (Units) |
|---|---|
| Red blood cell count | RBC (E6/UL or $X10^6/\mu L$) |
| Hemoglobin | HGB (G/DL) |
| Hematocrit | HCT (%) |
| Mean corpuscular volume | MCV (FL) |
| Mean corpuscular hemoglobin | MCH (PG) |
| Mean corpuscular hemoglobin concentration | MCHC (%) |
| Platelet count | PLT (E3/UL or $X10^3/\mu L$) |
| Mean platelet volume | MPV (FL) |
| Reticulocyte count | RETIC (%) |
| Absolute reticulocyte count | RETIC (E3/UL or $X10^3/\mu L$) |
| Heinz body count | HEINZ (%) |
| Erythrocyte sedimentation rate | ESR (MM/HR) |
| Prothrombin time | PT (SEC) |
| Activated partial thromboplastin time | PTT (SEC) |
| Thrombin time | TT (SEC) |
| Activated coagulation time | ACT (SEC) |
| Fibrinogen | FBR (MG/DL) |
| Fibrin/fibrinogen degradation products | FDP (UG/ML) |
| Platelet aggregation | |
| Collagen | PAGG/COL (%) |
| Adenosine diphosphate | PAGG/ADP (%) |
| Alpha 2-antiplasmin | ANTIPLAS (%) |
| Bleeding time | BLE TIME (SEC) |
| Methemoglobin | METHGB (%) |
| Plasma hemoglobin | PLA HGB (MG/DL) |
| Myeloid/erythroid ratio | M/E RATIO |
| Estimated myeloid/erythroid ratio | EST M/E RATIO |
| White blood cell count | WBC (E3/UL or $X10^3/\mu L$) |
| Differential blood cell count | |
| Nucleated red blood cell count | NRBC (/100 WBC) |
| Corrected white blood cell count | COR WBC (E3/UL or $X10^3/\mu L$) |
| Segmented neutrophil count | N-SEG (E3/UL or $X10^3/\mu L$) and % |
| Band neutrophil count | N-BAND (E3/UL or $X10^3/\mu L$) and % |
| Lymphocyte count | LYMPH (E3/UL or $X10^3/\mu L$) and % |
| Monocyte count | MONO (E3/UL or $X10^3/\mu L$) and % |
| Eosinophil count | EOSIN (E3/UL or $X10^3/\mu L$) and % |
| Basophil count | BASO (E3/UL or $X10^3/\mu L$) and % |
| Anisocytosis | ANISO (-,1,2,3) |
| Polychromasia | POLY (-,1,2,3) |

**Abbreviations and Units for Clinical Hematology (Continued)**

| Test | Abbreviation (Units) |
|---|---|
| Poikilocytosis | POIK (-,1,2,3) |
| Hypochromasia | HYPO (-,1,2,3) |
| Howell-Jolly bodies | HJBODY (-,1,2,3,4) |
| Basophilic stippling | BASTIP (-,1,2,3) |
| Toxic neutrophils | TOXNEUT (-,1,2,3,4) |
| Atypical lymphocytes | ATYPLYM (-,1,2,3,4) |
| Aqueous white blood cell count (right eye) | R EYE (WBC/UL) |
| Aqueous white blood cell count (left eye) | L EYE (WBC/UL) |

## Abbreviations and Units for Clinical Chemistry

| Test | Abbreviation (Units) |
|---|---|
| Glucose | GLU (MG/DL) |
| Urea nitrogen | UN (MG/DL) |
| Urea | UREA (MG/DL) |
| Creatinine | CREAT (MG/DL) |
| Total protein | T PRO (G/DL) |
| Albumin | ALB (G/DL) |
| Globulin | GLOB (G/DL) |
| Albumin/globulin ratio | A/G RATIO |
| Total bilirubin | T BILI (MG/DL) |
| Direct bilirubin | D BILI (MG/DL) |
| Indirect bilirubin | I BILI (MG/DL) |
| Cholesterol | CHOL (MG/DL) |
| Triglyceride | TRIG (MG/DL) |
| Urea nitrogen/creatinine ratio | UN/CREAT (RATIO) |
| Total lipids | T LIPIDS (MG/DL) |
| Phospholipids | P LIPIDS (MG/DL) |
| High-density lipoprotein cholesterol | HDL (MG/DL) |
| Low-density lipoprotein cholesterol | LDL (MG/DL) |
| Uric acid | UA (MG/DL) |
| Aspartate aminotransferase | AST/SGOT (IU/L) |
| Alanine aminotransferase | ALT/SGPT (IU/L) |
| Alkaline phosphatase | ALK PHOS (IU/L) |
| Gamma glutamyl transferase | GGT (IU/L) |
| Sorbitol dehydrogenase | SDH (IU/L) |
| Lactate dehydrogenase | LDH (IU/L) |
| Creatine kinase | CK (IU/L) |
| Amylase | AMYLASE (IU/L) |
| Lipase | LIPASE (IU/L) |
| Palmitoyl CoA oxidase | PCOAO (IU/G) |
| Calcium | CA (MG/DL) |
| Ionized calcium | ION CA (MG/DL) |
| Inorganic phosphorus | I PHOS (MG/DL) |
| Sodium | NA (MMOL/L) |
| Potassium | K (MMOL/L) |
| Chloride | CL (MMOL/L) |
| Magnesium | MG (MEQ/L or MG/DL) |
| Zinc | ZN (MG/L or PPM) |
| Strontium | SR (MG/L or PPM) |
| Iron | FE (UG/DL) |

**Abbreviations and Units for Clinical Chemistry (Continued)**

| Test | Abbreviation (Units) |
|---|---|
| Excess iron | EX FE (UG/DL) |
| Total iron binding capacity | TIBC (UG/DL) |
| Unbound iron binding capacity | UIBC (UG/DL) |
| Percent iron saturation | FE %SAT (%) |
| Plasma cholinesterase | CHEP (MU/ML) |
| Red blood cell cholinesterase | CHER (MU/ML) |
| Brain cholinesterase | CHEB (MU/ML) |
| Caudate putamen | CAUD PUT (UMOL/G) |
| Hippocampus | HIPPOCAM (UMOL/G) |
| Frontal cortex | F CORTEX (UMOL/G) |
| Cerebellum | CEREBELL (UMOL/G) |
| Bicarbonate | BICARB (MMOL/L) |
| Serum hemoglobin | SER HGB (MG/DL) |
| Serum bile acids | SBA (UMOL/L or MG/DL) |
| Fecal bile acids | FBA (UG/ML) |
| Average fecal weight | FCC WGT (G) |
| Fecal bile acids (calculation) | FBA (MG/Day) |
| Osmolality | OSMO (MOSM/KG) |
| Electrophoresis | |
| Albumin | E ALB (G/DL) |
| Alpha-1-globulin | E A-1 (G/DL) |
| Alpha-2-globulin | E A-2 (G/DL) |
| Beta globulin | E BETA (G/DL) |
| Gamma globulin | E GAMMA (G/DL) |
| High-density lipoprotein | E-HDL (%) |
| Low-density lipoprotein | E-LDL (%) |
| Very-low-density lipoprotein | E-VLDL (%) |
| Insulin | INSULIN (UU/ML) |
| Adrenocorticotropic hormone | ACTH (PG/ML) |
| Cortisol | CORTISOL (UG/ML) |
| Glucagon | GLUCAGON (PG/ML) |
| Creatine kinase isoenzymes | |
| BB | CK-BB (U/L) |
| MB | CK-MB (U/L) |
| MM | CK-MM (U/L) |
| Pancreatic-specific amylase | P AMYL (U/L) |

## Codes for Anatomical Pathology

## MICROSCOPIC CODES

**Distribution of Findings**
Focal
Diffuse
Multifocal

**Grades for Severity or Amount**

| | |
|---|---|
| 1 | Minimal - the least amount of change that can be observed with the light microscope |
| 2 | Slight - less than average amount of change, but readily discernible as abnormal |
| 3 | Moderate - the average amount of change that is expected for a lesion |
| 4 | Moderately severe (marked) - a marked amount of change with possible loss of function of the affected cells or organs |
| 5 | Severe - a great amount of change with probable loss of function of the affected cell or organs and frequently involves large areas of the organ |

## TISSUE ABBREVIATIONS

| Abbreviation | Definition |
|---|---|
| STOMACH, GL | Glandular stomach |

Table 1

Summary of Clinical Observations
A.M./Weekly

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS



NUMBER OF ANIMALS AFFECTED

DAYS 1-30

| | SEX: | -----MALE---- | | |
|---|---|---|---|---|
| | GROUP: | 1 | 2 | 3 |
| CATEGORY | DOSE: | 0 | 2 | 20 |
| KEYWORD | | | | |
| QUALIFIER | NUMBER: | 2 | 3 | 3 |
| *** TOP OF LIST *** | | | | |
| EXCRETION | | | | |
| NO FECES | | 0 | 0 | 1 |
| SKIN & PELAGE | | | | |
| BROKEN SKIN | | | | |
| THIGH-LEFT | | 0 | 1 | 0 |
| SCAB(S) | | | | |
| LIMB-HIND-LEFT | | 0 | 1 | 0 |
| QUALITATIVE FOOD CONSUMPTION | | | | |
| LOW | | 1 | 1 | 1 |
| NONE | | 0 | 0 | 1 |
| *** END OF LIST *** | | | | |

Table 2

Summary of Clinical Observations
30 Minutes Postdose

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS



NUMBER OF ANIMALS AFFECTED

DAYS 1-29

| | SEX: | -----MALE---- | | |
|---|---|---|---|---|
| | GROUP: | 1 | 2 | 3 |
| CATEGORY | DOSE: | 0 | 2 | 20 |
| KEYWORD<br>QUALIFIER | NUMBER: | 2 | 3 | 3 |
| *** TOP OF LIST *** | | | | |
| SKIN & PELAGE | | | | |
| BROKEN SKIN | | | | |
| DIGIT(S)-HIND-RIGHT | | 1 | 0 | 0 |
| THIGH-LEFT | | 0 | 1 | 0 |
| SCAB(S) | | | | |
| LIMB-HIND-LEFT | | 0 | 1 | 0 |
| *** END OF LIST *** | | | | |

Table 3

Summary of Clinical Observations
60 Minutes Postdose

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS



NUMBER OF ANIMALS AFFECTED

DAYS 1-29

| CATEGORY / KEYWORD / QUALIFIER | SEX: | -----MALE---- | | |
|---|---|---|---|---|
| | GROUP: | 1 | 2 | 3 |
| | DOSE: | 0 | 2 | 20 |
| | NUMBER: | 2 | 3 | 3 |
| *** TOP OF LIST *** | | | | |
| SKIN & PELAGE | | | | |
| BROKEN SKIN | | | | |
| DIGIT(S)-HIND-RIGHT | | 1 | 0 | 0 |
| THIGH-LEFT | | 0 | 1 | 0 |
| SCAB(S) | | | | |
| LIMB-HIND-LEFT | | 0 | 1 | 0 |
| *** END OF LIST *** | | | | |

Table 4

Summary of Clinical Observations
90 Minutes Postdose



NUMBER OF ANIMALS AFFECTED

DAYS 1-29

| CATEGORY / KEYWORD / QUALIFIER | SEX: | -----MALE---- | | |
|---|---|---|---|---|
| | GROUP: | 1 | 2 | 3 |
| | DOSE: | 0 | 2 | 20 |
| | NUMBER: | 2 | 3 | 3 |
| *** TOP OF LIST *** | | | | |
| APPEARANCE | | | | |
| UNDER EFFECTS OF ANESTHESIA | | 0 | 1 | 0 |
| SKIN & PELAGE | | | | |
| BROKEN SKIN | | | | |
| DIGIT(S)-HIND-RIGHT | | 1 | 0 | 0 |
| THIGH-LEFT | | 0 | 1 | 0 |
| SCAB(S) | | | | |
| LIMB-HIND-LEFT | | 0 | 1 | 0 |
| *** END OF LIST *** | | | | |

Table 5

Summary of Body Weight Data (kg)

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS



| SEX: | | MALE | | |
|---|---|---|---|---|
| GROUP: | | 1 | 2 | 3 |
| DOSE: | | 0 | 2 | 20 |
| WEEK | UNITS: | MG/KG/DAY | MG/KG/DAY | MG/KG/DAY |
| -2 | N | 2 | 3 | 3 |
| | MEAN | 3.0 | 2.8 | 2.7 |
| | S.D. | 0.71 | 0.70 | 0.50 |
| -1[a] | N | 2 | 3 | 3 |
| | MEAN | 3.0 | 2.9 | 2.8 |
| | S.D. | 0.71 | 0.75 | 0.50 |
| -1[b] | N | 2 | 3 | 3 |
| | MEAN | 3.1 | 2.8 | 2.8 |
| | S.D. | 0.78 | 0.75 | 0.50 |
| 1 | N | 2 | 3 | 3 |
| | MEAN | 3.1 | 2.9 | 2.8 |
| | S.D. | 0.71 | 0.75 | 0.55 |
| 2 | N | 2 | 3 | 3 |
| | CAM | 2.9 | 2.9 | 2.9 |
| | MEAN | 3.1 | 2.9 | 2.8 |
| | S.D. | 0.57 | 0.70 | 0.56 |
| 3 | N | 2 | 3 | 3 |
| | CAM | 2.9 | 3.0 | 2.9 |
| | MEAN | 3.2 | 2.9 | 2.8 |
| | S.D. | 0.64 | 0.70 | 0.56 |
| 4 | N | 2 | 3 | 3 |
| | CAM | 2.9 | 2.9 | 2.9 |
| | MEAN | 3.1 | 2.9 | 2.8 |
| | S.D. | 0.64 | 0.70 | 0.50 |
| 5 | N | 2 | 3 | 3 |
| | CAM | 2.9 | 3.0 | 3.0 |
| | MEAN | 3.2 | 3.0 | 2.9 |
| | S.D. | 0.64 | 0.75 | 0.56 |

a Day -7.
b Day -1.

Table 6

Summary of Clinical Hematology Data

Males Day -8

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| DOSE mg/kg/day | | RBC $X10^6/\mu L$ | HGB G/DL | HCT % | MCV FL | MCH PG | MCHC % | PLT $X10^3/\mu L$ | RETIC % | RETIC $X10^3/\mu L$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | | | | | | | | | |
| | MEAN | 5.96 | 12.6 | 40.5 | 70.4 | 22.2 | 31.3 | 430 | .4 | 19 |
| | S.D. | 2.157 | .92 | 6.65 | 14.28 | 6.43 | 2.83 | 75.7 | .42 | 17.0 |
| | N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | | | | | | | | | | |
| | MEAN | 6.85 | 13.6 | 44.2 | 65.8 | 20.3 | 30.7 | 422 | .6 | 34 |
| | S.D. | 1.196 | .57 | 1.21 | 11.04 | 4.63 | 1.80 | 41.1 | .57 | 30.0 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 20 | | | | | | | | | | |
| | MEAN | 6.39 | 13.3 | 42.3 | 67.2 | 21.2 | 31.4 | 522 | .3 | 20 |
| | S.D. | .925 | .96 | .72 | 10.84 | 4.78 | 2.22 | 131.7 | .23 | 10.7 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Table 6

Summary of Clinical Hematology Data

Males Day -8

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| DOSE mg/kg/day | | WBC $X10^3/\mu L$ | N-SEG $X10^3/\mu L$ | LYMPH $X10^3/\mu L$ | MONO $X10^3/\mu L$ | EOSIN $X10^3/\mu L$ | BASO $X10^3/\mu L$ | N-SEG% | LYMPH% | MONO% | EOSIN% | BASO% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | | | | | | | | | | | |
| | MEAN | 10.4 | 1.8 | 7.4 | .6 | .6 | .0 | 17 | 72 | 6 | 6 | 0 |
| | S.D. | 1.63 | 1.13 | .64 | .28 | .71 | .00 | 8.5 | 17.0 | 2.1 | 6.4 | .0 |
| | N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | | | | | | | | | | | | |
| | MEAN | 9.4 | 2.6 | 5.9 | .9 | .1 | .0 | 27 | 63 | 9 | 1 | 0 |
| | S.D. | 1.01 | 1.51 | 1.40 | .42 | .06 | .00 | 15.5 | 18.1 | 3.2 | .6 | .0 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 20 | | | | | | | | | | | | |
| | MEAN | 9.6 | 2.8 | 6.0 | .5 | .3 | .0 | 28 | 63 | 6 | 2 | 0 |
| | S.D. | 1.10 | 1.83 | 1.38 | .15 | .15 | .06 | 16.6 | 18.1 | 1.2 | 1.5 | .6 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Table 7

Summary of Clinical Hematology Data

Males Day -5

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| DOSE mg/kg/day | | RBC $X10^6/\mu L$ | HGB G/DL | HCT % | MCV FL | MCH PG | MCHC % | PLT $X10^3/\mu L$ | RETIC % | RETIC $X10^3/\mu L$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | | | | | | | | | |
| | MEAN | 5.47 | 11.5 | 37.3 | 70.6 | 22.0 | 31.0 | 404 | .6 | 26 |
| | S.D. | 1.711 | .35 | 4.45 | 14.00 | 6.29 | 2.83 | 61.5 | .85 | 36.1 |
| | N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | | | | | | | | | | |
| | MEAN | 5.91 | 11.8 | 38.4 | 66.3 | 20.6 | 30.8 | 395 | .9 | 51 |
| | S.D. | 1.061 | .49 | 1.17 | 10.81 | 4.93 | 2.22 | 22.1 | .61 | 24.5 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 20 | | | | | | | | | | |
| | MEAN | 5.75 | 11.9 | 37.9 | 67.3 | 21.2 | 31.4 | 500 | .2 | 9 |
| | S.D. | 1.057 | .42 | 1.48 | 10.68 | 4.83 | 2.20 | 101.4 | .12 | 4.4 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Table 7

Summary of Clinical Hematology Data

Males Day -5

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| DOSE mg/kg/day | | WBC $X10^3/\mu L$ | N-SEG $X10^3/\mu L$ | LYMPH $X10^3/\mu L$ | MONO $X10^3/\mu L$ | EOSIN $X10^3/\mu L$ | BASO $X10^3/\mu L$ | N-SEG% | LYMPH% | MONO% | EOSIN% | BASO% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | | | | | | | | | | | |
| | MEAN | 11.1 | 2.0 | 7.8 | .7 | .6 | .0 | 18 | 72 | 6 | 4 | 0 |
| | S.D. | 1.56 | 1.13 | .49 | .14 | .64 | .07 | 7.8 | 14.1 | .7 | 4.9 | .7 |
| | N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | | | | | | | | | | | | |
| | MEAN | 10.7 | 4.3 | 5.6 | .7 | .2 | .0 | 40 | 52 | 6 | 2 | 0 |
| | S.D. | 3.18 | 1.81 | 1.76 | .12 | .10 | .00 | 12.1 | 12.6 | .6 | .6 | .0 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 20 | | | | | | | | | | | | |
| | MEAN | 10.5 | 4.1 | 5.4 | .6 | .2 | .0 | 38 | 54 | 5 | 2 | 0 |
| | S.D. | 3.10 | 1.89 | .76 | .32 | .21 | .00 | 9.0 | 12.7 | 2.1 | 1.7 | .0 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Table 8

Summary of Clinical Hematology Data

Males Day 30

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| DOSE mg/kg/day | | RBC $X10^6/\mu L$ | HGB G/DL | HCT % | MCV FL | MCH PG | MCHC % | PLT $X10^3/\mu L$ | RETIC % | RETIC $X10^3/\mu L$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | | | | | | | | | |
| | MEAN | 5.96 | 12.4 | 40.8 | 70.1 | 21.6 | 30.5 | 461 | .2 | 14 |
| | S.D. | 1.457 | .14 | 2.26 | 13.44 | 5.52 | 2.05 | 66.5 | .21 | 9.2 |
| | N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | | | | | | | | | | |
| | MEAN | 6.74 | 13.6 | 44.3 | 66.5 | 20.5 | 30.6 | 407 | .2 | 10 |
| | S.D. | .824 | 1.19 | 1.51 | 11.02 | 4.52 | 1.63 | 4.2 | .21 | 11.8 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 20 | | | | | | | | | | |
| | MEAN | 6.24 | 13.0 | 41.9 | 68.1 | 21.3 | 31.1 | 489 | .2 | 14 |
| | S.D. | .814 | 1.11 | 1.31 | 10.91 | 4.78 | 2.25 | 116.0 | .21 | 12.1 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Table 8

Summary of Clinical Hematology Data

Males Day 30

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| DOSE mg/kg/day | | WBC $X10^3/\mu L$ | N-SEG $X10^3/\mu L$ | LYMPH $X10^3/\mu L$ | MONO $X10^3/\mu L$ | EOSIN $X10^3/\mu L$ | BASO $X10^3/\mu L$ | N-SEG% | LYMPH% | MONO% | EOSIN% | BASO% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | | | | | | | | | | | |
| | MEAN | 9.8 | 1.6 | 7.2 | .6 | .4 | .0 | 16 | 74 | 6 | 4 | 0 |
| | S.D. | .07 | .64 | .78 | .21 | .35 | .07 | 7.1 | 7.8 | 2.1 | 3.5 | .7 |
| | N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | | | | | | | | | | | | |
| | MEAN | 8.0 | 2.2 | 5.4 | .3 | .0 | .0 | 27 | 67 | 5 | 1 | 0 |
| | S.D. | 2.29 | .79 | 1.60 | .15 | .06 | .00 | 4.6 | 6.6 | 2.9 | .0 | .0 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 20 | | | | | | | | | | | | |
| | MEAN | 8.3 | 3.1 | 4.4 | .6 | .1 | .0 | 38 | 53 | 7 | 2 | 1 |
| | S.D. | .85 | 1.11 | 1.31 | .10 | .12 | .00 | 12.7 | 14.6 | 1.5 | .6 | .6 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Table 9

Summary of Clinical Chemistry Data

Males Day -8

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| DOSE mg/kg/day | | GLU MG/DL | UN MG/DL | CREAT MG/DL | T PRO G/DL | ALB G/DL | GLOB G/DL | T BILI MG/DL | SBA umol/L | CHOL MG/DL | TRIG MG/DL |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | | | | | | | | | | |
| | MEAN | 74 | 22 | 1.0 | 8.7 | 5.2 | 3.5 | .4 | 9 | 146 | 34 |
| | S.D. | 20.5 | 7.8 | .21 | 2.40 | 1.13 | 1.27 | .14 | 5.7 | 9.2 | 7.8 |
| | N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | | | | | | | | | | | |
| | MEAN | 76 | 18 | 1.1 | 8.4 | 5.1 | 3.3 | .3 | 9 | 174 | 36 |
| | S.D. | 14.2 | 2.0 | .26 | 1.30 | .30 | 1.00 | .12 | 2.5 | 19.9 | 6.7 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 20 | | | | | | | | | | | |
| | MEAN | 64 | 23 | 1.2 | 8.0 | 4.8 | 3.3 | .2 | 12 | 141 | 31 |
| | S.D. | 5.1 | 2.3 | .25 | .55 | .21 | .75 | .06 | .6 | 12.7 | 6.7 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Table 9

Summary of Clinical Chemistry Data

Males Day -8

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| DOSE mg/kg/day | | AST/SGOT IU/L | ALT/SGPT IU/L | ALK PHOS IU/L | GGT IU/L | SDH IU/L | CK IU/L | AMYLASE IU/L | LIPASE IU/L | P AMYL U/L |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | | | | | | | | | |
| | MEAN | 41 | 45 | 847 | 122 | 2 | 348 | 492 | 43 | 252 |
| | S.D. | 1.4 | 9.9 | 420.0 | 11.3 | .0 | 244.0 | 70.0 | 1.4 | 33.2 |
| | N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | | | | | | | | | | |
| | MEAN | 48 | 53 | 1013 | 159 | 5 | 369 | 522 | 46 | 250 |
| | S.D. | 14.7 | 22.2 | 106.1 | 45.1 | 4.4 | 159.3 | 174.5 | 23.4 | 41.7 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 20 | | | | | | | | | | |
| | MEAN | 52 | 59 | 948 | 136 | 5 | 195 | 496 | 43 | 239 |
| | S.D. | 6.7 | 26.7 | 65.5 | 35.5 | 1.2 | 40.3 | 32.0 | 8.5 | 37.3 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Table 9

Summary of Clinical Chemistry Data

Males Day -8

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS

| DOSE mg/kg/day | | CA MG/DL | I PHOS MG/DL | NA MMOL/L | K MMOL/L | CL MMOL/L |
|---|---|---|---|---|---|---|
| 0 | | | | | | |
| | MEAN | 11.4 | 7.2 | 158 | 6.6 | 112 |
| | S.D. | 1.70 | .99 | 11.3 | .71 | 1.4 |
| | N | 2 | 2 | 2 | 2 | 2 |
| 2 | | | | | | |
| | MEAN | 11.0 | 7.5 | 158 | 5.6 | 108 |
| | S.D. | .35 | 1.11 | 4.6 | .35 | 1.2 |
| | N | 3 | 3 | 3 | 3 | 3 |
| 20 | | | | | | |
| | MEAN | 10.9 | 7.0 | 154 | 6.1 | 108 |
| | S.D. | .53 | .29 | 1.5 | .40 | .6 |
| | N | 3 | 3 | 3 | 3 | 3 |

Table 10

Summary of Clinical Chemistry Data

Males Day -5

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| DOSE mg/kg/day | | GLU MG/DL | UN MG/DL | CREAT MG/DL | T PRO G/DL | ALB G/DL | GLOB G/DL | T BILI MG/DL | SBA umol/L | CHOL MG/DL | TRIG MG/DL |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | | | | | | | | | | |
| | MEAN | 84 | 18 | 1.2 | 8.4 | 5.0 | 3.4 | .6 | 4 | 138 | 28 |
| | S.D. | 41.0 | 9.2 | .14 | 1.70 | .78 | .92 | .07 | 2.1 | 26.2 | .7 |
| | N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | | | | | | | | | | | |
| | MEAN | 66 | 15 | 1.1 | 8.0 | 4.8 | 3.2 | .3 | 5 | 155 | 34 |
| | S.D. | 6.5 | 1.0 | .21 | .85 | .10 | .90 | .17 | 4.0 | 12.3 | 3.8 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 20 | | | | | | | | | | | |
| | MEAN | 67 | 20 | 1.1 | 8.0 | 4.7 | 3.4 | .4 | 1 | 141 | 39 |
| | S.D. | 14.4 | 5.1 | .15 | .57 | .25 | .75 | .31 | 1.0 | 13.5 | 11.0 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Table 10

Summary of Clinical Chemistry Data

Males Day -5

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| DOSE mg/kg/day | | AST/SGOT IU/L | ALT/SGPT IU/L | ALK PHOS IU/L | GGT IU/L | SDH IU/L | CK IU/L | AMYLASE IU/L | LIPASE IU/L | P AMYL U/L |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | | | | | | | | | |
| | MEAN | 38 | 43 | 788 | 112 | 3 | 494 | 495 | 40 | 252 |
| | S.D. | 4.9 | 12.7 | 393.9 | 6.4 | .0 | 478.7 | 76.4 | 2.8 | 26.2 |
| | N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | | | | | | | | | | |
| | MEAN | 39 | 49 | 930 | 137 | 3 | 279 | 520 | 56 | 252 |
| | S.D. | 13.3 | 15.2 | 83.5 | 39.7 | 1.0 | 227.6 | 174.8 | 9.2 | 37.2 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 20 | | | | | | | | | | |
| | MEAN | 48 | 51 | 896 | 121 | 3 | 171 | 466 | 57 | 229 |
| | S.D. | 9.8 | 14.5 | 54.7 | 38.2 | 1.0 | 29.3 | 15.0 | 13.5 | 18.4 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Table 10

Summary of Clinical Chemistry Data

Males Day -5

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS

| DOSE mg/kg/day | | CA MG/DL | I PHOS MG/DL | NA MMOL/L | K MMOL/L | CL MMOL/L |
|---|---|---|---|---|---|---|
| 0 | | | | | | |
| | MEAN | 10.9 | 6.8 | 156 | 5.4 | 108 |
| | S.D. | 1.27 | 1.27 | 9.2 | .92 | 2.1 |
| | N | 2 | 2 | 2 | 2 | 2 |
| 2 | | | | | | |
| | MEAN | 10.4 | 6.4 | 155 | 5.2 | 110 |
| | S.D. | .61 | 1.04 | 3.5 | .75 | 2.6 |
| | N | 3 | 3 | 3 | 3 | 3 |
| 20 | | | | | | |
| | MEAN | 10.4 | 6.2 | 152 | 5.0 | 107 |
| | S.D. | .51 | .17 | 2.6 | .58 | 1.7 |
| | N | 3 | 3 | 3 | 3 | 3 |

Table 11

Summary of Clinical Chemistry Data

Males Day 2

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| DOSE mg/kg/day | | GLU MG/DL | UN MG/DL | CREAT MG/DL | T PRO G/DL | ALB G/DL | GLOB G/DL | T BILI MG/DL | SBA umol/L | CHOL MG/DL | TRIG MG/DL |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | MEAN | 76 | 21 | 1.0 | 9.0 | 5.2 | 3.9 | .4 | 10 | 152 | 28 |
| | S.D. | 4.2 | 7.1 | .21 | 2.19 | .92 | 1.27 | .21 | 4.9 | 7.8 | 7.8 |
| | N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | MEAN | 65 * | 20 | 1.0 | 8.4 | 4.9 | 3.5 | .3 | 9 | 169 | 52 |
| | S.D. | 4.2 | 4.9 | .21 | .65 | .10 | .75 | .06 | 3.0 | 22.3 | 8.3 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 20 | MEAN | 68 | 23 | 1.0 | 8.3 | 4.8 | 3.5 | .1 * | 14 | 132 | 45 |
| | S.D. | 2.0 | 1.0 | .12 | .51 | .21 | .70 | .06 | 4.4 | 10.0 | 11.1 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Table 11

Summary of Clinical Chemistry Data

Males Day 2

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| DOSE mg/kg/day | | AST/SGOT IU/L | ALT/SGPT IU/L | ALK PHOS IU/L | GGT IU/L | SDH IU/L | CK IU/L | AMYLASE IU/L | LIPASE IU/L | P AMYL U/L |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | | | | | | | | | |
| | MEAN | 47 | 53 | 872 | 125 | 1 | 225 | 477 | 29 | 249 |
| | S.D. | 2.8 | 25.5 | 355.7 | 5.7 | .0 | 62.2 | 59.4 | 1.4 | 29.7 |
| | N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | | | | | | | | | | |
| | MEAN | 53 | 52 | 1029 | 148 | 2 | 794 | 483 | 44 | 244 |
| | S.D. | 32.7 | 26.8 | 113.3 | 44.9 | 1.5 | 1173.2 | 144.3 | 7.5 | 42.7 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 20 | | | | | | | | | | |
| | MEAN | 55 | 56 | 921 | 126 | 1 | 165 | 479 | 33 | 234 |
| | S.D. | 4.9 | 22.4 | 124.2 | 39.1 | 1.0 | 44.4 | 40.1 | 12.2 | 32.5 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Table 11

Summary of Clinical Chemistry Data

Males Day 2

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS

| DOSE mg/kg/day | | CA MG/DL | I PHOS MG/DL | NA MMOL/L | K MMOL/L | CL MMOL/L |
|---|---|---|---|---|---|---|
| 0 | | | | | | |
| | MEAN | 11.6 | 8.8 | 162 | 6.2 | 110 |
| | S.D. | 1.63 | .28 | 12.0 | .99 | 4.9 |
| | N | 2 | 2 | 2 | 2 | 2 |
| 2 | | | | | | |
| | MEAN | 10.5 | 8.0 | 155 | 5.3 | 109 |
| | S.D. | .57 | 1.30 | 2.5 | .44 | 3.8 |
| | N | 3 | 3 | 3 | 3 | 3 |
| 20 | | | | | | |
| | MEAN | 10.7 | 7.4 | 156 | 5.5 | 109 |
| | S.D. | .83 | .90 | 4.2 | .67 | 1.5 |
| | N | 3 | 3 | 3 | 3 | 3 |

Table 12

Summary of Clinical Chemistry Data

Males Day 30

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| DOSE mg/kg/day | | GLU MG/DL | UN MG/DL | CREAT MG/DL | T PRO G/DL | ALB G/DL | GLOB G/DL | T BILI MG/DL | SBA umol/L | CHOL MG/DL | TRIG MG/DL |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | | | | | | | | | | |
| | MEAN | 84 | 19 | 1.0 | 8.6 | 5.1 | 3.4 | .5 | 4 | 150 | 32 |
| | S.D. | 9.2 | 5.7 | .07 | 1.77 | .71 | 1.06 | .28 | 3.5 | 31.8 | .7 |
| | N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | | | | | | | | | | | |
| | MEAN | 76 | 17 | 1.1 | 8.3 | 5.0 | 3.3 | .1 | 4 | 147 | 43 |
| | S.D. | 3.2 | 2.1 | .06 | .60 | .17 | .75 | .06 | 1.5 | 9.2 | 3.2 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 20 | | | | | | | | | | | |
| | MEAN | 65 * | 20 | 1.2 | 7.8 | 4.5 | 3.3 | .1 | 6 | 138 | 52 |
| | S.D. | 2.1 | 3.5 | .35 | .47 | .23 | .70 | .15 | 4.5 | 31.5 | 10.6 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Table 12

Summary of Clinical Chemistry Data

Males Day 30

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| DOSE mg/kg/day | | AST/SGOT IU/L | ALT/SGPT IU/L | ALK PHOS IU/L | GGT IU/L | SDH IU/L | CK IU/L | AMYLASE IU/L | LIPASE IU/L | P AMYL U/L | PCOAO IU/G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | | | | | | | | | | | |
| | MEAN | 52 | 59 | 917 | 110 | 2 | 511 | 471 | 42 | 242 | 2 |
| | S.D. | .7 | 24.0 | 335.2 | 11.3 | 1.4 | 446.9 | 28.3 | 16.3 | 6.4 | .7 |
| | N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | | | | | | | | | | | |
| | MEAN | 50 | 75 | 1111 | 136 | 2 | 324 | 500 | 38 | 244 | 1 |
| | S.D. | 19.7 | 33.3 | 88.9 | 35.8 | 1.0 | 220.4 | 155.0 | 7.8 | 49.9 | 1.7 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 20 | | | | | | | | | | | |
| | MEAN | 55 | 83 | 704 | 101 | 2 | 207 | 441 | 39 | 218 | 6 |
| | S.D. | 11.5 | 50.0 | 87.8 | 33.2 | 1.5 | 9.0 | 71.8 | 11.6 | 52.8 | 3.1 |
| | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Table 12

Summary of Clinical Chemistry Data

Males Day 30

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS

| DOSE mg/kg/day | | CA MG/DL | I PHOS MG/DL | NA MMOL/L | K MMOL/L | CL MMOL/L |
|---|---|---|---|---|---|---|
| 0 | | | | | | |
| | MEAN | 10.7 | 7.2 | 153 | 5.4 | 106 |
| | S.D. | .71 | 1.77 | 5.7 | .49 | 2.8 |
| | N | 2 | 2 | 2 | 2 | 2 |
| 2 | | | | | | |
| | MEAN | 10.8 | 6.7 | 155 | 5.2 | 109 |
| | S.D. | .53 | 1.06 | 2.1 | .90 | 2.9 |
| | N | 3 | 3 | 3 | 3 | 3 |
| 20 | | | | | | |
| | MEAN | 10.2 | 5.5 | 151 | 5.2 | 109 |
| | S.D. | .70 | .31 | 5.1 | .66 | 2.1 |
| | N | 3 | 3 | 3 | 3 | 3 |

Incidence of Macroscopic Observations

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS



TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=ALL;SUBSET=ALL

| ORGAN AND KEYWORD(S) OR PHRASE | | --- NUMBER-OF-ANIMALS-AFFECTED --- | | |
|---|---|---|---|---|
| SEX: | | -----MALE---- | | |
| GROUP: | | -1- | -2- | -3- |
| NUMBER: | | 2 | 3 | 3 |
| ** TOP OF LIST ** | | | | |
| GENERAL COMMENT (GC) | NUMBER EXAMINED: | 2 | 3 | 3 |
| EYES - DAVIDSONS | | 2 | 3 | 3 |
| NO MACROSCOPIC LESIONS | | 0 | 3 | 1 |
| KIDNEY (KD) | NUMBER EXAMINED: | 2 | 3 | 3 |
| | NOT REMARKABLE: | 1 | 3 | 3 |
| LIGHT FOCUS(I)/AREA(S) | | 1 | 0 | 0 |
| LUNG (LU) | NUMBER EXAMINED: | 2 | 3 | 3 |
| | NOT REMARKABLE: | 2 | 3 | 2 |
| ADHESION(S) | | 0 | 0 | 1 |
| STOMACH, GL (ST) | NUMBER EXAMINED: | 2 | 3 | 3 |
| | NOT REMARKABLE: | 1 | 3 | 1 |
| RED FOCUS(I)/AREA(S) | | 1 | 0 | 2 |
| ** END OF LIST ** | | | | |

Incidence of Microscopic Observations

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS 

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=ALL
DEATH=ALL;FIND=ALL;SUBSET=ALL

| ORGAN AND FINDING DESCRIPTION | | --- N U M B E R - O F - A N I M A L S - A F F E C T E D | | |
|---|---|---|---|---|
| SEX: | | -----MALE---- | | |
| GROUP: | | -1- | -2- | -3- |
| NUMBER: | | 2 | 3 | 3 |
| ** TOP OF LIST ** | | | | |
| LIVER (LI) | NUMBER EXAMINED: | 2 | 3 | 3 |
| | NOT REMARKABLE: | 2 | 3 | 3 |
| SPLEEN (SP) | NUMBER EXAMINED: | 2 | 3 | 3 |
| | NOT REMARKABLE: | 2 | 3 | 3 |
| PANCREAS (PA) | NUMBER EXAMINED: | 2 | 3 | 3 |
| | NOT REMARKABLE: | 2 | 3 | 3 |
| TESTIS (TE) | NUMBER EXAMINED: | 2 | 3 | 3 |
| | NOT REMARKABLE: | 2 | 2 | 2 |
| --IMMATURE | | 0 | 1 | 1 |
| ADRENAL (AD) | NUMBER EXAMINED: | 2 | 3 | 3 |
| | NOT REMARKABLE: | 2 | 2 | 3 |
| --MINERALIZATION | | 0 | 1 | 0 |
| ** END OF LIST ** | | | | |

## APPENDIX 1

Protocol
Protocol Amendment No. 1
Protocol Amendment No. 2
Protocol Amendment No. 3
Material Safety Data Sheet
Certificate of Analysis

COVANCE
THE DEVELOPMENT SERVICES COMPANY

**Sponsors:**

APME Ad-Hoc APFO Toxicology Working Group

# PROTOCOL

**Study Title:**

4-Week Capsule Toxicity Study with Ammonium Perfluorooctonoate (APFO) in Cynomolgus Monkeys

**Date:**

June 11, 1998

**Performing Laboratory:**

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704-2595

**Laboratory Study Identification:**

Proposal No. w6806a

Covance 6329-230

**Study**

4-Week Capsule Toxicity Study with Ammonium Perfluorooctonoate (APFO) in Cynomolgus Monkeys

**Purpose**

To provide data for determining an estimated maximum-tolerated dose and lower dose levels to be used in a chronic toxicity study. In addition, the effect on critical enzyme levels, hormones, and other selected biochemical parameters will be determined.

**Sponsors**

APME Ad-Hoc APFO Toxicology Working Group

**Study Monitor**

Paul Lieder, PhD, DABT
3M
Toxicology Services
Building 220-2E-02, 3M Center
St. Paul, Minnesota, 55144-1000
Telephone No.: 612.737.2678
Facsimile No.: 612.733.1773

**Alternate Study Monitor**

John Butenhoff, PhD, DABT
3M
Toxicology Services
Building 220-2E-02, 3M Center
St. Paul, Minnesota, 55144-1000
Telephone No.: 612.733.1962
Facsimile No.: 612.733.1733

**Study Location**

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704-2595

Mailing Address: PO Box 7545
Madison, Wisconsin 53707-7454

**Study Director**

Peter J. Thomford, PhD
Covance Laboratories Inc.
Telephone No.: 608.241.7207
Facsimile No.: 608.242.2736

**Toxicologist**

Dale Aldridge, BS
Covance Laboratories Inc.

**Proposed Study Timetable**

Study Initiation Date: June 11, 1998
In-Life (Experimental) Start Date: June 17, 1998
In-Life Termination Date: July 17, 1998
Experimental Termination Date: To be determined
Audited Draft Report Date: November 3, 1998

**Regulatory Compliance**

This study will be conducted in compliance with the Environmental Protection Agency Good Laboratory Practice Regulations as set forth in Title 40 of the US Code of Federal Regulations, Part 792, issued November 29, 1983 (effective December 29, 1983), and with any applicable amendments.

**Animal Care and Use Statement**

All procedures in this protocol are in compliance with the Animal Welfare Act Regulations, 9 CFR 1-4. In the opinion of the Sponsor and study director, the study does not unnecessarily duplicate any previous work.

**Quality Assurance**

The protocol, study conduct, and final report will be audited by the Covance Quality Assurance Unit (QAU). The proliferation cell nuclear antigen evaluation data and report will be audited by the QAU of Pathology Associates International. The serum and liver APFO analysis and report will be audited by the QAU of 3M Environmental Laboratories. The blood hormone determinations and report will be audited by the QAUs of Ani Lytics Inc and DuPont, as appropriate. The receptor level determinations and report will be audited by the QAU of the University of Dundee.

**Test Material**

**Identification**

Ammonium Perfluorooctonoate (APFO)

**Lot Number**

The lot number will be maintained in the raw data.

**Purity**

Responsibility of the Sponsor

**Stability**

Responsibility of the Sponsor

**Storage Conditions**

At room temperature

**Characteristics**

Information on synthesis methods, composition, or other characteristics that define the test material is on file with the Sponsor.

**Gelatin Capsules**

Capsules (Size No. 2) obtained from Torpac, Inc. (Fairfield, New Jersey); lot number will be supplied by the manufacturer. Gelatin capsules will be stored at room temperature. A copy of the Certificate of Analysis provided by the manufacturer will be maintained in the data.

**Reserve (Archive) Samples**

A reserve sample (1 g each) of each lot of test material will be taken and stored at room temperature. These samples will be transferred to the Sponsor after completion of the in-life phase.

**Disposition of Test Material**

Any remaining test material will be returned after authorization from the Sponsor.

**Animals**

**Species**

Cynomolgus monkey

**Source**

Covance Research Products Inc.

**Age at Initiation of Treatment**

Young adult/adult

**Weight at Initiation of Treatment**

2 to 4 kg

**Number and Sex**

Eight males

**Identification**

Collar tag

**Husbandry**

**Housing**

Individual. Animals will be housed in suspended, stainless steel cages.

**Diet**

Certified primate diet (#8726C, Harlan Teklad) once or twice daily. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Results of specified nutrient and contaminant analyses are on file at Covance-Madison. Fruit, vegetables, or other supplements may be provided but will not require analysis.

**Water**

*Ad libitum.* Samples of the water are routinely analyzed for specified microorganisms and environmental contaminants. The results are on file at Covance-Madison.

**Contaminants**

There are no known contaminants in the diet or water at levels that might interfere with this study.

**Environment**

Environmental controls for the animal room will be set to maintain 18 to 29°C, a relative humidity of 30 to 70%, and a 12-hour light/12-hour dark cycle.

**Acclimation**

Minimum of 4 weeks

**Randomization**

Animals will be weighed, stratified by body weight, and allocated to the number of blocks equal to the number of animals to be selected for each group. Animals in each block will then be assigned to groups using computer-generated random numbers.

**Justification**

APFO is a known hepatic peroxisome proliferator (PP) in the rat. When exposed to PP, nonhuman primates (such as the cynomolgus monkey) respond similarly to humans (i.e., low to no hepatic response) and therefore are an appropriate human surrogate species.

**Group Designations and Dose Levels**

| Group | Dose Level (mg/kg/day) | Total Material Dose Level (mg/kg/day) | Number of Males |
|---|---|---|---|
| 1 (control) | 0[a] | 0[a] | 2 |
| 2 (low-dose) | 2 | 2 | 3 |
| 3 (high-dose) | 20 | 20 | 3 |

a The control group (Group 1) will receive empty gelatin capsules

**Dosing Procedures**

**Method of Administration**

Orally by gelatin capsules, daily for at least 28 days

**Reason for Dosing Route**

To compare with previously conducted toxicology studies using the oral route, which is the route of most likely exposure.

**Dose Preparation**

Capsules will be prepared at least weekly. The size and number of capsules will depend on the physical characteristics of the test material, the dose level, and the weight of the monkey. Individual daily doses will be based on the most recent individual body weights, with the exception of body weight collection days when the previous body weight will be used. Dose levels will be based on the test material as supplied. All dose preparations will be stored at room temperature until dosed.

**Dose Analysis**

Because the test material is not being mixed with a vehicle, dose analyses are not required.

**Observation of Animals**

**Clinical Observations**

Each animal will be observed twice daily (a.m. and p.m.) for mortality and moribundity; findings will be recorded as they are observed.

Each animal will be observed daily, and food consumption will be assessed qualitatively; abnormal findings will be recorded. Once weekly, each animal will be observed; abnormal findings, or an indication of normal will be recorded. During treatment, each animal will be observed for signs of poor health or abnormal behavior approximately 30, 60, and 90 minutes postdose.

Additional findings will be recorded as they are observed.

**Body Weights**
Weekly before initiation of treatment, Day -1 (for the Day 1 dose calculations), on the first day of treatment, and weekly thereafter.

## Clinical Pathology

### Frequency

**Unscheduled Collections**
When possible, blood will be collected for clinical pathology tests from animals sacrificed at unscheduled intervals

**Scheduled Collections**
Hematology and clinical chemistry will be done twice before initiation of treatment and after at least 28 days of treatment; clinical chemistry will also be done on Day 2 (approximately 24 hours after the first dose).

**Method of Collection**
Animals will be fasted overnight; blood will be collected from a femoral vein (an alternative vein may be used if necessary). Anticoagulant will be potassium EDTA for hematology tests. No anticoagulant is used for clinical chemistry tests.

**Tests**

**Hematology**

| | |
|---|---|
| red blood cell (erythrocyte) count | platelet count |
| hemoglobin | white blood cell (leukocyte) count |
| hematocrit | differential blood cell count |
| mean corpuscular volume | blood cell morphology |
| mean corpuscular hemoglobin | reticulocyte count |
| mean corpuscular hemoglobin concentration | |

**Clinical Chemistry**

| | |
|---|---|
| glucose | aspartate aminotransferase |
| urea nitrogen | gamma glutamyltransferase |
| creatinine | sorbitol dehydrogenase |
| total protein | creatine kinase |
| albumin | calcium |
| globulin | inorganic phosphorus |
| total bilirubin | sodium |
| direct bilirubin (if total bilirubin is greater than 2.0 mg/dL) | potassium |
| | chloride |
| cholesterol | bile acids |
| triglycerides | amylase |
| alanine aminotransferase | lipase |
| alkaline phosphatase | pancreatic-specific amylase |

**Blood Hormone Determination**

**Frequency**

Before initiation of treatment and after 28 days of treatment; blood will be collected between 7:00 a.m. and 9:00 a.m.

**Number of Animals**

All

**Method of Collection**

Animals will be fasted overnight; blood will be collected from a femoral vein (an alternative vein may be used if necessary). Approximately 5 mL of blood for plasma samples will be collected with EDTA as anticoagulant. Approximately 5 mL of blood for serum samples will be collected without anticoagulant and allowed to clot.

**Serum Sample Handling**

Samples for serum will be centrifuged within 1 hour after collection, and serum will be harvested. Serum will be divided into two approximately equal aliquots and stored in a freezer set to maintain -60 to -80°C until packed on dry ice and shipped to:

Dr. Saroj Das
Ani Lytics Inc.
200 Girard Street, Suite 200
Gaithersburg, Maryland 20877
Telephone No.: 301.921.0168
Facsimile No.: 301.977.0433

Ani Lytics Inc. will be notified regarding shipment of samples.

**Serum Tests**

Samples will be analyzed by Ani Lytics Inc., for estradiol, estrone, estriol, thyroid stimulating hormone, triiodothyronine (T3), and thyroxin (T4). Results will be provided for inclusion in the final report.

**Plasma Sample Handling**

Samples for plasma will be centrifuged within 1 hour after collection. Plasma will be harvested and stored in a freezer set to maintain -60 to -80°C until packed on dry ice and shipped to:

Jon C. Cook, PhD, DABT
E. I. du Pont de Nemours & Company, Inc.
Haskell Laboratory for Toxicology and Industrial Medicine
Building 14
Elkton Road
Newark, Delaware 19714-0500
Telephone No.: 302.366.5495
Facsimile No.: 302.366.5003

DuPont will be notified regarding shipment of samples.

**Plasma Analysis**

Samples will be analyzed by DuPont Haskell Company for cholecystokinin. Results will be provided for inclusion in the final report.

## Serum APFO Level Determination

**Frequency**

Once before initiation of treatment and after at least 28 days of treatment

**Number of Animals**

All

**Method of Collection**

Animals will be fasted overnight; blood (approximately 2 mL) will be collected from a femoral vein (an alternative vein may be used if necessary) without an anticoagulant.

**Sample Handling**

Samples will be centrifuged within 1 hour after collection, and serum will be harvested. Serum samples will be stored in a freezer set to maintain -10 to -30°C.

**Sample Shipping**

Serum samples will be packed on dry ice and shipped to:

Kris J. Hansen, PhD
3M E.T. & S
Bldg. 2-3E-09
935 Bush Avenue
St. Paul, Minnesota 55106

Kris J. Hansen, PhD or her designee will be notified regarding the shipment of the samples. Results will be provided for inclusion in the final report.

**Additional Blood Collection**

At scheduled and unscheduled necropsies, blood (as much as possible, up to 20 mL) will be collected from animals at the time of exsanguination. Blood from each animal will be transferred into containers (approximately 5 mL each) and stored in a freezer set to maintain -60 to -80°C until shipped to the Sponsor for possible future analysis.

**Termination**

**Unscheduled Sacrifices and Deaths**

Necropsies will be done. Animals to be sacrificed will be anesthetized with ketamine and xylazine, weighed, bled for the additional blood collection, and exsanguinated.

**Scheduled Sacrifice**

After at least 4 weeks of treatment, all surviving animals will be fasted overnight, then anesthetized with ketamine and xylazine, weighed, bled for the additional blood collection, exsanguinated, and necropsied.

**Postmortem Procedures**

**Necropsy**

The necropsy will include examination of:

all orifices
cranial cavity
external surface of the brain; the external surface of the spinal cord and cut surfaces of the brain and spinal cord will be examined whenever tissue trimming is performed

cervical tissues and organs
thoracic, abdominal, and pelvic cavities and viscera
external surface of the body
nasal cavity and paranasal sinuses

**Palmitoyl CoA Oxidase Determinations**

Representative samples of the right lateral lobe of liver will be collected from each animal at the scheduled sacrifice, weighed, flash-frozen in liquid nitrogen, and stored in a freezer set to maintain -60 to -80°C until analyzed for palmitoyl CoA oxidase activity.

**Cell Proliferation Evaluation**

Representative samples of the left lateral lobe of the liver, left and right testes, and pancreas will be collected and preserved in zinc formalin.

After fixation, samples for proliferation cell nuclear antigen (PCNA) evaluation will be embedded in paraffin and shipped to:

Sandra R. Eldridge, PhD
Pathology Associates International
15 Worman's Mill Court
Suite I
Frederick, Maryland 21701
Telephone No.: 301.663.1644 ext. 2201
Facsimile No: 301.663.8994

Pathology Associates International will be notified regarding shipment of samples. Results of PCNA analyses will be provided for inclusion in the final report.

**Receptor Level Determination**

All available bile (up to 5 mL) will be collected from each animal at the scheduled sacrifice, flash-frozen in liquid nitrogen, and stored in a freezer set to maintain -60 to -80°C. Samples (approximately 2 g) of liver and pancreas will be collected from each animal at the scheduled sacrifice, flash-frozen in liquid nitrogen, and stored in a freezer set to maintain -60 to -80°C. Bile, liver, and pancreas samples will be packed on dry ice and shipped to:

Dr. Cliff Elcombe
Biomedical Research Center
University of Dundee
Dundee, Scotland
Telephone No.: 011 44 1382 632641
Facsimile No.: 011 44 1382 668278

Results of analyses will be provided for inclusion in the final report.

**Liver APFO Determination**

A section (approximately 20 g) of liver will be collected from each animal at the scheduled sacrifice, weighed, flash-frozen in liquid nitrogen, and stored in a freezer set to maintain -60 to -80°C until shipped with plasma samples to 3M (Kris Hansen) for analysis. Results will be provided for inclusion in the final report.

**Tissue Preservation**

The following tissues (when present) from each animal will be preserved in 10% neutral-buffered formalin, unless otherwise specified, for possible future microscopic examination:

adrenal (2)
aorta
brain
cecum
colon
duodenum
epididymis (2)
esophagus
eye [to be preserved in Davidsons fixative for sacrificed animals only (2)]
femur with bone marrow (articular surface of the distal end)
gallbladder
heart
ileum
jejunum
kidney (2)
lesions
liver
lung
mammary gland
mesenteric lymph node
pancreas
pituitary
prostate
rectum
salivary gland [mandibular (2)]
sciatic nerve
seminal vesicle (2)
skeletal muscle (thigh)
skin
spinal cord (cervical, thoracic, and lumbar)
spleen
sternum with bone marrow
stomach
testis (2)
thymus
thyroid (2) with parathyroid
trachea
urinary bladder

**Histopathology**

The pancreas, liver, adrenals, spleen, and testes from each animal will be embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically.

**Reports**

A draft report that includes the following information will be prepared and submitted:

**Experimental Design and Methods**

**Results**

mortality
clinical observations
body weights
food consumption
clinical pathology results
palmitoyl CoA oxidase activities
hormone analyses (provided by Ani Lytics and DuPont Haskell Company)
receptor determination (provided by the Sponsor's designee)
serum and liver APFO levels (provided by 3M)
macroscopic observations
microscopic observations
cell proliferation evaluations (provided by the Sponsor's designee)

**Statistical Evaluation**

Levene's test will be done to test for variance homogeneity. In the case of heterogeneity of variance at $p \leq 0.05$, transformations will be used to stabilize the variance. Comparison tests will take variance heterogeneity into consideration.

One-way analysis of variance (ANOVA) will be used (if applicable) to analyze initial body weights, continuous clinical pathology values, and palmitoyl CoA oxidase activities. If the ANOVA is significant, Dunnett's t-test will be used for control versus treated group comparisons.

One-way analysis of covariance (ANCOVA) will be used to analyze body weights, with initial body weights as the covariate. If the ANCOVA is significant, covariate-adjusted means will be used for control versus treated group comparisons.

Group comparisons (Groups 2 and 3 versus Group 1) will be evaluated at the 5.0% two-tailed probability level. Only data collected on or after the first day of treatment will be analyzed statistically.

At the end of 1 year after issuance of the audited draft report, if no requested revisions or instructions to finalize have been communicated by the Sponsor, then the audited draft report will be considered 'final' and issued as the final report, signed by the study director, and submitted to the Sponsor.

Any modifications or changes to the audited draft report requested 1 year after issuance will be performed at additional cost to the Sponsor.

**Record Retention**

All raw data, documentation, records, protocol, specimens, and final report generated as a result of this study will be archived in the storage facilities of Covance-Madison for a period of 1 year following submission of the final report to the Sponsor. One year after submission of the final report, all of the aforementioned materials will be sent to the Sponsor, and a return fee will be charged. The Sponsor may elect to have the materials retained in the Covance archives for an additional period of time, and Covance will charge a storage fee. If the Sponsor chooses to have Covance dispose of the materials, a disposal fee will be charged. All raw data stored on magnetic media will be retained by Covance.

## PROTOCOL APPROVAL

Paul N. Lieder — 6/15/98

Paul Lieder, PhD
Diplomate, ABT
Study Monitor
3M

Date

6/11/98

Peter J. Thomford, PhD
Study Director
Covance Laboratories Inc.

Date

COVANCE
THE DEVELOPMENT SERVICES COMPANY

# PROTOCOL AMENDMENT NO. 1

Covance 6329-230

4-Week Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO) in Cynomolgus Monkeys

| | |
|---|---|
| Sponsor: | APME Ad-Hoc APFO Toxicology Working Group |
| Study Monitor: | Paul Lieder, PhD, DABT |
| Testing Facility: | Covance Laboratories Inc., Madison, Wisconsin |
| Study Director: | Peter J. Thomford, PhD |

This amendment modifies the following portions of the protocol:

**Effective June 11, 1998**

1. **Page 1, Study Title and all other occurrences of the name of test material in the protocol.** To correct the spelling of the test material, delete the name of the test material and replace with the following:

   Ammonium Perfluorooctanoate (APFO)

2. **Page 3, Regulatory Compliance.** Because bile acid determinations done by the University of Dundee will not be in compliance with GLPs, delete the text in this section and replace with the following:

   This study will be conducted in compliance with the Environmental Protection Agency Good Laboratory Practice Regulations as set forth in Title 40 of the US Code of Federal Regulations, Part 792, issued November 29, 1983 (effective December 29, 1983), and with any applicable amendments except that bile acid determinations done by University of Dundee will not be done in compliance with GLPs.

3. **Page 4, Quality Assurance, Sentence 5.** Because receptor level analysis will be done by DuPont Haskell Company, delete this sentence and replace with the following:

   The receptor level determinations and report will be audited by the QAU of the DuPont Haskell Company. Bile acid determinations done by the University of Dundee will not be audited.

4. **Page 7, Dosing Procedures, Reason for Dosing Route.** To correct the reason for the dosing route, delete the text in this section and replace with the following:

   To compare with previously conducted toxicology studies using the oral route

5. **Page 10, Blood Hormone Determination, Method of Collection.** To increase the volume of the blood collected for serum and plasma samples, to specify the use of serum separation tubes, and to specify the storage conditions of blood samples before serum or plasma samples are prepared, delete the text in this section and replace with the following:

   Animals will be fasted overnight; blood will be collected from a femoral vein (an alternative vein may be used if necessary). Approximately 6 mL of blood for plasma samples will be collected into tubes with potassium EDTA as the anticoagulant. Approximately 6 mL of blood for serum samples will be collected without anticoagulant and allowed to clot. Blood samples will be maintained at room temperature until serum is harvested. Blood samples will be maintained chilled until plasma is harvested.

6. **Page 10, Blood Hormone Determination, Serum Tests, Sentence 1.** To specify total and free triiodothyronine (T3) and total and free thyroxin (T4) tests that will be done, delete this sentence and replace with the following:

   Samples will be analyzed by Ani Lytics Inc., for estradiol, estrone, estriol, thyroid stimulating hormone, total and free triiodothyronine (T3), and total and free thyroxin (T4).

7. **Page 11, Blood Hormone Determination, Plasma Sample Handling, Paragraph 2.** To correct the shipping address, delete this paragraph and replace with the following:

Jon C. Cook, PhD, DABT
E. I. du Pont de Nemours & Company, Inc.
Haskell Laboratory for Toxicology and Industrial Medicine
Building 11 Room 1325
1090 Elkton Road
Newark, Delaware 19711-0500
Telephone No.: 302.366.5495
Facsimile No.: 302.366.5003

8. **Page 12, Additional Blood Collection, Sentence 2.** To clarify the processing and disposition of the additional blood samples, delete this sentence and replace with the following:

Approximately equally sized samples of serum (collected without anticoagulant) and whole blood and plasma (both collected using potassium EDTA as the anticoagulant) from each animal will be transferred into containers (approximately 5 mL each). Whole blood, serum, and plasma samples will be stored in a freezer set to maintain -60 to -80°C until shipped to the Sponsor for possible future analysis.

9. **Page 13, Receptor Level Determination.** To correct and clarify the process, delete this section and replace with the following:

**Receptor Level Determination**
Samples (approximately 2 g) of liver and pancreas will be collected from each animal at the scheduled sacrifice, flash-frozen in liquid nitrogen, and stored in a freezer set to maintain -60 to -80°C. Liver and pancreas samples will be packed on dry ice and shipped to:

Jon C. Cook, PhD, DABT
E. I. du Pont de Nemours & Company, Inc.
Haskell Laboratory for Toxicology and Industrial Medicine
Building 11 Room 1325
1090 Elkton Road
Newark, Delaware 19711-0500
Telephone No.: 302.366.5495
Facsimile No.: 302.366.5003

Results of receptor level determinations will be provided for inclusion in the final report.

**Bile Acid Determination**

All available bile (up to 5 mL) will be collected from each animal at the scheduled sacrifice, flash-frozen in liquid nitrogen, and stored in a freezer set to maintain -60 to -80°C. Bile samples will be packed on dry ice and shipped to:

Biomedical Research Centre
Level 5
Ninewells Hospital and Medical School
University of Dundee
Dundee DD1 9SY
UK
Dr Cliff Elcombe
Biomedical Research Centre
Ninewells Hospital and Medical School
University of Dundee
Dundee DD1 9SY, UK
Telephone: +44 (0)1382-632641
Fax: +44 (0)1382-425586

Results of bile acid determinations will be provided for inclusion in the final report.

10. **Page 15, Reports, Results.** To include bile acid determinations in the list of items to be reported, add the following:

Bile Acid Determinations (provided by the University of Dundee)

11. **Page 16, Record Retention.** To clarify the record retention requirements for the Sponsor's designees, add the following:

Within 1 year after submission of the final report, all of the aforementioned materials from the Sponsor's designees (Ani Lytics Inc., E. I. du Pont de Nemours & Company, Inc., 3M E.T. & S, Pathology Associates International, and the University of Dundee) will be sent to the Sponsor (Paul Lieder, PhD, DABT, 3M).

**AMENDMENT APPROVAL**

Paul H. Lieder — 9/25/98

Paul Lieder, PhD
Diplomate, ABT
Study Monitor
3M

Date

Peter J. Thomford — 9/23/98

Peter J. Thomford, PhD
Study Director
Covance Laboratories Inc.

Date

COVANCE
THE DEVELOPMENT SERVICES COMPANY

# PROTOCOL AMENDMENT NO. 2

Covance 6329-230

4-Week Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO) in Cynomolgus Monkeys

| | |
|---|---|
| Sponsor: | APME Ad-Hoc APFO Toxicology Working Group |
| Study Monitor: | Paul Lieder, PhD, DABT |
| Testing Facility: | Covance Laboratories Inc., Madison, Wisconsin |
| Study Director: | Peter J. Thomford, PhD |

This amendment modifies the following portions of the protocol.

**Effective December 15, 1998**

1. **Page 13, Postmortem Procedures, Cell Proliferation Evaluation, Paragraph 3, Sentence 2.** To include evaluation of slides stained with hematoxylin and eosin in the PCNA evaluation, delete this sentence and replace with the following.

   PCNA evaluation (including examination of slides stained with hematoxylin and eosin) will be done on the samples and results will be provided for inclusion in the final report.

**Effective April 8, 1999**

2. **Page 16, Record Retention, Paragraph 2.** To modify the record retention requirements for Pathology Associates International, delete this paragraph and replace with the following.

   Within 1 year after submission of the final report, all of the aforementioned materials from the Sponsor's designees (Ani Lytics Inc., E. I. du Pont de Nemours & Company, Inc., 3M E.T. & S, and the University of Dundee) will be sent to the Sponsor (Paul Lieder, PhD, DABT, 3M). Pathology Associates International (PAI) is responsible for the maintenance of any raw data or specimens produced by PAI.

## AMENDMENT APPROVAL

Paul W. Lieder

Paul Lieder, PhD
Diplomate, ABT
Study Monitor
3M

24 June 1999
Date

Peter J. Thomford, PhD
Study Director
Covance Laboratories Inc.

18 June 1999
Date

COVANCE
THE DEVELOPMENT SERVICES COMPANY

**PROTOCOL AMENDMENT NO. 3**

Covance 6329-230

4-Week Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO) in Cynomolgus Monkeys

| | |
|---|---|
| Sponsor: | APME Ad-Hoc APFO Toxicology Working Group |
| Study Monitor: | Paul Lieder, PhD, DABT |
| Testing Facility: | Covance Laboratories Inc., Madison, Wisconsin |
| Study Director: | Peter J. Thomford, PhD |

This amendment modifies the following portions of the protocol.

**Effective October 21, 1999**

1. **Page 11, Blood Hormone Determination, Plasma Analysis.** To reflect the Sponsor's decision to retain these samples for possible future analysis, delete the text in this section and replace with the following.

   Plasma samples for cholecystokinin determination will be stored in a freezer set to maintain –60 to -80°C for possible future analysis.

2. **Page 11, Serum APFO Level Determination, Sample Shipping, Paragraph 2, Sentence 2.** To reflect the Sponsor's decision to report the results of specified analyses separately, delete this sentence and replace with the following.

   Results will be reported separately.

3. **Page 13, Postmortem Procedures, Receptor Level Determination, Paragraph 2, Sentence 1.** To reflect the Sponsor's decision to retain these samples for possible future analysis, delete this sentence and replace with the following.

Liver and pancreas samples for receptor level determination will be stored in a freezer set to maintain -60 to -80°C for possible future analysis.

4. **Page 14, Postmortem Procedures, Bile Acid Determination, Paragraph 2, Sentence 1.** To reflect the Sponsor's decision to report the results of specified analyses separately, delete this sentence and replace with the following.

   Results will be reported separately.

5. **Page 14, Postmortem Procedures, Liver APFO Determination, Sentence 2.** To reflect the Sponsor's decision to report the results of specified analyses separately, delete this sentence and replace with the following.

   Results will be reported separately.

6. **Page 15, Report, Results.** To reflect the Sponsor's decision to report the results of specified analyses separately, delete this sentence and replace with the following.

   mortality
   clinical observations
   body weights
   food consumption
   clinical pathology results
   palmitoyl CoA oxidase activities
   hormone analyses (provided by Ani Lytics)
   macroscopic observations
   microscopic observations
   cell proliferation evaluations (provided by the Sponsor's designee)

## AMENDMENT APPROVAL

Paul W. Lieder

Paul Lieder, PhD
Diplomate, ABT
Study Monitor
3M

14 June 2000
Date

Peter J. Thomford, PhD
Study Director
Covance Laboratories Inc.

09 June 2000
Date

MATERIAL SAFETY DATA SHEET

3M
3M Center
St. Paul, Minnesota
55144-1000
1-800-364-3577 or (612) 737-6501 (24 hours)



DIVISION: 3M CHEMICALS
TRADE NAME:
FC-143 FLUORAD Brand Fluorochemical Surfactant
ID NUMBER/U.P.C.:

| | | | |
|---|---|---|---|
| 98-0211-0008-0 | 00-51135-09138-8 | 98-0211-0891-9 | 00-51135-09365-8 |
| 98-0211-5489-7 | 00-51135-02941-1 | 98-0211-6581-0 | 00-51135-10405-7 |
| 98-0211-7394-7 | 00-51135-10762-1 | ZF-0002-0378-4 | - - - |

ISSUED: January 29, 1998
SUPERSEDES: November 05, 1997
DOCUMENT: 10-3808-2

## 1. INGREDIENT

| INGREDIENT | C.A.S. NO. | PERCENT |
|---|---|---|
| AMMONIUM PERFLUOROOCTANOATE | 3825-26-1 | 93 - 97 |
| AMMONIUM PERFLUOROHEPTANOATE | 6130-43-4 | 1 - 3 |
| AMMONIUM PERFLUOROPENTANOATE | 68259-11-0 | 1 - 3 |
| AMMONIUM PERFLUOROHEXANOATE | 21615-47-4 | 0.1 - 1 |

## 2. PHYSICAL DATA

BOILING POINT: N/A
VAPOR PRESSURE: N/A
VAPOR DENSITY: N/A
EVAPORATION RATE: N/A
SOLUBILITY IN WATER: apprec.
SPECIFIC GRAVITY: 0.4 - 0.5 Water=1 (Bulk)
PERCENT VOLATILE: N/A
pH: ca. 5 (0.5% Aqueous)
VISCOSITY: N/D
MELTING POINT: N/A

APPEARANCE AND ODOR:
White colored powder; slight odor.

## 3. FIRE AND EXPLOSION HAZARD DATA

FLASH POINT:..... Non-flammable
FLAMMABLE LIMITS - LEL:..... N/A
FLAMMABLE LIMITS - UEL:..... N/A
AUTOIGNITION TEMPERATURE:..... N/A

EXTINGUISHING MEDIA:
Water, Carbon dioxide, Dry chemical, Foam

SPECIAL FIRE FIGHTING PROCEDURES:
Wear full protective clothing, including helmet, self-contained, positive pressure or pressure demand breathing apparatus, bunker coat and pants, bands around arms, waist and legs, face mask, and protective covering for exposed areas of the head.

UNUSUAL FIRE AND EXPLOSION HAZARDS:
See Hazardous Decomposition section for products of combustion.

## 4. REACTIVITY DATA

STABILITY: Stable

INCOMPATIBILITY - MATERIALS/CONDITIONS TO AVOID:
Not Applicable

HAZARDOUS POLYMERIZATION: Hazardous polymerization will not occur.

HAZARDOUS DECOMPOSITION PRODUCTS:
Carbon Monoxide and Carbon Dioxide, Oxides of Nitrogen, Hydrogen Fluoride, Ammonia.

## 5. ENVIRONMENTAL INFORMATION

SPILL RESPONSE:
Observe precautions from other sections. Collect spilled material. Use wet sweeping compound or water to avoid dusting. Clean up residue. Place in a closed container.

RECOMMENDED DISPOSAL:
Incinerate in an industrial or commercial facility in the presence of a combustible material. Combustion products will include HF. Disposal alternative: Dispose of waste product in a facility permitted to accept chemical waste.

. FC-143 FLUORAD Brand Fluorochemical Surfactant
,nuary 29, 1998 PAGE 3

5. ENVIRONMENTAL INFORMATION (continued)

ENVIRONMENTAL DATA:
SUPPORTING DATA:

BIODEGRADATION:
Theoretical Oxygen Demand (ThOD): 0.320 g/g
Chemical Oxygen Demand (COD): 0.0007 g/g
20-Day Biochemical Oxygen Demand (BOD20): Nil

AQUATIC TOXICITY:
Fathead minnow (Pimephales promelas) 96-hr LC50: 766 mg/L
Water flea (Daphnia magna) 48-hr EC50: 632 mg/L
Bluegill sunfish (Lepomis macrochirus) 96-hr EC50: 569 mg/L
Green Algae (Selenastrum capricornutum) 14-Day EC50:
>1000 mg/L
Microtox (Photobacterium phosphoreum) 30-min EC50: 730 mg/L

BIONCONCENTRATION:
BCF = 1.8

REGULATORY INFORMATION:
Volatile Organic Compounds: N/A.
VOC Less H2O & Exempt Solvents: N/A.

Since regulations vary, consult applicable regulations or authorities before disposal. U.S. EPA Hazardous Waste Number = None (Not U.S. EPA Hazardous).

The components of this product are in compliance with the chemical registration requirements of TSCA, EINECS, CDSL, AICS, MITI and KTCCL.

OTHER ENVIRONMENTAL INFORMATION:
This substance did not degrade significantly in a ready biodegradation test.

This compound is completely fluorinated (perfluorinated), or it contains perfluorinated portions. Perfluoroalkyl groups resist degradation in most natural environments.

This substance has minimal toxicity to aquatic organisms (100 mg/L < Lowest LC50, EC50, or IC50 < or = 1000 mg/L).

No data are available on the toxicity effects of this substance on wastewater treatment system organisms.

This substance has been found to not bioconcentrate or accumulate significantly in one or more living organisms.

EPCRA HAZARD CLASS:
FIRE HAZARD: No PRESSURE: No REACTIVITY: No ACUTE: Yes CHRONIC: Yes

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6. SUGGESTED FIRST AID

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EYE CONTACT:
Immediately flush eyes with large amounts of water for at least 15 minutes. Get immediate medical attention.

SKIN CONTACT:
Flush skin with large amounts of water. If irritation persists, get medical attention.

INHALATION:
If signs/symptoms occur, remove person to fresh air. If signs/symptoms continue, call a physician.

IF SWALLOWED:
Do not induce vomiting. Drink two glasses of water. Call a physician.

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7. PRECAUTIONARY INFORMATION

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EYE PROTECTION:
Avoid eye contact. Wear vented goggles.

SKIN PROTECTION:
Avoid skin contact. Wear appropriate gloves when handling this material. A pair of gloves made from the following material(s) are recommended: butyl rubber. Use one or more of the following personal protection items as necessary to prevent skin contact: head covering, coveralls. Protective garments (other than gloves) should be made of either of the following materials: polyethylene/polyvinylidene chloride (Saranex).

RECOMMENDED VENTILATION:
Use with appropriate local exhaust ventilation. Provide sufficient ventilation to maintain emissions below recommended exposure limits. If exhaust ventilation is not adequate, use appropriate respiratory protection.

RESPIRATORY PROTECTION:
Avoid breathing of airborne material. Select one of the following NIOSH approved respirators based on airborne concentration of contaminants and in accordance with OSHA regulations: full-face high-efficiency filter respirator, full-face supplied air respirator.

PREVENTION OF ACCIDENTAL INGESTION:
Do not eat, drink or smoke when using this product. Wash exposed areas thoroughly with soap and water. Wash hands after handling and before eating.

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7. PRECAUTIONARY INFORMATION (continued)

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RECOMMENDED STORAGE:
Do not store containers on their sides. Store at room temperature. Keep container dry. Keep container closed when not in use.

**PREVENT MOISTURE CONTAMINATION TO KEEP POWDER FREE FLOWING.**

FIRE AND EXPLOSION AVOIDANCE:
Keep container tightly closed. No smoking while handling this material.

HMIS HAZARD RATINGS: HEALTH: 3 FLAMMABILITY: 0 REACTIVITY: 0
PERSONAL PROTECTION: X (See precautions, section 7.)

EXPOSURE LIMITS

| INGREDIENT | VALUE | UNIT | TYPE | AUTH | SKIN* |
|---|---|---|---|---|---|
| AMMONIUM PERFLUOROOCTANOATE..... | 0.01 | MG/M3 | TWA | ACGIH | Y |
| AMMONIUM PERFLUOROHEPTANOATE..... | 0.1 | MG/M3 | TWA | 3M | Y |
| AMMONIUM PERFLUOROPENTANOATE..... | 0.1 | MG/M3 | TWA | 3M | Y |
| MONIUM PERFLUOROHEXANOATE..... | 0.1 | MG/M3 | TWA | 3M | Y |

KIN NOTATION: Listed substances indicated with 'Y' under SKIN refer to e potential contribution to the overall exposure by the cutaneous route including mucous membrane and eye, either by airborne or, more particularly, by direct contact with the substance. Vehicles can alter skin absorption.

SOURCE OF EXPOSURE LIMIT DATA:
- ACGIH: American Conference of Governmental Industrial Hygienists
- 3M: 3M Recommended Exposure Guidelines

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8. HEALTH HAZARD DATA

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EYE CONTACT:
Moderate Eye Irritation: signs/symptoms can include redness, swelling, pain, tearing, and hazy vision.

Airborne product may cause eye injury consisting of corneal opacity.

SKIN CONTACT:
Product is not expected to be irritating to the skin.

Mild Skin Irritation (after prolonged or repeated contact): signs/symptoms can include redness, swelling, and itching.

.ay be absorbed through the skin and persist in the body for an extended time.

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8. HEALTH HAZARD DATA (continued)

INHALATION:
Illness requiring medical attention may result from a single exposure by inhalation to moderate quantities of this material.

May be absorbed by inhalation and persist in the body for an extended time.

Single overexposure, above recommended guidelines, may cause:

Irritation (upper respiratory): signs/symptoms can include soreness of the nose and throat, coughing and sneezing.

Prolonged or repeated overexposure, above recommended guidelines, may cause:

Liver Effects: signs/symptoms can include yellow skin(jaundice) and tenderness of upper abdomen.

Repeated inhalation of airborne product above the exposure guideline can result in elevated organofluoride levels in the blood.

IF SWALLOWED:
Ingestion is not a likely route of exposure to this product.

Illness may result from a single swallowing of a moderate quantity of this material.

CANCER:
A mixture of ammonium perfluorooctanoate, ammonium perfluoroheptanoate, ammonium perfluoropentanoate and ammonium perfluorohexanoate, that was 93 to 97% AMMONIUM PERFLUOROOCTANOATE (3825-26-1) was fed to albino rats for 2 years, no compound induced carcinogenicity was found in the study. There were statistically significant compound related benign testicular tumors. In a second two-year study there were statistically significant compound related benign tumors in the liver, pancreas, and testis when compared to ad libitum and pair-fed controls. Based on the current knowledge, these findings have no human health implications. (3825-26-1) (1983 and 1993 studies conducted jointly by 3M and DuPont).

MUTAGENICITY:
Not mutagenic in invitro mutagenicity assays. Did not cause cell transformation in a mammalian cell transformation assay.

REPRODUCTIVE/DEVELOPMENTAL TOXINS:
Not teratogenic in rabbits by oral administration. Not teratogenic to rats by gavage or inhalation exposures.

8. HEALTH HAZARD DATA (continued)

OTHER HEALTH HAZARD INFORMATION:
This product is not known to contain any substances regulated under California Proposition 65.

A Product Toxicity Summary Sheet is available.

SECTION CHANGE DATES

| HEADING | SECTION CHANGED SINCE November 05, 1997 | ISSUE |
|---|---|---|

Abbreviations: N/D - Not Determined N/A - Not Applicable CA - Approximately

**Centre Analytical Laboratories, Inc.**
3048 Research Drive State College, PA 16801
Phone: (814) 231-8032 Fax: (814) 231-1253 or (814) 231-1580

# *INTERIM CERTIFICATE OF ANALYSIS*

**Centre Analytical Laboratories COA Reference #: 023-033**
**3M Product: Ammonium Perfluorooctanoate**
**Test Control Reference #: TCR-99131-37, Lot #: 332**
**Purity: 95.2%**

| Test Name | Specifications | Result |
|---|---|---|
| **Purity**[1] | | **95.2%** |
| Appearance | White, crystalline solid | Conforms |
| Identification<br>NMR | | Positive |
| Metals (ICP/MS)<br>1. Calcium<br>2. Magnesium<br>3. Sodium<br>4. Potassium<br>5. Nickel<br>6. Iron<br>7. Manganese | | <br>1. 0.001 wt./wt.%<br>2. <0.001 wt./wt.%<br>3. 0.005 wt./wt.%<br>4. <0.001 wt./wt.%<br>5. <0.001 wt./wt.%<br>6. <0.001 wt./wt.%<br>7. <0.001 wt./wt.% |
| Total % Impurity (NMR) | | 0.34 wt./wt.% |
| Total % Impurity (LC/MS) | | 4.49 wt./wt.% |
| Total % Impurity (GC/MS) | | None Quantified |
| Residual Solvents (TGA) | | None Detected |
| Purity by DSC | | 99.7% |
| Inorganic Anions (IC)<br>1. Chloride<br>2. Fluoride<br>3. Bromide<br>4. Nitrate<br>5. Nitrite<br>6. Phosphate<br>7. Sulfate | | <br>1. <0.015 wt./wt.%<br>2. <0.005 wt./wt.%<br>3. <0.040 wt./wt.%<br>4. <0.009 wt./wt.%<br>5. <0.006 wt./wt.%<br>6. <0.006 wt./wt.%<br>7. <0.040 wt./wt.% |
| Organic Acids[2] (IC)<br>1. TFA<br>2. PFPA<br>3. HFBA<br>4. NFPA | | <br>1. <0.1 wt./wt.%<br>2. <0.1 wt./wt.%<br>3. <0.1 wt./wt.%<br>4. <0.25 wt./wt.% |
| Elemental Analysis[3]:<br>1. Carbon<br>2. Hydrogen<br>3. Nitrogen<br>4. Sulfur<br>5. Fluorine | <br>Theoretical Value = 22.3%<br>Theoretical Value = 0.935%<br>Theoretical Value = 3.25%<br>Theoretical Value = 0%<br>Theoretical Value = 66.1% | <br>1. 18.9 wt./wt.%<br>2. 1.31 wt./wt.%<br>3. 3.75 wt./wt.%<br>4. 4.34 wt./wt.%<br>5. 63.2 wt./wt.% |
| Ammonium Analysis[3]<br>Ion Selective Electrode | Theoretical Value = 4.18% | 3.49 wt./wt. % |

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# *INTERIM CERTIFICATE OF ANALYSIS*

**Centre Analytical Laboratories COA Reference #: 023-033**
**3M Product: Ammonium Perfluorooctanoate**
**Test Control Reference #: TCR-99131-37, Lot #: 332**

Date of Last Analysis: 12/15/00

Expiration Date: 12/15/01

Storage Conditions: < -10 °C

Re-assessment Date: 12/15/01

[1]Purity = 100% - (Total Metal impurities, 0.006% + Total NMR impurities, 0.34% + Total LC/MS impurities, 4.49%)

Total impurity from all tests = 4.84%
Purity = 100% - 4.84% = 95.2%

[2] TFA Trifluoroacetic acid
HFBA Heptafluorobutyric acid
NFPA Nonafluoropentanoic acid
PFPA Pentafluoropropanoic acid

[3]Theoretical value calculations based on the empirical formula, $C_8F_{15}O_2^{(-)}NH_4^{(+)}$ (MW=431.1)

LC/MS Purity Profile:

| Peak # | Retention Time (min) | Mass(s) | Identity | Area | % Area |
|---|---|---|---|---|---|
| 1 | 12.140 | 269 | $C_6$ | 167596 | 0.73 |
| 2 | 13.504 | 331, 319 | $C_7$ homologue/$F_{13}$ | 860991 | 3.76 |
| 3 | 14.099 | 369 | PFOA | 21861700 | - |
| Total | - | - | - | 22890287 | 4.49 |

This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 160).

Prepared By: _____ 1/09/01
David S. Bell Date
Scientist, Centre Analytical Laboratories

Reviewed By: _____ 1/9/01
John Flaherty Date
Laboratory Manager, Centre Analytical Laboratories

# APPENDIX 2

Individual Animal Fate Data
Individual Clinical Observations

APPENDIX 2

Individual Animal Fate Data

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS



| ANIMAL NUMBER | DOSE GROUP | SEX | DEATH CODE | TYPE OF DEATH | DESCRIPTION OF DEATH | DATE OF DEATH | DAY OF STUDY | WEEK OF STUDY |
|---|---|---|---|---|---|---|---|---|
| I05353 | 1 | MALE | T | SCHEDULED | TERMINAL SACRIFICE | 07/16/98 | 30 | 5 |
| I05417 | 1 | MALE | T | SCHEDULED | TERMINAL SACRIFICE | 07/16/98 | 30 | 5 |
| I05231 | 2 | MALE | T | SCHEDULED | TERMINAL SACRIFICE | 07/16/98 | 30 | 5 |
| I05352 | 2 | MALE | T | SCHEDULED | TERMINAL SACRIFICE | 07/16/98 | 30 | 5 |
| I05419 | 2 | MALE | T | SCHEDULED | TERMINAL SACRIFICE | 07/16/98 | 30 | 5 |
| I05235 | 3 | MALE | T | SCHEDULED | TERMINAL SACRIFICE | 07/16/98 | 30 | 5 |
| I05351 | 3 | MALE | T | SCHEDULED | TERMINAL SACRIFICE | 07/16/98 | 30 | 5 |
| I05416 | 3 | MALE | T | SCHEDULED | TERMINAL SACRIFICE | 07/16/98 | 30 | 5 |

Appendix 2

Individual Clinical Observations

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS



| ANIMAL NUMBER | DEATH CODE | WK OF DEATH | CATEGORY KEYWORD / QUALIFIER (GROUP: M1 DOSE: 0 MG/KG/DAY, DAYS 1-30) | DAY | 'C' - COMMENTS LISTED AT END OF OBSERVATIONS FOR SEX/GROUP OBS | 30 MIN | 60 MIN | 90 MIN | DISPATCH |
|---|---|---|---|---|---|---|---|---|---|
| I05353 | T | 5 | SKIN & PELAGE | | | | | | |
| | | | BROKEN SKIN | | | | | | |
| | | | DIGIT(S)-HIND-RIGHT | 18 | - | P | P | P | - |
| | | | NORMAL | | | | | | |
| | | | NO REMARKABLE OBSERVATIONS | 1 | P | - | - | - | - |
| | | | | 8 | P | - | - | - | - |
| | | | | 15 | P | - | - | - | - |
| | | | | 22 | P | - | - | - | - |
| | | | | 29 | P | - | - | - | - |
| | | | | 30 | P | - | - | - | P |
| I05417 | T | 5 | NORMAL | | | | | | |
| | | | NO REMARKABLE OBSERVATIONS | 1 | P | - | - | - | - |
| | | | | 8 | P | - | - | - | - |
| | | | | 15 | P | - | - | - | - |
| | | | | 22 | P | - | - | - | - |
| | | | | 29 | P | - | - | - | - |
| | | | | 30 | P | - | - | - | P |
| | | | QUALITATIVE FOOD CONSUMPTION | | | | | | |
| | | | LOW | 21 | P | - | - | - | - |

Appendix 2

Individual Clinical Observations



CATEGORY GROUP: M2 DOSE: 2 MG/KG/DAY 'C' - COMMENTS LISTED AT END OF OBSERVATIONS FOR SEX/GROUP

| ANIMAL NUMBER | DEATH CODE | WK OF DEATH | KEYWORD / QUALIFIER (DAYS 1-30) | DAY | OBS | 30 MIN | 60 MIN | 90 MIN | DISPATCH |
|---|---|---|---|---|---|---|---|---|---|
| I05231 | T | 5 | NORMAL | | | | | | |
| | | | NO REMARKABLE OBSERVATIONS | 1 | P | - | - | - | - |
| | | | | 8 | P | - | - | - | - |
| | | | | 15 | P | - | - | - | - |
| | | | | 22 | P | - | - | - | - |
| | | | | 29 | P | - | - | - | - |
| | | | | 30 | P | - | - | - | P |
| I05352 | T | 5 | NORMAL | | | | | | |
| | | | NO REMARKABLE OBSERVATIONS | 1 | P | - | - | - | - |
| | | | | 8 | P | - | - | - | - |
| | | | | 15 | P | - | - | - | - |
| | | | | 22 | P | - | - | - | - |
| | | | | 29 | P | - | - | - | - |
| | | | | 30 | P | - | - | - | P |
| I05419 | T | 5 | APPEARANCE | | | | | | |
| | | | UNDER EFFECTS OF ANESTHESIA | 23 | - | - | - | P | - |
| | | | SKIN & PELAGE | | | | | | |
| | | | BROKEN SKIN | | | | | | |
| | | | THIGH-LEFT | 24 | P | P | P | P | - |
| | | | | 25 | - | P | P | P | - |
| | | | SCAB(S) | | | | | | |
| | | | LIMB-HIND-LEFT | 26 | P | P | P | P | - |
| | | | | 27 | P | P | P | P | - |
| | | | | 28 | P | P | P | P | - |
| | | | | 29 | P | - | - | - | - |
| | | | | 30 | P | - | - | - | P |
| | | | NORMAL | | | | | | |
| | | | NO REMARKABLE OBSERVATIONS | 1 | P | - | - | - | - |

Appendix 2

Individual Clinical Observations



| ANIMAL NUMBER | DEATH CODE | WK OF DEATH | CATEGORY KEYWORD QUALIFIER | GROUP: M2 DOSE: 2 MG/KG/DAY 'C' - DAYS 1-30 | DAY | OBS | COMMENTS LISTED AT END OF OBSERVATIONS FOR SEX/GROUP 30 MIN | 60 MIN | 90 MIN | DISPATCH |
|---|---|---|---|---|---|---|---|---|---|---|
| (CONTINUED FROM PREVIOUS PAGE) | | | | | | | | | | |
| I05419 | T | 5 | NORMAL | | | | | | | |
| | | | NO REMARKABLE OBSERVATIONS | | 8 | P | - | - | - | - |
| | | | | | 15 | P | - | - | - | - |
| | | | | | 22 | P | - | - | - | - |
| | | | QUALITATIVE FOOD CONSUMPTION | | | | | | | |
| | | | LOW | | 24 | P | - | - | - | - |

Appendix 2

Individual Clinical Observations



| ANIMAL NUMBER | DEATH CODE | WK OF DEATH | CATEGORY KEYWORD QUALIFIER | GROUP: M3 DOSE: 20 MG/KG/DAY 'C' - DAYS 1-30 | DAY | COMMENTS | OBS | 30 MIN | 60 MIN | 90 MIN | LISTED AT END OF OBSERVATIONS FOR SEX/GROUP DISPATCH |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I05235 | T | 5 | EXCRETION | | | | | | | | |
| | | | NO FECES | | 17 | P | | - | - | - | - |
| | | | NORMAL | | | | | | | | |
| | | | NO REMARKABLE OBSERVATIONS | | 1 | P | | - | - | - | - |
| | | | | | 8 | P | | - | - | - | - |
| | | | | | 15 | P | | - | - | - | - |
| | | | | | 22 | P | | - | - | - | - |
| | | | | | 29 | P | | - | - | - | - |
| | | | | | 30 | P | | - | - | - | P |
| | | | QUALITATIVE FOOD CONSUMPTION | | | | | | | | |
| | | | LOW | | 5 | P | | - | - | - | - |
| | | | | | 7 | P | | - | - | - | - |
| | | | | | 11 | P | | - | - | - | - |
| | | | | | 14 | P | | - | - | - | - |
| | | | | | 16 | P | | - | - | - | - |
| | | | | | 17 | P | | - | - | - | - |
| | | | | | 24 | P | | - | - | - | - |
| | | | NONE | | 12 | P | | - | - | - | - |
| I05351 | T | 5 | NORMAL | | | | | | | | |
| | | | NO REMARKABLE OBSERVATIONS | | 1 | P | | - | - | - | - |
| | | | | | 8 | P | | - | - | - | - |
| | | | | | 15 | P | | - | - | - | - |
| | | | | | 22 | P | | - | - | - | - |
| | | | | | 29 | P | | - | - | - | - |
| | | | | | 30 | P | | - | - | - | P |

Appendix 2

Individual Clinical Observations



CATEGORY GROUP: M3 DOSE: 20 MG/KG/DAY 'C' - COMMENTS LISTED AT END OF OBSERVATIONS FOR SEX/GROUP

| ANIMAL NUMBER | DEATH CODE | WK OF DEATH | KEYWORD QUALIFIER | DAYS 1-30 | DAY | OBS | 30 MIN | 60 MIN | 90 MIN | DISPATCH |
|---|---|---|---|---|---|---|---|---|---|---|
| I05416 | T | 5 | NORMAL | | | | | | | |
| | | | NO REMARKABLE OBSERVATIONS | | 1 | P | - | - | - | - |
| | | | | | 8 | P | - | - | - | - |
| | | | | | 15 | P | - | - | - | - |
| | | | | | 22 | P | - | - | - | - |
| | | | | | 29 | P | - | - | - | - |
| | | | | | 30 | P | - | - | - | P |

# APPENDIX 3

Individual Body Weight Data (kg)

Appendix 3

Individual Body Weight Data (kg)



| ANIMAL NUMBER | WEEK -2 | WEEK[a] -1 | WEEK[b] -1 | WEEK 1 | WEEK 2 | WEEK 3 | WEEK 4 | WEEK 5 |
|---|---|---|---|---|---|---|---|---|
| GROUP: MALE 1 - 0 MG/KG/DAY | | | | | | | | |
| I05353 | 2.5 | 2.5 | 2.5 | 2.6 | 2.7 | 2.7 | 2.6 | 2.7 |
| I05417 | 3.5 | 3.5 | 3.6 | 3.6 | 3.5 | 3.6 | 3.5 | 3.6 |
| GROUP: MALE 2 - 2 MG/KG/DAY | | | | | | | | |
| I05231 | 2.7 | 2.9 | 2.8 | 2.9 | 2.9 | 3.0 | 2.9 | 3.0 |
| I05352 | 2.1 | 2.1 | 2.1 | 2.1 | 2.2 | 2.2 | 2.2 | 2.2 |
| I05419 | 3.5 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 | 3.7 |
| GROUP: MALE 3 - 20 MG/KG/DAY | | | | | | | | |
| I05235 | 2.7 | 2.8 | 2.7 | 2.8 | 2.7 | 2.7 | 2.8 | 2.8 |
| I05351 | 2.2 | 2.3 | 2.3 | 2.2 | 2.3 | 2.3 | 2.3 | 2.4 |
| I05416 | 3.2 | 3.3 | 3.3 | 3.3 | 3.4 | 3.4 | 3.3 | 3.5 |

a Day -7.
b Day -1.

# APPENDIX 4

Individual Clinical Hematology Data
Individual Clinical Chemistry Data

Appendix 4

Individual Clinical Hematology Data

Males Day -8

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| ANIMAL NUMBER | RBC $X10^6/\mu L$ | HGB G/DL | HCT % | MCV FL | MCH PG | MCHC % | PLT $X10^3/\mu L$ | RETIC % | RETIC $X10^3/\mu L$ |
|---|---|---|---|---|---|---|---|---|---|
| Group: 1 | Dose Level: 0 | | Dosage Unit: mg/kg/day | | | | | | |
| I05353 | 4.44 | 11.9 | 35.8 | 80.5 | 26.8 | 33.3 | 376 | .7 | 31 |
| I05417 | 7.49 | 13.2 | 45.2 | 60.3 | 17.7 | 29.3 | 483 | .1 | 7 |
| MEAN | 5.96 | 12.6 | 40.5 | 70.4 | 22.2 | 31.3 | 430 | .4 | 19 |
| S.D. | 2.157 | .92 | 6.65 | 14.28 | 6.43 | 2.83 | 75.7 | .42 | 17.0 |
| N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Group: 2 | Dose Level: 2 | | Dosage Unit: mg/kg/day | | | | | | |
| I05231 | 7.08 | 13.1 | 43.5 | 61.5 | 18.5 | 30.0 | 455 | .4 | 28 |
| I05352 | 5.56 | 14.2 | 43.5 | 78.3 | 25.6 | 32.7 | 376 | 1.2 | 67 |
| I05419 | 7.92 | 13.4 | 45.6 | 57.5 | 16.9 | 29.3 | 435 | .1 | 8 |
| MEAN | 6.85 | 13.6 | 44.2 | 65.8 | 20.3 | 30.7 | 422 | .6 | 34 |
| S.D. | 1.196 | .57 | 1.21 | 11.04 | 4.63 | 1.80 | 41.1 | .57 | 30.0 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Group: 3 | Dose Level: 20 | | Dosage Unit: mg/kg/day | | | | | | |
| I05235 | 7.18 | 12.4 | 42.7 | 59.4 | 17.2 | 29.0 | 530 | .2 | 14 |
| I05351 | 5.37 | 14.3 | 42.8 | 79.6 | 26.5 | 33.4 | 649 | .6 | 32 |
| I05416 | 6.61 | 13.1 | 41.5 | 62.7 | 19.9 | 31.7 | 386 | .2 | 13 |
| MEAN | 6.39 | 13.3 | 42.3 | 67.2 | 21.2 | 31.4 | 522 | .3 | 20 |
| S.D. | .925 | .96 | .72 | 10.84 | 4.78 | 2.22 | 131.7 | .23 | 10.7 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Appendix 4

Individual Clinical Hematology Data

Males Day -8

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| ANIMAL NUMBER | WBC $X10^3/\mu L$ | N-SEG $X10^3/\mu L$ | LYMPH $X10^3/\mu L$ | MONO $X10^3/\mu L$ | EOSIN $X10^3/\mu L$ | BASO $X10^3/\mu L$ | N-SEG% | LYMPH% | MONO% | EOSIN% | BASO% |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Group: 1 | Dose Level: 0 | | Dosage Unit: mg/kg/day | | | | | | | | |
| I05353 | 9.2 | 1.0 | 7.8 | .4 | .1 | .0 | 11 | 84 | 4 | 1 | 0 |
| I05417 | 11.5 | 2.6 | 6.9 | .8 | 1.1 | .0 | 23 | 60 | 7 | 10 | 0 |
| MEAN | 10.4 | 1.8 | 7.4 | .6 | .6 | .0 | 17 | 72 | 6 | 6 | 0 |
| S.D. | 1.63 | 1.13 | .64 | .28 | .71 | .00 | 8.5 | 17.0 | 2.1 | 6.4 | .0 |
| N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Group: 2 | Dose Level: 2 | | Dosage Unit: mg/kg/day | | | | | | | | |
| I05231 | 9.3 | 4.0 | 4.3 | 1.0 | .1 | .0 | 43 | 46 | 10 | 1 | 0 |
| I05352 | 8.5 | 1.0 | 7.0 | .4 | .1 | .0 | 12 | 82 | 5 | 1 | 0 |
| I05419 | 10.5 | 2.8 | 6.3 | 1.2 | .2 | .0 | 27 | 60 | 11 | 2 | 0 |
| MEAN | 9.4 | 2.6 | 5.9 | .9 | .1 | .0 | 27 | 63 | 9 | 1 | 0 |
| S.D. | 1.01 | 1.51 | 1.40 | .42 | .06 | .00 | 15.5 | 18.1 | 3.2 | .6 | .0 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Group: 3 | Dose Level: 20 | | Dosage Unit: mg/kg/day | | | | | | | | |
| I05235 | 9.0 | 3.2 | 5.0 | .4 | .4 | .0 | 35 | 56 | 5 | 4 | 0 |
| I05351 | 9.0 | .8 | 7.6 | .5 | .1 | .1 | 9 | 84 | 5 | 1 | 1 |
| I05416 | 10.9 | 4.4 | 5.5 | .7 | .3 | .0 | 40 | 50 | 7 | 2 | 0 |
| MEAN | 9.6 | 2.8 | 6.0 | .5 | .3 | .0 | 28 | 63 | 6 | 2 | 0 |
| S.D. | 1.10 | 1.83 | 1.38 | .15 | .15 | .06 | 16.6 | 18.1 | 1.2 | 1.5 | .6 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Appendix 4

Individual Clinical Hematology Data

Males Day -8

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS

| ANIMAL NUMBER | ANISO | POLY | POIK | HYPO | TOXNEUT |
|---|---|---|---|---|---|
| Group: 1 | Dose Level: 0 | | Dosage Unit: mg/kg/day | | |
| I05353 | - | - | - | - | - |
| I05417 | - | - | - | - | - |
| Group: 2 | Dose Level: 2 | | Dosage Unit: mg/kg/day | | |
| I05231 | - | - | - | - | - |
| I05352 | - | - | - | - | - |
| I05419 | - | - | - | - | - |
| Group: 3 | Dose Level: 20 | | Dosage Unit: mg/kg/day | | |
| I05235 | - | - | - | - | - |
| I05351 | - | - | - | - | - |
| I05416 | - | - | - | - | - |

Appendix 4

Individual Clinical Hematology Data

Males Day -5

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| ANIMAL NUMBER | RBC $X10^6/\mu L$ | HGB G/DL | HCT % | MCV FL | MCH PG | MCHC % | PLT $X10^3/\mu L$ | RETIC % | RETIC $X10^3/\mu L$ |
|---|---|---|---|---|---|---|---|---|---|
| Group: 1 | Dose Level: 0 | | Dosage Unit: mg/kg/day | | | | | | |
| I05353 | 4.26 | 11.3 | 34.2 | 80.5 | 26.5 | 33.0 | 360 | 1.2 | 51 |
| I05417 | 6.68 | 11.8 | 40.5 | 60.7 | 17.6 | 29.0 | 447 | .0 | 0 |
| MEAN | 5.47 | 11.5 | 37.3 | 70.6 | 22.0 | 31.0 | 404 | .6 | 26 |
| S.D. | 1.711 | .35 | 4.45 | 14.00 | 6.29 | 2.83 | 61.5 | .85 | 36.1 |
| N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Group: 2 | Dose Level: 2 | | Dosage Unit: mg/kg/day | | | | | | |
| I05231 | 6.27 | 11.5 | 38.9 | 62.0 | 18.3 | 29.5 | 418 | .8 | 50 |
| I05352 | 4.72 | 12.4 | 37.1 | 78.6 | 26.3 | 33.4 | 393 | 1.6 | 76 |
| I05419 | 6.75 | 11.6 | 39.3 | 58.3 | 17.3 | 29.6 | 374 | .4 | 27 |
| MEAN | 5.91 | 11.8 | 38.4 | 66.3 | 20.6 | 30.8 | 395 | .9 | 51 |
| S.D. | 1.061 | .49 | 1.17 | 10.81 | 4.93 | 2.22 | 22.1 | .61 | 24.5 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Group: 3 | Dose Level: 20 | | Dosage Unit: mg/kg/day | | | | | | |
| I05235 | 6.61 | 11.4 | 39.2 | 59.3 | 17.3 | 29.2 | 516 | .1 | 7 |
| I05351 | 4.57 | 12.2 | 36.3 | 79.4 | 26.6 | 33.6 | 593 | .3 | 14 |
| I05416 | 6.07 | 12.0 | 38.3 | 63.1 | 19.7 | 31.3 | 392 | .1 | 6 |
| MEAN | 5.75 | 11.9 | 37.9 | 67.3 | 21.2 | 31.4 | 500 | .2 | 9 |
| S.D. | 1.057 | .42 | 1.48 | 10.68 | 4.83 | 2.20 | 101.4 | .12 | 4.4 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Appendix 4

Individual Clinical Hematology Data

Males Day -5

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| ANIMAL NUMBER | WBC $X10^3/\mu L$ | N-SEG $X10^3/\mu L$ | LYMPH $X10^3/\mu L$ | MONO $X10^3/\mu L$ | EOSIN $X10^3/\mu L$ | BASO $X10^3/\mu L$ | N-SEG% | LYMPH% | MONO% | EOSIN% | BASO% |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Group: 1 | Dose Level: 0 | | Dosage Unit: mg/kg/day | | | | | | | | |
| I05353 | 10.0 | 1.2 | 8.2 | .6 | .1 | .1 | 12 | 82 | 6 | 1 | 1 |
| I05417 | 12.2 | 2.8 | 7.5 | .8 | 1.0 | .0 | 23 | 62 | 7 | 8 | 0 |
| MEAN | 11.1 | 2.0 | 7.8 | .7 | .6 | .0 | 18 | 72 | 6 | 4 | 0 |
| S.D. | 1.56 | 1.13 | .49 | .14 | .64 | .07 | 7.8 | 14.1 | .7 | 4.9 | .7 |
| N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Group: 2 | Dose Level: 2 | | Dosage Unit: mg/kg/day | | | | | | | | |
| I05231 | 9.0 | 4.6 | 3.7 | .6 | .2 | .0 | 51 | 40 | 7 | 2 | 0 |
| I05352 | 8.8 | 2.4 | 5.8 | .6 | .1 | .0 | 27 | 65 | 6 | 1 | 0 |
| I05419 | 14.4 | 6.0 | 7.2 | .8 | .3 | .0 | 42 | 50 | 6 | 2 | 0 |
| MEAN | 10.7 | 4.3 | 5.6 | .7 | .2 | .0 | 40 | 52 | 6 | 2 | 0 |
| S.D. | 3.18 | 1.81 | 1.76 | .12 | .10 | .00 | 12.1 | 12.6 | .6 | .6 | .0 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Group: 3 | Dose Level: 20 | | Dosage Unit: mg/kg/day | | | | | | | | |
| I05235 | 11.1 | 4.8 | 5.1 | .7 | .3 | .0 | 44 | 46 | 7 | 3 | 0 |
| I05351 | 7.1 | 2.0 | 4.9 | .2 | .0 | .0 | 28 | 69 | 3 | 0 | 0 |
| I05416 | 13.2 | 5.6 | 6.3 | .8 | .4 | .0 | 43 | 48 | 6 | 3 | 0 |
| MEAN | 10.5 | 4.1 | 5.4 | .6 | .2 | .0 | 38 | 54 | 5 | 2 | 0 |
| S.D. | 3.10 | 1.89 | .76 | .32 | .21 | .00 | 9.0 | 12.7 | 2.1 | 1.7 | .0 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Appendix 4

Individual Clinical Hematology Data

Males Day -5

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| ANIMAL NUMBER | ANISO | POLY | POIK | HYPO | TOXNEUT |
|---|---|---|---|---|---|
| Group: 1 | Dose Level: 0 | | Dosage Unit: mg/kg/day | | |
| I05353 | - | - | - | - | - |
| I05417 | - | - | - | - | - |
| Group: 2 | Dose Level: 2 | | Dosage Unit: mg/kg/day | | |
| I05231 | - | - | - | - | - |
| I05352 | - | - | - | - | - |
| I05419 | - | - | - | - | - |
| Group: 3 | Dose Level: 20 | | Dosage Unit: mg/kg/day | | |
| I05235 | - | - | - | - | - |
| I05351 | - | - | - | - | - |
| I05416 | - | - | - | - | - |

Appendix 4

Individual Clinical Hematology Data

Males Day 30

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| ANIMAL NUMBER | RBC $X10^6/\mu L$ | HGB G/DL | HCT % | MCV FL | MCH PG | MCHC % | PLT $X10^3/\mu L$ | RETIC % | RETIC $X10^3/\mu L$ |
|---|---|---|---|---|---|---|---|---|---|
| Group: 1 | Dose Level: 0 | | Dosage Unit: mg/kg/day | | | | | | |
| I05353 | 4.93 | 12.5 | 39.2 | 79.6 | 25.5 | 32.0 | 414 | .4 | 20 |
| I05417 | 6.99 | 12.3 | 42.4 | 60.6 | 17.7 | 29.1 | 508 | .1 | 7 |
| MEAN | 5.96 | 12.4 | 40.8 | 70.1 | 21.6 | 30.5 | 461 | .2 | 14 |
| S.D. | 1.457 | .14 | 2.26 | 13.44 | 5.52 | 2.05 | 66.5 | .21 | 9.2 |
| N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Group: 2 | Dose Level: 2 | | Dosage Unit: mg/kg/day | | | | | | |
| I05231 | 7.03 | 13.2 | 43.6 | 62.0 | 18.7 | 30.3 | 412 | .1 | 7 |
| I05352 | 5.81 | 14.9 | 46.0 | 79.1 | 25.6 | 32.4 | 404 | .4 | 23 |
| I05419 | 7.38 | 12.6 | 43.2 | 58.5 | 17.1 | 29.2 | 406 | .0 | 0 |
| MEAN | 6.74 | 13.6 | 44.3 | 66.5 | 20.5 | 30.6 | 407 | .2 | 10 |
| S.D. | .824 | 1.19 | 1.51 | 11.02 | 4.52 | 1.63 | 4.2 | .21 | 11.8 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Group: 3 | Dose Level: 20 | | Dosage Unit: mg/kg/day | | | | | | |
| I05235 | 6.95 | 12.0 | 42.0 | 60.5 | 17.3 | 28.6 | 489 | .3 | 21 |
| I05351 | 5.35 | 14.2 | 43.1 | 80.6 | 26.6 | 33.0 | 605 | .4 | 21 |
| I05416 | 6.41 | 12.8 | 40.5 | 63.2 | 20.0 | 31.6 | 373 | .0 | 0 |
| MEAN | 6.24 | 13.0 | 41.9 | 68.1 | 21.3 | 31.1 | 489 | .2 | 14 |
| S.D. | .814 | 1.11 | 1.31 | 10.91 | 4.78 | 2.25 | 116.0 | .21 | 12.1 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Appendix 4

Individual Clinical Hematology Data

Males Day 30

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| ANIMAL NUMBER | WBC $X10^3/\mu L$ | N-SEG $X10^3/\mu L$ | LYMPH $X10^3/\mu L$ | MONO $X10^3/\mu L$ | EOSIN $X10^3/\mu L$ | BASO $X10^3/\mu L$ | N-SEG% | LYMPH% | MONO% | EOSIN% | BASO% |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Group: 1 | Dose Level: 0 | | Dosage Unit: mg/kg/day | | | | | | | | |
| I05353 | 9.9 | 1.1 | 7.8 | .7 | .2 | .1 | 11 | 79 | 7 | 2 | 1 |
| I05417 | 9.8 | 2.0 | 6.7 | .4 | .7 | .0 | 21 | 68 | 4 | 7 | 0 |
| MEAN | 9.8 | 1.6 | 7.2 | .6 | .4 | .0 | 16 | 74 | 6 | 4 | 0 |
| S.D. | .07 | .64 | .78 | .21 | .35 | .07 | 7.1 | 7.8 | 2.1 | 3.5 | .7 |
| N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Group: 2 | Dose Level: 2 | | Dosage Unit: mg/kg/day | | | | | | | | |
| I05231 | 6.2 | 1.9 | 3.8 | .5 | .0 | .0 | 30 | 61 | 8 | 1 | 0 |
| I05352 | 7.3 | 1.6 | 5.4 | .2 | .0 | .0 | 22 | 74 | 3 | 1 | 0 |
| I05419 | 10.6 | 3.1 | 7.0 | .3 | .1 | .0 | 30 | 66 | 3 | 1 | 0 |
| MEAN | 8.0 | 2.2 | 5.4 | .3 | .0 | .0 | 27 | 67 | 5 | 1 | 0 |
| S.D. | 2.29 | .79 | 1.60 | .15 | .06 | .00 | 4.6 | 6.6 | 2.9 | .0 | .0 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Group: 3 | Dose Level: 20 | | Dosage Unit: mg/kg/day | | | | | | | | |
| I05235 | 9.2 | 4.1 | 4.3 | .6 | .2 | .0 | 45 | 47 | 7 | 2 | 0 |
| I05351 | 8.2 | 1.9 | 5.8 | .5 | .0 | .0 | 23 | 70 | 6 | 1 | 1 |
| I05416 | 7.5 | 3.3 | 3.2 | .7 | .2 | .0 | 45 | 43 | 9 | 2 | 1 |
| MEAN | 8.3 | 3.1 | 4.4 | .6 | .1 | .0 | 38 | 53 | 7 | 2 | 1 |
| S.D. | .85 | 1.11 | 1.31 | .10 | .12 | .00 | 12.7 | 14.6 | 1.5 | .6 | .6 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Appendix 4

Individual Clinical Hematology Data

Males Day 30

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| ANIMAL NUMBER | ANISO | POLY | POIK | HYPO | TOXNEUT |
|---|---|---|---|---|---|
| Group: 1 | Dose Level: 0 | | Dosage Unit: mg/kg/day | | |
| I05353 | - | - | - | - | - |
| I05417 | - | - | - | - | - |
| Group: 2 | Dose Level: 2 | | Dosage Unit: mg/kg/day | | |
| I05231 | - | - | - | - | - |
| I05352 | - | - | - | - | - |
| I05419 | - | - | - | - | - |
| Group: 3 | Dose Level: 20 | | Dosage Unit: mg/kg/day | | |
| I05235 | - | - | - | - | - |
| I05351 | - | - | - | - | - |
| I05416 a | - | - | - | - | - |

a Plasmodium was observed.

Appendix 4

Individual Clinical Chemistry Data

Males Day -8

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| ANIMAL NUMBER | GLU MG/DL | UN MG/DL | CREAT MG/DL | T PRO G/DL | ALB G/DL | GLOB G/DL | T BILI MG/DL | SBA UMOL/L | CHOL MG/DL | TRIG MG/DL |
|---|---|---|---|---|---|---|---|---|---|---|
| Group: 1 | Dose Level: 0 | | Dosage Unit: mg/kg/day | | | | | | | |
| I05353 | 59 | 28 | .9 | 7.0 | 4.4 | 2.6 | .3 | 5 | 152 | 29 |
| I05417 | 88 | 17 | 1.2 | 10.4 | 6.0 | 4.4 | .5 | 13 | 139 | 40 |
| MEAN | 74 | 22 | 1.0 | 8.7 | 5.2 | 3.5 | .4 | 9 | 146 | 34 |
| S.D. | 20.5 | 7.8 | .21 | 2.40 | 1.13 | 1.27 | .14 | 5.7 | 9.2 | 7.8 |
| N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Group: 2 | Dose Level: 2 | | Dosage Unit: mg/kg/day | | | | | | | |
| I05231 | 60 | 16 | .9 | 8.3 | 5.1 | 3.2 | .2 | 11 | 157 | 40 |
| I05352 | 85 | 18 | 1.0 | 7.1 | 4.8 | 2.3 | .4 | 6 | 170 | 28 |
| I05419 | 84 | 20 | 1.4 | 9.7 | 5.4 | 4.3 | .2 | 9 | 196 | 39 |
| MEAN | 76 | 18 | 1.1 | 8.4 | 5.1 | 3.3 | .3 | 9 | 174 | 36 |
| S.D. | 14.2 | 2.0 | .26 | 1.30 | .30 | 1.00 | .12 | 2.5 | 19.9 | 6.7 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Group: 3 | Dose Level: 20 | | Dosage Unit: mg/kg/day | | | | | | | |
| I05235 | 63 | 24 | 1.4 | 8.3 | 4.6 | 3.7 | .2 | 12 | 126 | 39 |
| I05351 | 70 | 20 | .9 | 7.4 | 5.0 | 2.4 | .1 | 11 | 149 | 27 |
| I05416 | 60 | 24 | 1.2 | 8.4 | 4.7 | 3.7 | .2 | 12 | 147 | 28 |
| MEAN | 64 | 23 | 1.2 | 8.0 | 4.8 | 3.3 | .2 | 12 | 141 | 31 |
| S.D. | 5.1 | 2.3 | .25 | .55 | .21 | .75 | .06 | .6 | 12.7 | 6.7 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Appendix 4

Individual Clinical Chemistry Data

Males Day -8

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS

| ANIMAL NUMBER | AST/SGOT IU/L | ALT/SGPT IU/L | ALK PHOS IU/L | GGT IU/L | SDH IU/L | CK IU/L | AMYLASE IU/L | LIPASE IU/L | P AMYL U/L |
|---|---|---|---|---|---|---|---|---|---|
| Group: 1 | Dose Level: 0 | | Dosage Unit: mg/kg/day | | | | | | |
| I05353 | 40 | 38 | 1144 | 130 | 2 | 520 | 442 | 42 | 228 |
| I05417 | 42 | 52 | 550 | 114 | 2 | 175 | 541 | 44 | 275 |
| MEAN | 41 | 45 | 847 | 122 | 2 | 348 | 492 | 43 | 252 |
| S.D. | 1.4 | 9.9 | 420.0 | 11.3 | .0 | 244.0 | 70.0 | 1.4 | 33.2 |
| N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Group: 2 | Dose Level: 2 | | Dosage Unit: mg/kg/day | | | | | | |
| I05231 | 43 | 33 | 1122 | 209 | 10 | 307 | 415 | 51 | 223 |
| I05352 | 65 | 77 | 910 | 122 | 2 | 550 | 427 | 21 | 229 |
| I05419 | 37 | 50 | 1007 | 145 | 3 | 250 | 723 | 67 | 298 |
| MEAN | 48 | 53 | 1013 | 159 | 5 | 369 | 522 | 46 | 250 |
| S.D. | 14.7 | 22.2 | 106.1 | 45.1 | 4.4 | 159.3 | 174.5 | 23.4 | 41.7 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Group: 3 | Dose Level: 20 | | Dosage Unit: mg/kg/day | | | | | | |
| I05235 | 45 | 89 | 985 | 116 | 4 | 210 | 461 | 44 | 207 |
| I05351 | 58 | 38 | 986 | 115 | 4 | 225 | 524 | 51 | 280 |
| I05416 | 54 | 50 | 872 | 177 | 6 | 149 | 502 | 34 | 230 |
| MEAN | 52 | 59 | 948 | 136 | 5 | 195 | 496 | 43 | 239 |
| S.D. | 6.7 | 26.7 | 65.5 | 35.5 | 1.2 | 40.3 | 32.0 | 8.5 | 37.3 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Appendix 4

Individual Clinical Chemistry Data

Males Day -8

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| ANIMAL NUMBER | CA MG/DL | I PHOS MG/DL | NA MMOL/L | K MMOL/L | CL MMOL/L |
|---|---|---|---|---|---|
| Group: 1 | Dose Level: 0 | | Dosage Unit: mg/kg/day | | |
| I05353 | 10.2 | 7.9 | 150 | 6.1 | 111 |
| I05417 | 12.6 | 6.5 | 166 | 7.1 | 113 |
| MEAN | 11.4 | 7.2 | 158 | 6.6 | 112 |
| S.D. | 1.70 | .99 | 11.3 | .71 | 1.4 |
| N | 2 | 2 | 2 | 2 | 2 |
| Group: 2 | Dose Level: 2 | | Dosage Unit: mg/kg/day | | |
| I05231 | 11.3 | 8.7 | 159 | 5.6 | 109 |
| I05352 | 10.6 | 7.4 | 153 | 6.0 | 107 |
| I05419 | 11.0 | 6.5 | 162 | 5.3 | 109 |
| MEAN | 11.0 | 7.5 | 158 | 5.6 | 108 |
| S.D. | .35 | 1.11 | 4.6 | .35 | 1.2 |
| N | 3 | 3 | 3 | 3 | 3 |
| Group: 3 | Dose Level: 20 | | Dosage Unit: mg/kg/day | | |
| I05235 | 11.3 | 7.2 | 156 | 6.1 | 108 |
| I05351 | 11.1 | 7.2 | 154 | 6.5 | 109 |
| I05416 | 10.3 | 6.7 | 153 | 5.7 | 108 |
| MEAN | 10.9 | 7.0 | 154 | 6.1 | 108 |
| S.D. | .53 | .29 | 1.5 | .40 | .6 |
| N | 3 | 3 | 3 | 3 | 3 |

Appendix 4

Individual Clinical Chemistry Data

Males Day -5

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| ANIMAL NUMBER | GLU MG/DL | UN MG/DL | CREAT MG/DL | T PRO G/DL | ALB G/DL | GLOB G/DL | T BILI MG/DL | SBA UMOL/L | CHOL MG/DL | TRIG MG/DL |
|---|---|---|---|---|---|---|---|---|---|---|
| Group: 1 | Dose Level: 0 | | Dosage Unit: mg/kg/day | | | | | | | |
| I05353 | 55 | 24 | 1.1 | 7.2 | 4.4 | 2.8 | .6 | 6 | 156 | 28 |
| I05417 | 113 | 11 | 1.3 | 9.6 | 5.5 | 4.1 | .7 | 3 | 119 | 29 |
| MEAN | 84 | 18 | 1.2 | 8.4 | 5.0 | 3.4 | .6 | 4 | 138 | 28 |
| S.D. | 41.0 | 9.2 | .14 | 1.70 | .78 | .92 | .07 | 2.1 | 26.2 | .7 |
| N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Group: 2 | Dose Level: 2 | | Dosage Unit: mg/kg/day | | | | | | | |
| I05231 | 66 | 14 | 1.0 | 8.1 | 4.9 | 3.2 | .1 | 1 | 141 | 31 |
| I05352 | 60 | 15 | .9 | 7.1 | 4.8 | 2.3 | .4 | 5 | 160 | 32 |
| I05419 | 73 | 16 | 1.3 | 8.8 | 4.7 | 4.1 | .4 | 9 | 164 | 38 |
| MEAN | 66 | 15 | 1.1 | 8.0 | 4.8 | 3.2 | .3 | 5 | 155 | 34 |
| S.D. | 6.5 | 1.0 | .21 | .85 | .10 | .90 | .17 | 4.0 | 12.3 | 3.8 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Group: 3 | Dose Level: 20 | | Dosage Unit: mg/kg/day | | | | | | | |
| I05235 | 78 | 14 | 1.1 | 8.2 | 4.4 | 3.8 | .1 | 1 | 128 | 30 |
| I05351 | 51 | 24 | .9 | 7.4 | 4.9 | 2.5 | .7 | 2 | 155 | 51 |
| I05416 | 73 | 21 | 1.2 | 8.5 | 4.7 | 3.8 | .3 | 0 | 140 | 35 |
| MEAN | 67 | 20 | 1.1 | 8.0 | 4.7 | 3.4 | .4 | 1 | 141 | 39 |
| S.D. | 14.4 | 5.1 | .15 | .57 | .25 | .75 | .31 | 1.0 | 13.5 | 11.0 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Appendix 4

Individual Clinical Chemistry Data

Males Day -5

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| ANIMAL NUMBER | AST/SGOT IU/L | ALT/SGPT IU/L | ALK PHOS IU/L | GGT IU/L | SDH IU/L | CK IU/L | AMYLASE IU/L | LIPASE IU/L | P AMYL U/L |
|---|---|---|---|---|---|---|---|---|---|
| Group: 1 | Dose Level: 0 | | Dosage Unit: mg/kg/day | | | | | | |
| I05353 | 42 | 34 | 1066 | 117 | 3 | 833 | 441 | 42 | 233 |
| I05417 | 35 | 52 | 509 | 108 | 3 | 156 | 549 | 38 | 270 |
| MEAN | 38 | 43 | 788 | 112 | 3 | 494 | 495 | 40 | 252 |
| S.D. | 4.9 | 12.7 | 393.9 | 6.4 | .0 | 478.7 | 76.4 | 2.8 | 26.2 |
| N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Group: 2 | Dose Level: 2 | | Dosage Unit: mg/kg/day | | | | | | |
| I05231 | 31 | 35 | 1026 | 182 | 4 | 141 | 395 | 64 | 220 |
| I05352 | 54 | 65 | 872 | 108 | 3 | 542 | 446 | 46 | 244 |
| I05419 | 31 | 46 | 893 | 120 | 2 | 155 | 720 | 58 | 293 |
| MEAN | 39 | 49 | 930 | 137 | 3 | 279 | 520 | 56 | 252 |
| S.D. | 13.3 | 15.2 | 83.5 | 39.7 | 1.0 | 227.6 | 174.8 | 9.2 | 37.2 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Group: 3 | Dose Level: 20 | | Dosage Unit: mg/kg/day | | | | | | |
| I05235 | 37 | 66 | 945 | 106 | 2 | 181 | 451 | 57 | 213 |
| I05351 | 54 | 37 | 906 | 92 | 3 | 194 | 467 | 71 | 249 |
| I05416 | 54 | 51 | 837 | 164 | 4 | 138 | 481 | 44 | 224 |
| MEAN | 48 | 51 | 896 | 121 | 3 | 171 | 466 | 57 | 229 |
| S.D. | 9.8 | 14.5 | 54.7 | 38.2 | 1.0 | 29.3 | 15.0 | 13.5 | 18.4 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Appendix 4

Individual Clinical Chemistry Data

Males Day -5

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS

| ANIMAL NUMBER | CA MG/DL | I PHOS MG/DL | NA MMOL/L | K MMOL/L | CL MMOL/L |
|---|---|---|---|---|---|
| Group: 1 | Dose Level: 0 | | Dosage Unit: mg/kg/day | | |
| I05353 | 10.0 | 7.7 | 149 | 4.8 | 107 |
| I05417 | 11.8 | 5.9 | 162 | 6.1 | 110 |
| MEAN | 10.9 | 6.8 | 156 | 5.4 | 108 |
| S.D. | 1.27 | 1.27 | 9.2 | .92 | 2.1 |
| N | 2 | 2 | 2 | 2 | 2 |
| Group: 2 | Dose Level: 2 | | Dosage Unit: mg/kg/day | | |
| I05231 | 11.1 | 6.9 | 159 | 5.6 | 113 |
| I05352 | 10.3 | 7.1 | 153 | 5.6 | 109 |
| I05419 | 9.9 | 5.2 | 153 | 4.3 | 108 |
| MEAN | 10.4 | 6.4 | 155 | 5.2 | 110 |
| S.D. | .61 | 1.04 | 3.5 | .75 | 2.6 |
| N | 3 | 3 | 3 | 3 | 3 |
| Group: 3 | Dose Level: 20 | | Dosage Unit: mg/kg/day | | |
| I05235 | 10.8 | 6.1 | 154 | 5.3 | 108 |
| I05351 | 10.5 | 6.4 | 153 | 5.3 | 108 |
| I05416 | 9.8 | 6.1 | 149 | 4.3 | 105 |
| MEAN | 10.4 | 6.2 | 152 | 5.0 | 107 |
| S.D. | .51 | .17 | 2.6 | .58 | 1.7 |
| N | 3 | 3 | 3 | 3 | 3 |

Appendix 4

Individual Clinical Chemistry Data

Males Day 2

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| ANIMAL NUMBER | GLU MG/DL | UN MG/DL | CREAT MG/DL | T PRO G/DL | ALB G/DL | GLOB G/DL | T BILI MG/DL | SBA UMOL/L | CHOL MG/DL | TRIG MG/DL |
|---|---|---|---|---|---|---|---|---|---|---|
| Group: 1 | Dose Level: 0 | | Dosage Unit: mg/kg/day | | | | | | | |
| I05353 | 73 | 26 | .8 | 7.5 | 4.5 | 3.0 | .3 | 13 | 157 | 23 |
| I05417 | 79 | 16 | 1.1 | 10.6 | 5.8 | 4.8 | .6 | 6 | 146 | 34 |
| MEAN | 76 | 21 | 1.0 | 9.0 | 5.2 | 3.9 | .4 | 10 | 152 | 28 |
| S.D. | 4.2 | 7.1 | .21 | 2.19 | .92 | 1.27 | .21 | 4.9 | 7.8 | 7.8 |
| N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Group: 2 | Dose Level: 2 | | Dosage Unit: mg/kg/day | | | | | | | |
| I05231 | 64 | 17 | .8 | 8.4 | 4.9 | 3.5 | .4 | 6 | 143 | 49 |
| I05352 | 70 | 26 | .9 | 7.7 | 5.0 | 2.7 | .3 | 9 | 183 | 45 |
| I05419 | 62 | 18 | 1.2 | 9.0 | 4.8 | 4.2 | .3 | 12 | 180 | 61 |
| MEAN | 65 | 20 | 1.0 | 8.4 | 4.9 | 3.5 | .3 | 9 | 169 | 52 |
| S.D. | 4.2 | 4.9 | .21 | .65 | .10 | .75 | .06 | 3.0 | 22.3 | 8.3 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Group: 3 | Dose Level: 20 | | Dosage Unit: mg/kg/day | | | | | | | |
| I05235 | 68 | 24 | 1.1 | 8.4 | 4.6 | 3.8 | .1 | 11 | 121 | 35 |
| I05351 | 70 | 22 | .9 | 7.7 | 5.0 | 2.7 | .2 | 12 | 136 | 57 |
| I05416 | 66 | 23 | .9 | 8.7 | 4.7 | 4.0 | .1 | 19 | 140 | 43 |
| MEAN | 68 | 23 | 1.0 | 8.3 | 4.8 | 3.5 | .1 | 14 | 132 | 45 |
| S.D. | 2.0 | 1.0 | .12 | .51 | .21 | .70 | .06 | 4.4 | 10.0 | 11.1 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Appendix 4

Individual Clinical Chemistry Data

Males Day 2

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| ANIMAL NUMBER | AST/SGOT IU/L | ALT/SGPT IU/L | ALK PHOS IU/L | GGT IU/L | SDH IU/L | CK IU/L | AMYLASE IU/L | LIPASE IU/L | P AMYL U/L |
|---|---|---|---|---|---|---|---|---|---|
| Group: 1 | Dose Level: 0 | | Dosage Unit: mg/kg/day | | | | | | |
| I05353 | 45 | 35 | 1123 | 129 | 1 | 181 | 435 | 28 | 228 |
| I05417 | 49 | 71 | 620 | 121 | 1 | 269 | 519 | 30 | 270 |
| MEAN | 47 | 53 | 872 | 125 | 1 | 225 | 477 | 29 | 249 |
| S.D. | 2.8 | 25.5 | 355.7 | 5.7 | .0 | 62.2 | 59.4 | 1.4 | 29.7 |
| N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Group: 2 | Dose Level: 2 | | Dosage Unit: mg/kg/day | | | | | | |
| I05231 | 33 | 31 | 1151 | 200 | 1 | 113 | 366 | 45 | 204 |
| I05352 | 91 | 82 | 927 | 119 | 4 | 2149 | 438 | 36 | 239 |
| I05419 | 36 | 42 | 1009 | 126 | 2 | 121 | 644 | 51 | 289 |
| MEAN | 53 | 52 | 1029 | 148 | 2 | 794 | 483 | 44 | 244 |
| S.D. | 32.7 | 26.8 | 113.3 | 44.9 | 1.5 | 1173.2 | 144.3 | 7.5 | 42.7 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Group: 3 | Dose Level: 20 | | Dosage Unit: mg/kg/day | | | | | | |
| I05235 | 49 | 80 | 1062 | 110 | 2 | 175 | 433 | 41 | 205 |
| I05351 | 58 | 36 | 827 | 98 | 0 | 203 | 503 | 39 | 269 |
| I05416 | 57 | 51 | 875 | 171 | 1 | 116 | 502 | 19 | 227 |
| MEAN | 55 | 56 | 921 | 126 | 1 | 165 | 479 | 33 | 234 |
| S.D. | 4.9 | 22.4 | 124.2 | 39.1 | 1.0 | 44.4 | 40.1 | 12.2 | 32.5 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Appendix 4

Individual Clinical Chemistry Data

Males Day 2

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| ANIMAL NUMBER | CA MG/DL | I PHOS MG/DL | NA MMOL/L | K MMOL/L | CL MMOL/L |
|---|---|---|---|---|---|
| Group: 1 | Dose Level: 0 | | Dosage Unit: mg/kg/day | | |
| I05353 | 10.4 | 9.0 | 153 | 5.5 | 106 |
| I05417 | 12.7 | 8.6 | 170 | 6.9 | 113 |
| MEAN | 11.6 | 8.8 | 162 | 6.2 | 110 |
| S.D. | 1.63 | .28 | 12.0 | .99 | 4.9 |
| N | 2 | 2 | 2 | 2 | 2 |
| Group: 2 | Dose Level: 2 | | Dosage Unit: mg/kg/day | | |
| I05231 | 11.0 | 8.1 | 158 | 5.5 | 113 |
| I05352 | 10.7 | 9.3 | 155 | 5.6 | 106 |
| I05419 | 9.9 | 6.7 | 153 | 4.8 | 107 |
| MEAN | 10.5 | 8.0 | 155 | 5.3 | 109 |
| S.D. | .57 | 1.30 | 2.5 | .44 | 3.8 |
| N | 3 | 3 | 3 | 3 | 3 |
| Group: 3 | Dose Level: 20 | | Dosage Unit: mg/kg/day | | |
| I05235 | 11.0 | 7.5 | 159 | 5.7 | 110 |
| I05351 | 11.4 | 8.3 | 157 | 6.1 | 107 |
| I05416 | 9.8 | 6.5 | 151 | 4.8 | 109 |
| MEAN | 10.7 | 7.4 | 156 | 5.5 | 109 |
| S.D. | .83 | .90 | 4.2 | .67 | 1.5 |
| N | 3 | 3 | 3 | 3 | 3 |

Appendix 4

Individual Clinical Chemistry Data

Males Day 30

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| ANIMAL NUMBER | GLU MG/DL | UN MG/DL | CREAT MG/DL | T PRO G/DL | ALB G/DL | GLOB G/DL | T BILI MG/DL | SBA UMOL/L | CHOL MG/DL | TRIG MG/DL |
|---|---|---|---|---|---|---|---|---|---|---|
| Group: 1 | Dose Level: 0 | | Dosage Unit: mg/kg/day | | | | | | | |
| I05353 | 77 | 23 | .9 | 7.3 | 4.6 | 2.7 | .7 | 6 | 172 | 33 |
| I05417 | 90 | 15 | 1.0 | 9.8 | 5.6 | 4.2 | .3 | 1 | 127 | 32 |
| MEAN | 84 | 19 | 1.0 | 8.6 | 5.1 | 3.4 | .5 | 4 | 150 | 32 |
| S.D. | 9.2 | 5.7 | .07 | 1.77 | .71 | 1.06 | .28 | 3.5 | 31.8 | .7 |
| N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Group: 2 | Dose Level: 2 | | Dosage Unit: mg/kg/day | | | | | | | |
| I05231 | 75 | 15 | 1.1 | 8.4 | 5.1 | 3.3 | .2 | 2 | 137 | 45 |
| I05352 | 80 | 16 | 1.2 | 7.7 | 5.1 | 2.6 | .1 | 4 | 149 | 39 |
| I05419 | 74 | 19 | 1.1 | 8.9 | 4.8 | 4.1 | .1 | 5 | 155 | 44 |
| MEAN | 76 | 17 | 1.1 | 8.3 | 5.0 | 3.3 | .1 | 4 | 147 | 43 |
| S.D. | 3.2 | 2.1 | .06 | .60 | .17 | .75 | .06 | 1.5 | 9.2 | 3.2 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Group: 3 | Dose Level: 20 | | Dosage Unit: mg/kg/day | | | | | | | |
| I05235 | 64 | 16 | 1.5 | 8.0 | 4.4 | 3.6 | .1 | 10 | 107 | 50 |
| I05351 | 63 | 22 | 1.2 | 7.3 | 4.8 | 2.5 | .0 | 1 | 138 | 63 |
| I05416 | 67 | 22 | .8 | 8.2 | 4.4 | 3.8 | .3 | 6 | 170 | 42 |
| MEAN | 65 | 20 | 1.2 | 7.8 | 4.5 | 3.3 | .1 | 6 | 138 | 52 |
| S.D. | 2.1 | 3.5 | .35 | .47 | .23 | .70 | .15 | 4.5 | 31.5 | 10.6 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Appendix 4

Individual Clinical Chemistry Data

Males Day 30

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO)
IN CYNOMOLGUS MONKEYS

| ANIMAL NUMBER | AST/SGOT IU/L | ALT/SGPT IU/L | ALK PHOS IU/L | GGT IU/L | SDH IU/L | CK IU/L | AMYLASE IU/L | LIPASE IU/L | P AMYL U/L | PCOAO IU/G |
|---|---|---|---|---|---|---|---|---|---|---|
| Group: 1 | Dose Level: 0 | | Dosage Unit: mg/kg/day | | | | | | | |
| I05353 | 52 | 42 | 1154 | 118 | 1 | 827 | 451 | 53 | 238 | 2 |
| I05417 | 51 | 76 | 680 | 102 | 3 | 195 | 491 | 30 | 247 | 1 |
| MEAN | 52 | 59 | 917 | 110 | 2 | 511 | 471 | 42 | 242 | 2 |
| S.D. | .7 | 24.0 | 335.2 | 11.3 | 1.4 | 446.9 | 28.3 | 16.3 | 6.4 | .7 |
| N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Group: 2 | Dose Level: 2 | | Dosage Unit: mg/kg/day | | | | | | | |
| I05231 | 35 | 53 | 1155 | 177 | 1 | 145 | 344 | 36 | 186 | 0 |
| I05352 | 72 | 113 | 1009 | 115 | 2 | 570 | 501 | 32 | 273 | 0 |
| I05419 | 42 | 58 | 1170 | 115 | 3 | 256 | 654 | 47 | 272 | 3 |
| MEAN | 50 | 75 | 1111 | 136 | 2 | 324 | 500 | 38 | 244 | 1 |
| S.D. | 19.7 | 33.3 | 88.9 | 35.8 | 1.0 | 220.4 | 155.0 | 7.8 | 49.9 | 1.7 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Group: 3 | Dose Level: 20 | | Dosage Unit: mg/kg/day | | | | | | | |
| I05235 | 43 | 140 | 799 | 80 | 3 | 206 | 382 | 47 | 175 | 5 |
| I05351 | 66 | 60 | 626 | 83 | 2 | 216 | 521 | 45 | 277 | 3 |
| I05416 | 55 | 48 | 686 | 139 | 0 | 198 | 420 | 26 | 202 | 9 |
| MEAN | 55 | 83 | 704 | 101 | 2 | 207 | 441 | 39 | 218 | 6 |
| S.D. | 11.5 | 50.0 | 87.8 | 33.2 | 1.5 | 9.0 | 71.8 | 11.6 | 52.8 | 3.1 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Appendix 4

Individual Clinical Chemistry Data

Males Day 30

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS

| ANIMAL NUMBER | CA MG/DL | I PHOS MG/DL | NA MMOL/L | K MMOL/L | CL MMOL/L |
|---|---|---|---|---|---|
| Group: 1 | Dose Level: 0 | | Dosage Unit: mg/kg/day | | |
| I05353 | 10.2 | 8.4 | 149 | 5.0 | 104 |
| I05417 | 11.2 | 5.9 | 157 | 5.7 | 108 |
| MEAN | 10.7 | 7.2 | 153 | 5.4 | 106 |
| S.D. | .71 | 1.77 | 5.7 | .49 | 2.8 |
| N | 2 | 2 | 2 | 2 | 2 |
| Group: 2 | Dose Level: 2 | | Dosage Unit: mg/kg/day | | |
| I05231 | 11.2 | 7.1 | 157 | 5.2 | 112 |
| I05352 | 11.0 | 7.5 | 156 | 6.1 | 107 |
| I05419 | 10.2 | 5.5 | 153 | 4.3 | 107 |
| MEAN | 10.8 | 6.7 | 155 | 5.2 | 109 |
| S.D. | .53 | 1.06 | 2.1 | .90 | 2.9 |
| N | 3 | 3 | 3 | 3 | 3 |
| Group: 3 | Dose Level: 20 | | Dosage Unit: mg/kg/day | | |
| I05235 | 10.3 | 5.8 | 155 | 5.1 | 111 |
| I05351 | 10.9 | 5.6 | 152 | 5.9 | 107 |
| I05416 | 9.5 | 5.2 | 145 | 4.6 | 108 |
| MEAN | 10.2 | 5.5 | 151 | 5.2 | 109 |
| S.D. | .70 | .31 | 5.1 | .66 | 2.1 |
| N | 3 | 3 | 3 | 3 | 3 |

# APPENDIX 5

Individual Animal Pathology Data

APPENDIX 5

Individual Animal Pathology Data

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS 

ANIMAL NUMBER: I05353 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 07/16/98 STUDY DAY OF DEATH: 30 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 2500.0 GRAMS
DATE AND TIME OF NECROPSY: 07/16/98 11:10 PROSECTOR: KEVIN BILLINGS RECORDER: JILL PAUS
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. JAMES L. CARTER WEIGHER: NOT AVAILABLE

O B S E R V A T I O N S

NECROPSY

KIDNEY (KD) :
-LIGHT FOCUS(I)/AREA(S); RIGHT ON CORTICAL SURFACE, NEAR HILUS: SINGLE TAN AREA, 0.2 CM IN DIAMETER; COLLECTED ON ROUTINE SECTION
GENERAL COMMENT (GC) :
-EYES - DAVIDSONS

THE FOLLOWING ORGANS WERE UNREMARKABLE AT NECROPSY:
LIVER (LI), SPLEEN (SP), PANCREAS (PA), TESTIS (TE), BONE, FEMUR (FE), MARROW, FEMUR (FM), MARROW, STERNUM (SE), BONE, STERNUM (SB), EYE (EY), BRAIN (BR), LUNG (LU), HEART (HT), TRACHEA (TR), ESOPHAGUS (ES), THYROID (TY), PARATHYROID (PT), THYMUS (TH), LN, MESENTERIC (MS), AORTA (AO), PITUITARY (PI), SALIV GL, MANDIB (SG), MUSCLE, SKELETAL (SM), SPINAL CORD (SC), NERVE, SCIATIC (SN), STOMACH, GL (ST), DUODENUM (DU), GALLBLADDER (GB), JEJUNUM (JE), ILEUM (IL), CECUM (CE), COLON (CO), RECTUM (RE), SKIN (SK), MAMMARY, MALE (MM), URINARY BLADDER (UB), PROSTATE (PR), SEMINAL VESICLES (SV), EPIDIDYMIDES (EP), ADRENAL, CORTEX (AC), ADRENAL, MEDULLA (MA)

THE FOLLOWING TISSUES WERE UNREMARKABLE AT MICROSCOPIC EXAMINATION:
LIVER (LI), SPLEEN (SP), PANCREAS (PA), TESTIS (TE), ADRENAL (AD)

HISTOPATHOLOGY

KIDNEY (KD) :
>NOT REQUIRED TO BE EXAMINED FOR ANIMAL

APPENDIX 5

Individual Animal Pathology Data

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS 

ANIMAL NUMBER: I05417 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 07/16/98 STUDY DAY OF DEATH: 30 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 3325.0 GRAMS
DATE AND TIME OF NECROPSY: 07/16/98 11:15 PROSECTOR: JERALD W. JOHNSON RECORDER: JAMES T. CALLEN
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. JAMES L. CARTER WEIGHER: NOT AVAILABLE

O B S E R V A T I O N S

NECROPSY

STOMACH, GL (ST) :
-RED FOCUS(I)/AREA(S); MUCOSA: MULTIPLE RED AREAS, UP TO 0.8 X 0.5 CM; COLLECTED ON ROUTINE SECTION
GENERAL COMMENT (GC) :
-EYES - DAVIDSONS

HISTOPATHOLOGY

STOMACH, GL (ST) :
>NOT REQUIRED TO BE EXAMINED FOR ANIMAL

THE FOLLOWING ORGANS WERE UNREMARKABLE AT NECROPSY:
LIVER (LI), SPLEEN (SP), PANCREAS (PA), TESTIS (TE), BONE, FEMUR (FE), MARROW, FEMUR (FM), MARROW, STERNUM (SE), BONE, STERNUM (SB), EYE (EY), BRAIN (BR), KIDNEY (KD), LUNG (LU), HEART (HT), TRACHEA (TR), ESOPHAGUS (ES), THYROID (TY), PARATHYROID (PT), THYMUS (TH), LN, MESENTERIC (MS), AORTA (AO), PITUITARY (PI), SALIV GL, MANDIB (SG), MUSCLE, SKELETAL (SM), SPINAL CORD (SC), NERVE, SCIATIC (SN), DUODENUM (DU), GALLBLADDER (GB), JEJUNUM (JE), ILEUM (IL), CECUM (CE), COLON (CO), RECTUM (RE), SKIN (SK), MAMMARY, MALE (MM), URINARY BLADDER (UB), PROSTATE (PR), SEMINAL VESICLES (SV), EPIDIDYMIDES (EP), ADRENAL, CORTEX (AC), ADRENAL, MEDULLA (MA)

THE FOLLOWING TISSUES WERE UNREMARKABLE AT MICROSCOPIC EXAMINATION:
LIVER (LI), SPLEEN (SP), PANCREAS (PA), TESTIS (TE), ADRENAL (AD)

APPENDIX 5

Individual Animal Pathology Data

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS 

ANIMAL NUMBER: I05231 SEX: MALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 07/16/98 STUDY DAY OF DEATH: 30 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 2795.0 GRAMS
DATE AND TIME OF NECROPSY: 07/16/98 12:43 PROSECTOR: JERALD W. JOHNSON RECORDER: JAMES T. CALLEN
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. JAMES L. CARTER WEIGHER: NOT AVAILABLE

O B S E R V A T I O N S

NECROPSY

GENERAL COMMENT (GC) :
-EYES - DAVIDSONS
-NO MACROSCOPIC LESIONS

THE FOLLOWING ORGANS WERE UNREMARKABLE AT NECROPSY:
LIVER (LI), SPLEEN (SP), PANCREAS (PA), TESTIS (TE), BONE, FEMUR (FE), MARROW, FEMUR (FM), MARROW, STERNUM (SE), BONE, STERNUM (SB), EYE (EY), BRAIN (BR), KIDNEY (KD), LUNG (LU), HEART (HT), TRACHEA (TR), ESOPHAGUS (ES), THYROID (TY), PARATHYROID (PT), THYMUS (TH), LN, MESENTERIC (MS), AORTA (AO), PITUITARY (PI), SALIV GL, MANDIB (SG), MUSCLE, SKELETAL (SM), SPINAL CORD (SC), NERVE, SCIATIC (SN), STOMACH, GL (ST), DUODENUM (DU), GALLBLADDER (GB), JEJUNUM (JE), ILEUM (IL), CECUM (CE), COLON (CO), RECTUM (RE), SKIN (SK), MAMMARY, MALE (MM), URINARY BLADDER (UB), PROSTATE (PR), SEMINAL VESICLES (SV), EPIDIDYMIDES (EP), ADRENAL, CORTEX (AC), ADRENAL, MEDULLA (MA)

THE FOLLOWING TISSUES WERE UNREMARKABLE AT MICROSCOPIC EXAMINATION:
LIVER (LI), SPLEEN (SP), PANCREAS (PA), TESTIS (TE)

HISTOPATHOLOGY

ADRENAL (AD) :
-MINERALIZATION,-MINIMAL, MULTI-FOCAL

APPENDIX 5

Individual Animal Pathology Data

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS 

ANIMAL NUMBER: I05352 SEX: MALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 07/16/98 STUDY DAY OF DEATH: 30 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 1980.0 GRAMS
DATE AND TIME OF NECROPSY: 07/16/98 12:40 PROSECTOR: KEVIN BILLINGS RECORDER: JILL PAUS
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. JAMES L. CARTER WEIGHER: NOT AVAILABLE

O B S E R V A T I O N S

NECROPSY

HISTOPATHOLOGY

TESTIS (TE) :
-IMMATURE,-MODERATE, DIFFUSE

GENERAL COMMENT (GC) :
-EYES - DAVIDSONS
-NO MACROSCOPIC LESIONS

THE FOLLOWING ORGANS WERE UNREMARKABLE AT NECROPSY:
LIVER (LI), SPLEEN (SP), PANCREAS (PA), TESTIS (TE), BONE, FEMUR (FE), MARROW, FEMUR (FM), MARROW, STERNUM (SE), BONE, STERNUM (SB), EYE (EY), BRAIN (BR), KIDNEY (KD), LUNG (LU), HEART (HT), TRACHEA (TR), ESOPHAGUS (ES), THYROID (TY), PARATHYROID (PT), THYMUS (TH), LN, MESENTERIC (MS), AORTA (AO), PITUITARY (PI), SALIV GL, MANDIB (SG), MUSCLE, SKELETAL (SM), SPINAL CORD (SC), NERVE, SCIATIC (SN), STOMACH, GL (ST), DUODENUM (DU), GALLBLADDER (GB), JEJUNUM (JE), ILEUM (IL), CECUM (CE), COLON (CO), RECTUM (RE), SKIN (SK), MAMMARY, MALE (MM), URINARY BLADDER (UB), PROSTATE (PR), SEMINAL VESICLES (SV), EPIDIDYMIDES (EP), ADRENAL, CORTEX (AC), ADRENAL, MEDULLA (MA)

THE FOLLOWING TISSUES WERE UNREMARKABLE AT MICROSCOPIC EXAMINATION:
LIVER (LI), SPLEEN (SP), PANCREAS (PA), ADRENAL (AD)

APPENDIX 5

Individual Animal Pathology Data

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS



ANIMAL NUMBER: I05419 SEX: MALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 07/16/98 STUDY DAY OF DEATH: 30 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 3435.0 GRAMS
DATE AND TIME OF NECROPSY: 07/16/98 11:57 PROSECTOR: JERALD W. JOHNSON RECORDER: JAMES T. CALLEN
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. JAMES L. CARTER WEIGHER: NOT AVAILABLE

O B S E R V A T I O N S

NECROPSY

GENERAL COMMENT (GC) :
-EYES - DAVIDSONS
-NO MACROSCOPIC LESIONS
GENERAL INFORMATION (XX) :
>NOTE:>LEFT HIND LIMB, NEAR STIFLE JOINT: ANIMAL HAS SIX SUTURES; POSSIBLY CONSISTENT WITH CLINICAL OBSERVATIONS OF SCABS (HIND LEFT)

THE FOLLOWING ORGANS WERE UNREMARKABLE AT NECROPSY:
LIVER (LI), SPLEEN (SP), PANCREAS (PA), TESTIS (TE), BONE, FEMUR (FE), MARROW, FEMUR (FM), MARROW, STERNUM (SE), BONE, STERNUM (SB), EYE (EY), BRAIN (BR), KIDNEY (KD), LUNG (LU), HEART (HT), TRACHEA (TR), ESOPHAGUS (ES), THYROID (TY), PARATHYROID (PT), THYMUS (TH), LN, MESENTERIC (MS), AORTA (AO), PITUITARY (PI), SALIV GL, MANDIB (SG), MUSCLE, SKELETAL (SM), SPINAL CORD (SC), NERVE, SCIATIC (SN), STOMACH, GL (ST), DUODENUM (DU), GALLBLADDER (GB), JEJUNUM (JE), ILEUM (IL), CECUM (CE), COLON (CO), RECTUM (RE), SKIN (SK), MAMMARY, MALE (MM), URINARY BLADDER (UB), PROSTATE (PR), SEMINAL VESICLES (SV), EPIDIDYMIDES (EP), ADRENAL, CORTEX (AC), ADRENAL, MEDULLA (MA)

THE FOLLOWING TISSUES WERE UNREMARKABLE AT MICROSCOPIC EXAMINATION:
LIVER (LI), SPLEEN (SP), PANCREAS (PA), TESTIS (TE), ADRENAL (AD)

HISTOPATHOLOGY

APPENDIX 5

Individual Animal Pathology Data

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS



ANIMAL NUMBER: I05235 SEX: MALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 07/16/98 STUDY DAY OF DEATH: 30 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 2635.0 GRAMS
DATE AND TIME OF NECROPSY: 07/16/98 13:09 PROSECTOR: JERALD W. JOHNSON RECORDER: JAMES T. CALLEN
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. JAMES L. CARTER WEIGHER: NOT AVAILABLE

O B S E R V A T I O N S

| NECROPSY | HISTOPATHOLOGY |
|---|---|
| STOMACH, GL (ST) :<br>-RED FOCUS(I)/AREA(S); MUCOSA: MULTIPLE RED AREAS, UP TO 0.6 X 0.4 CM; COLLECTED ON ROUTINE SECTION | STOMACH, GL (ST) :<br>>NOT REQUIRED TO BE EXAMINED FOR ANIMAL |

GENERAL COMMENT (GC) :
-EYES - DAVIDSONS

THE FOLLOWING ORGANS WERE UNREMARKABLE AT NECROPSY:
LIVER (LI), SPLEEN (SP), PANCREAS (PA), TESTIS (TE), BONE, FEMUR (FE), MARROW, FEMUR (FM), MARROW, STERNUM (SE), BONE, STERNUM (SB), EYE (EY), BRAIN (BR), KIDNEY (KD), LUNG (LU), HEART (HT), TRACHEA (TR), ESOPHAGUS (ES), THYROID (TY), PARATHYROID (PT), THYMUS (TH), LN, MESENTERIC (MS), AORTA (AO), PITUITARY (PI), SALIV GL, MANDIB (SG), MUSCLE, SKELETAL (SM), SPINAL CORD (SC), NERVE, SCIATIC (SN), DUODENUM (DU), GALLBLADDER (GB), JEJUNUM (JE), ILEUM (IL), CECUM (CE), COLON (CO), RECTUM (RE), SKIN (SK), MAMMARY, MALE (MM), URINARY BLADDER (UB), PROSTATE (PR), SEMINAL VESICLES (SV), EPIDIDYMIDES (EP), ADRENAL, CORTEX (AC), ADRENAL, MEDULLA (MA)

THE FOLLOWING TISSUES WERE UNREMARKABLE AT MICROSCOPIC EXAMINATION:
LIVER (LI), SPLEEN (SP), PANCREAS (PA), TESTIS (TE), ADRENAL (AD)

APPENDIX 5

Individual Animal Pathology Data

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS 

ANIMAL NUMBER: I05351 SEX: MALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 07/16/98 STUDY DAY OF DEATH: 30 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 2185.0 GRAMS
DATE AND TIME OF NECROPSY: 07/16/98 11:56 PROSECTOR: KEVIN BILLINGS RECORDER: JILL PAUS
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. JAMES L. CARTER WEIGHER: NOT AVAILABLE

O B S E R V A T I O N S

NECROPSY

HISTOPATHOLOGY

TESTIS (TE) :
-IMMATURE,-MODERATE, DIFFUSE

GENERAL COMMENT (GC) :
-EYES - DAVIDSONS
-NO MACROSCOPIC LESIONS

THE FOLLOWING ORGANS WERE UNREMARKABLE AT NECROPSY:
LIVER (LI), SPLEEN (SP), PANCREAS (PA), TESTIS (TE), BONE, FEMUR (FE), MARROW, FEMUR (FM), MARROW, STERNUM (SE), BONE, STERNUM (SB), EYE (EY), BRAIN (BR), KIDNEY (KD), LUNG (LU), HEART (HT), TRACHEA (TR), ESOPHAGUS (ES), THYROID (TY), PARATHYROID (PT), THYMUS (TH), LN, MESENTERIC (MS), AORTA (AO), PITUITARY (PI), SALIV GL, MANDIB (SG), MUSCLE, SKELETAL (SM), SPINAL CORD (SC), NERVE, SCIATIC (SN), STOMACH, GL (ST), DUODENUM (DU), GALLBLADDER (GB), JEJUNUM (JE), ILEUM (IL), CECUM (CE), COLON (CO), RECTUM (RE), SKIN (SK), MAMMARY, MALE (MM), URINARY BLADDER (UB), PROSTATE (PR), SEMINAL VESICLES (SV), EPIDIDYMIDES (EP), ADRENAL, CORTEX (AC), ADRENAL, MEDULLA (MA)

THE FOLLOWING TISSUES WERE UNREMARKABLE AT MICROSCOPIC EXAMINATION:
LIVER (LI), SPLEEN (SP), PANCREAS (PA), ADRENAL (AD)

APPENDIX 5

Individual Animal Pathology Data



ANIMAL NUMBER: I05416 SEX: MALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 07/16/98 STUDY DAY OF DEATH: 30 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 3120.0 GRAMS
DATE AND TIME OF NECROPSY: 07/16/98 13:05 PROSECTOR: KEVIN BILLINGS RECORDER: JILL PAUS
POST-FIX WEIGHER: NOT AVAILABLE PATHOLOGIST: DR. JAMES L. CARTER WEIGHER: NOT AVAILABLE

O B S E R V A T I O N S

| NECROPSY | HISTOPATHOLOGY |
|---|---|
| LUNG (LU) : | LUNG (LU) : |
| -ADHESION(S); ALL LOBES TO EACH OTHER, PLEURA AND CAUDAL LOBES TO DIAPHRAGM: MULTIPLE TAN GRAY FIBROUS ADHESIONS; COLLECTED INTACT | >NOT REQUIRED TO BE EXAMINED FOR ANIMAL |
| STOMACH, GL (ST) : | STOMACH, GL (ST) : |
| -RED FOCUS(I)/AREA(S); MUCOSA: SINGLE AREA, 0.5 X 0.2 CM | >NOT REQUIRED TO BE EXAMINED FOR ANIMAL |
| GENERAL COMMENT (GC) : | |
| -EYES - DAVIDSONS | |

THE FOLLOWING ORGANS WERE UNREMARKABLE AT NECROPSY:
LIVER (LI), SPLEEN (SP), PANCREAS (PA), TESTIS (TE), BONE, FEMUR (FE), MARROW, FEMUR (FM), MARROW, STERNUM (SE), BONE, STERNUM (SB), EYE (EY), BRAIN (BR), KIDNEY (KD), HEART (HT), TRACHEA (TR), ESOPHAGUS (ES), THYROID (TY), PARATHYROID (PT), THYMUS (TH), LN, MESENTERIC (MS), AORTA (AO), PITUITARY (PI), SALIV GL, MANDIB (SG), MUSCLE, SKELETAL (SM), SPINAL CORD (SC), NERVE, SCIATIC (SN), DUODENUM (DU), GALLBLADDER (GB), JEJUNUM (JE), ILEUM (IL), CECUM (CE), COLON (CO), RECTUM (RE), SKIN (SK), MAMMARY, MALE (MM), URINARY BLADDER (UB), PROSTATE (PR), SEMINAL VESICLES (SV), EPIDIDYMIDES (EP), ADRENAL, CORTEX (AC), ADRENAL, MEDULLA (MA)

THE FOLLOWING TISSUES WERE UNREMARKABLE AT MICROSCOPIC EXAMINATION:
LIVER (LI), SPLEEN (SP), PANCREAS (PA), TESTIS (TE), ADRENAL (AD)

# APPENDIX 6

Quality Assurance Statement
Summary and Individual Hormone Analyses Data

Note: The data included in this appendix were supplied by Ani Lytics Inc. and have been reviewed by the Quality Assurance Unit of Ani Lytics Inc.

ANALYTICS INCORPORATED
200 Girard Street, Suite 200, Gaithersburg, MD 20877
301-921-0168 800-237-2815

The reports listed below was reviewed for compliance with the FDA Good Laboratory Practices and with the EPA Good Laboratory Practices. The final report and all associated raw data were reviewed for accuracy and consistency and the findings were reported to management.

The methods used were the methods described and the report accurately reflects the data. Therefore, these studies were done in compliance with the FDA and EPA Good Laboratory Practices.

Franklin B. Newman
QA Auditor

SPONSOR: COVANCE LABS STUDY: 6329-230

| REPORT TYPE: | AUDIT DATE: | REPORT TO MGMT: | AUDIT #: |
|---|---|---|---|
| E1, E2, E3, T3, T4 TSH, FT3, FT4 | 9-27-98 | 9-28-98 | 98-713 |

MANAGEMENT 9/28/98

Appendix 6

Summary and Individual Hormone Analyses Data

Day -8

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE (APFO) IN CYNOMOLGUS MONKEYS

| ANIMAL NUMBER | ESTRONE pg/mL | ESTRADIOL pg/mL | ESTRIOL ng/mL | THYROID STIMULATING µIU/mL | TOTAL TRIIODOTHYRONINE ng/dL | TOTAL THYROXIN µg/dL | FREE TRIIODOTHYRONINE pg/mL | FREE THYROXIN ng/dL |
|---|---|---|---|---|---|---|---|---|
| Group: 1 | Dose Level: 0 | | Dosage Unit: mg/kg/day | | | | | |
| I05353 | 163.53 | 12.39 | 0.00 | 1.40 | 205.95 | 5.11 | 5.08 | 1.08 |
| I05417 | 236.08 | 39.04 | 0.00 | 1.61 | 159.89 | 4.28 | 4.77 | 1.14 |
| MEAN | 199.81 | 25.72 | 0.00 | 1.51 | 182.92 | 4.70 | 4.93 | 1.11 |
| S.D. | 51.301 | 18.844 | 0.000 | 0.148 | 32.569 | 0.587 | 0.219 | 0.042 |
| N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Group: 2 | Dose Level: 2 | | Dosage Unit: mg/kg/day | | | | | |
| I05231 | 195.55 | 23.04 | 0.00 | 1.17 | 187.06 | 3.41 | 7.61 | 1.21 |
| I05352 | 187.55 | 30.44 | 0.00 | 1.18 | 174.59 | 5.71 | 2.96 | 1.17 |
| I05419 | 191.95 | 30.63 | 0.00 | 1.64 | 166.70 | 3.12 | 5.58 | 0.97 |
| MEAN | 191.68 | 28.04 | 0.00 | 1.33 | 176.12 | 4.08 | 5.38 | 1.12 |
| S.D. | 4.007 | 4.328 | 0.000 | 0.269 | 10.265 | 1.419 | 2.331 | 0.129 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Group: 3 | Dose Level: 20 | | Dosage Unit: mg/kg/day | | | | | |
| I05235 | 183.99 | 9.10 | 0.00 | 1.50 | 145.43 | 3.73 | 5.92 | 1.41 |
| I05351 | 186.13 | 14.61 | 0.00 | 0.74 | 176.53 | 5.99 | 3.75 | 2.06 |
| I05416 | 213.49 | 12.69 | 0.00 | 1.10 | 172.10 | 3.69 | 3.44 | 0.67 |
| MEAN | 194.54 | 12.13 | 0.00 | 1.11 | 164.69 | 4.47 | 4.37 | 1.38 |
| S.D. | 16.449 | 2.797 | 0.000 | 0.380 | 16.823 | 1.317 | 1.351 | 0.695 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

Appendix 6

Summary and Individual Hormone Analyses Data

Day 30

4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTANOATE
(APFO) IN CYNOMOLGUS MONKEYS

| ANIMAL NUMBER | ESTRONE pg/mL | ESTRADIOL pg/mL | ESTRIOL ng/mL | THYROID STIMULATING µIU/mL | TOTAL TRIIODOTHYRONINE ng/dL | TOTAL THYROXIN µg/dL | FREE TRIIODOTHYRONINE pg/mL | FREE THYROXIN ng/dL |
|---|---|---|---|---|---|---|---|---|
| Group: 1 | Dose Level: 0 | | Dosage Unit: mg/kg/day | | | | | |
| I05353 | 182.68 | 13.13 | 0.00 | 1.68 | 188.20 | 5.50 | 4.93 | 1.66 |
| I05417 | 48.21 | 34.20 | 0.00 | 1.55 | 137.22 | 4.31 | 4.72 | 1.43 |
| MEAN | 115.45 | 23.67 | 0.00 | 1.62 | 162.71 | 4.91 | 4.83 | 1.55 |
| S.D. | 95.085 | 14.899 | 0.000 | 0.092 | 36.048 | 0.841 | 0.148 | 0.163 |
| N | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Group: 2 | Dose Level: 2 | | Dosage Unit: mg/kg/day | | | | | |
| I05231 | 29.31 | 25.88 | 0.00 | 1.28 | 173.12 | 3.84 | 5.91 | 0.99 |
| I05352 | 30.04 | 16.03 | 0.00 | 1.17 | 150.56 | 3.60 | 4.98 | 1.14 |
| I05419 | 21.10 | 17.89 | 0.00 | 0.98 | 193.34 | 5.44 | 6.82 | 1.69 |
| MEAN | 26.82 | 19.93 | 0.00 | 1.14 | 172.34 | 4.29 | 5.90 | 1.27 |
| S.D. | 4.964 | 5.233 | 0.000 | 0.152 | 21.401 | 1.000 | 0.920 | 0.369 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Group: 3 | Dose Level: 20 | | Dosage Unit: mg/kg/day | | | | | |
| I05235 | 14.55 | 10.34 | 0.00 | 1.71 | 138.52 | 4.71 | 4.55 | 1.12 |
| I05351 | 28.80 | 11.50 | 0.00 | 1.06 | 143.31 | 5.00 | 4.67 | 1.19 |
| I05416 | 26.67 | 11.45 | 0.00 | 1.06 | 129.89 | 2.62 | 3.78 | 0.68 |
| MEAN | 23.34 | 11.10 | 0.00 | 1.28 | 137.24 | 4.11 | 4.33 | 1.00 |
| S.D. | 7.687 | 0.656 | 0.000 | 0.375 | 6.801 | 1.298 | 0.483 | 0.276 |
| N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

# APPENDIX 7

Cell Proliferation Report

Note: This appendix of the report contains information supplied by Pathology Associates, A Charles River Company and has been reviewed by the Quality Assurance Unit of Pathology Associates, A Charles River Company.

PATHOLOGY ASSOCIATES
A CHARLES RIVER COMPANY

**FINAL**
**CELL PROLIFERATION REPORT**

# 4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTONOATE (APFO) IN CYNOMOLGUS MONKEYS

**COVANCE STUDY NUMBER 6329-230**

**PREPARED FOR:**

**3M**
**TOXICOLOGY SERVICES**
**BUILDING 220-2E-02, 3M CENTER**
**ST. PAUL, MN 55144-1000**

**PREPARED BY:**

**PATHOLOGY ASSOCIATES – A CHARLES RIVER COMPANY**
**15 WORMAN'S MILL COURT, SUITE I**
**FREDERICK, MD 21701**

15 Worman's Mill Ct, Suite I* Frederick, MD 21071* (301) 663-1644 *Fax (301) 336-8994

# CELL PROLIFERATION REPORT

# 4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTONOATE (APFO) IN CYNOMOLGUS MONKEYS COVANCE STUDY NUMBER 6329-230

## PURPOSE

The purpose of this study was to provide data for determining an estimated maximum-tolerated dose and lower dose levels to be used in a chronic toxicity study. In addition, the effect on critical enzyme levels, hormones, and other selected biochemical parameters, such as cell proliferation, were determined.

This report, submitted by Pathology Associates –A Charles River Company (PAI) formally Pathology Associates International to the study Sponsor, APME Ad-Hoc APFO Toxicology Working Group, represents the cell proliferation findings and interpretation for Covance Study Number 6329-230 entitled "4-Week Capsule Toxicity Study with Ammonium Perfluorooctonoate (APFO) in Cynomolgus Monkeys". All aspects of the tasks associated with PAI's portion of this study were conducted in compliance with the Environmental Protection Agency Good Laboratory Practice (GLP) Regulations as set forth in title 40 of the US Code of Federal Regulations, Part 792, issued November 29, 1983 (effective December 29, 1983), and any applicable amendments.

## MATERIALS AND METHODS

### Tissue Collection for Cell Proliferation

Two animals in group 1, and three animals each in groups 2 and 3 were sacrificed at the end of week 4. A section of the left lateral lobe of the liver, left and right testes, and pancreas from each animal was fixed in zinc formalin, processed and embedded to paraffin block by Covance per protocol specifications. Tissue blocks were shipped to PAI for sectioning and staining. From each block, a slide was prepared for H&E evaluation and immunohistochemical detection of proliferating cell nuclear antigen (PCNA), a marker of cell proliferation.

### Immunohistochemistry for Cell Proliferation

Sections of paraffin-embedded tissues were cut at ≅ 5 μm and placed on positively charged slides (Sueprfrost Plus, fisher Scientific, Pittsburgh, PA) to ensure adhesion during processing for PCNA. Standard immunohistochemical methods were used to stain tissues for PCNA [PAI's Standard Operating Procedure for Immunohistochemical Staining (SOP #707]. Briefly, tissue sections were incubated with a monoclonal antibody to PCNA (DAKO, lot #016; PAI No. A1899) and reagents required for the avidin-biotin peroxidase (ABC Elite Kit; Vector, lot #PK-6100; PAI No. K 327) method for the detection of the antigen-antibody complex. PCNA expression in cells in all phases of the cell cycle ($G_1$, S, $G_2$ and M) was localized by the

chromagen 3,3'-diaminobenzidine (DAB; Sigma Chemical Co., lot #18H8201). Tissue sections were counterstained with hematoxylin.

**Cell Proliferation Measurements**

The percentage of proliferating cells (proliferating index, PI) was determined by scoring at least 3000 hepatocytes in 10 fields of liver, at least 500 Leydig cells of the testis (except for animal #IO5417 in which 250 cells were scored), and at least 2000 acinar cells of the pancreas per animal. Although both testes presented on the slide were perused, only one testis was scored for proliferation; no attempt was made to distinguish left from right testis. A negative control slide was included in the staining run and consisted of study tissue from animal #IO516 that was not incubated with the primary antibody.

For cell proliferation evaluations, slides were first perused at low magnification (100X) to judge quality of staining, processing and sectioning, potential patterns of cellular proliferation, and histomorphologic changes. Cell proliferation was then quantified at higher magnification (200X for liver, and 400X for testes and pancreas) as described above. Histomorphology was further assessed by evaluating the H&E slide prepared from the same tissue block for each animal evaluated for cell proliferation.

**Statistical Analysis**

Due to the small sample size, statistical analysis was not performed.

**RESULTS**

**Cell Proliferation**

Individual animal and group mean cell proliferation data are presented in Section II (Table 1) and graphically in Section III (Figures 1-3).

**Histopathology**

Sections from the same tissue blocks used for preparation of PCNA-stained slides were stained with hematoxylin and eosin (H&E) for histopathologic evaluation to facilitate the interpretation of the immunostained slides. Individual animal findings are presented in Appendix I.

The results showed that no significant changes were observed in either the liver, pancreas, or testicular tissues from male monkeys, which would alter the interpretation of the PCNA staining in this study.

**SUMMARY**

Although a glance at the cell proliferation data suggest a "dose-response" in terms of cell proliferation in the liver and testes, the sample size is extremely small. Furthermore, in the liver, only one PCNA-labeled cell was detected in those animals with a proliferating index greater than

zero percent. In the testes, only one control animal was able to be evaluated due to immaturity of the only other animal in that group, and the proliferating indices in the two treatment groups overlapped. Therefore, the data are considered to be inconclusive and do not support a cell proliferative response to the test material.

# II. TABLE

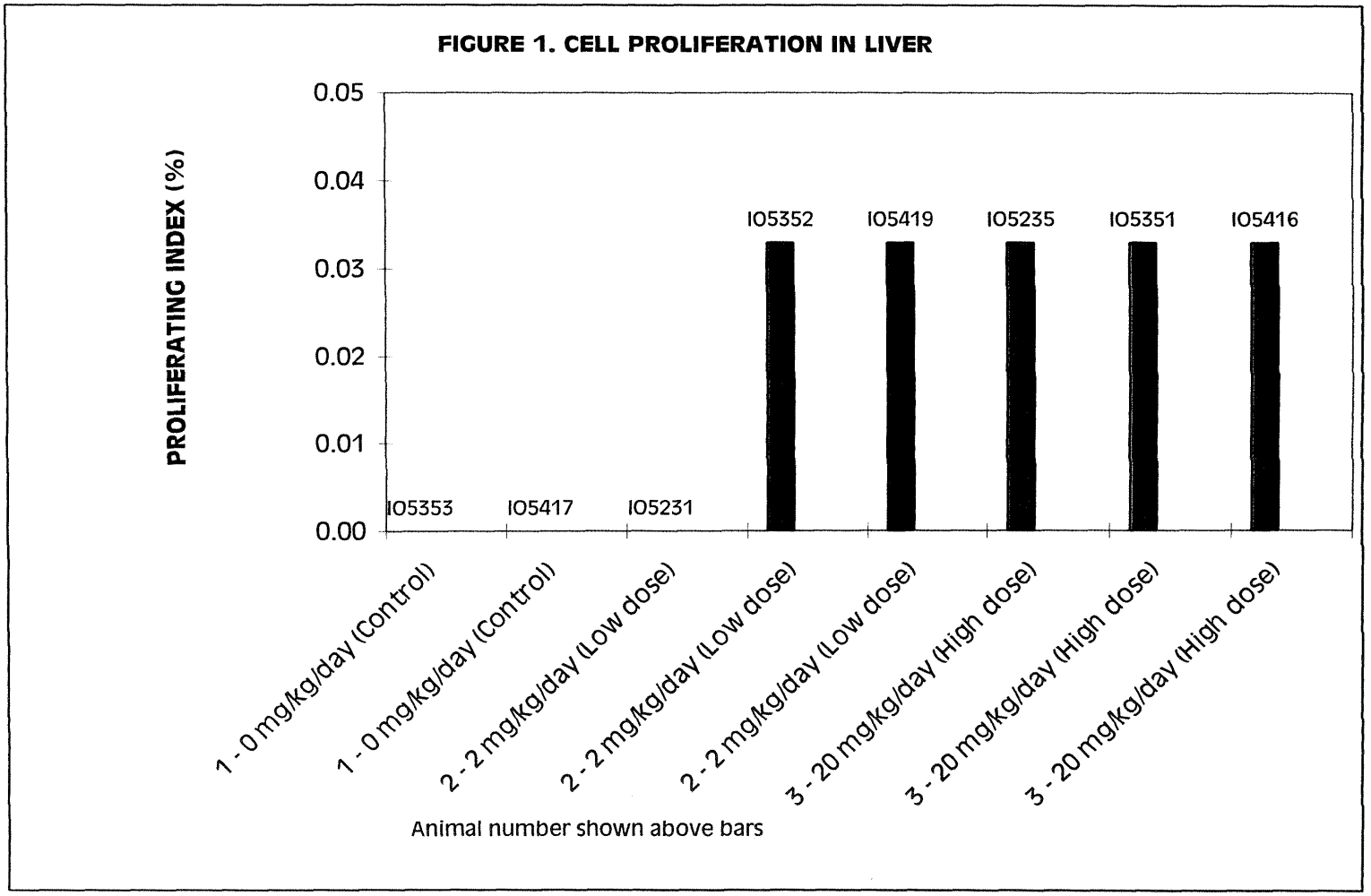
COVANCE STUDY NO. 6329-230
TABLE 1. CELL PROLIFERATION IN MONKEYS

| Dose Group | Animal Number | Proliferating Index Liver | Proliferating Index Testes | Proliferating Index Pancreas |
|---|---|---|---|---|
| 1 - 0 mg/kg/day (Control) | IO5353 | 0.000% | ND | 50.5% |
| 1 - 0 mg/kg/day (Control) | IO5417 | 0.000% | 9.6% | 51.5% |
| | **Mean** | **0.000%** | **9.6%** | **51.0%** |
| | **SEM** | **0.000%** | **Unable to calculate** | **0.5%** |
| 2 - 2 mg/kg/day (Low dose) | IO5231 | 0.000% | 19.6% | 49.7% |
| 2 - 2 mg/kg/day (Low dose) | IO5352 | 0.033% | ND | 49.6% |
| 2 - 2 mg/kg/day (Low dose) | IO5419 | 0.033% | 11.6% | 42.5% |
| | **Mean** | **0.022%** | **15.6%** | **47.3%** |
| | **SEM** | **0.011%** | **4.0%** | **2.4%** |
| 3 - 20 mg/kg/day (High dose) | IO5235 | 0.030% | 20.4% | 43.9% |
| 3 - 20 mg/kg/day (High dose) | IO5351 | 0.033% | ND | 35.6% |
| 3 - 20 mg/kg/day (High dose) | IO5416 | 0.033% | 15.6% | 49.7% |
| | **Mean** | **0.032%** | **18.0%** | **43.1%** |
| | **SEM** | **0.001%** | **2.4%** | **4.1%** |
| | | | | |
| ND, not determined due to immaturity | | | | |
| SEM, standard error of the mean | | | | |

# III. FIGURES

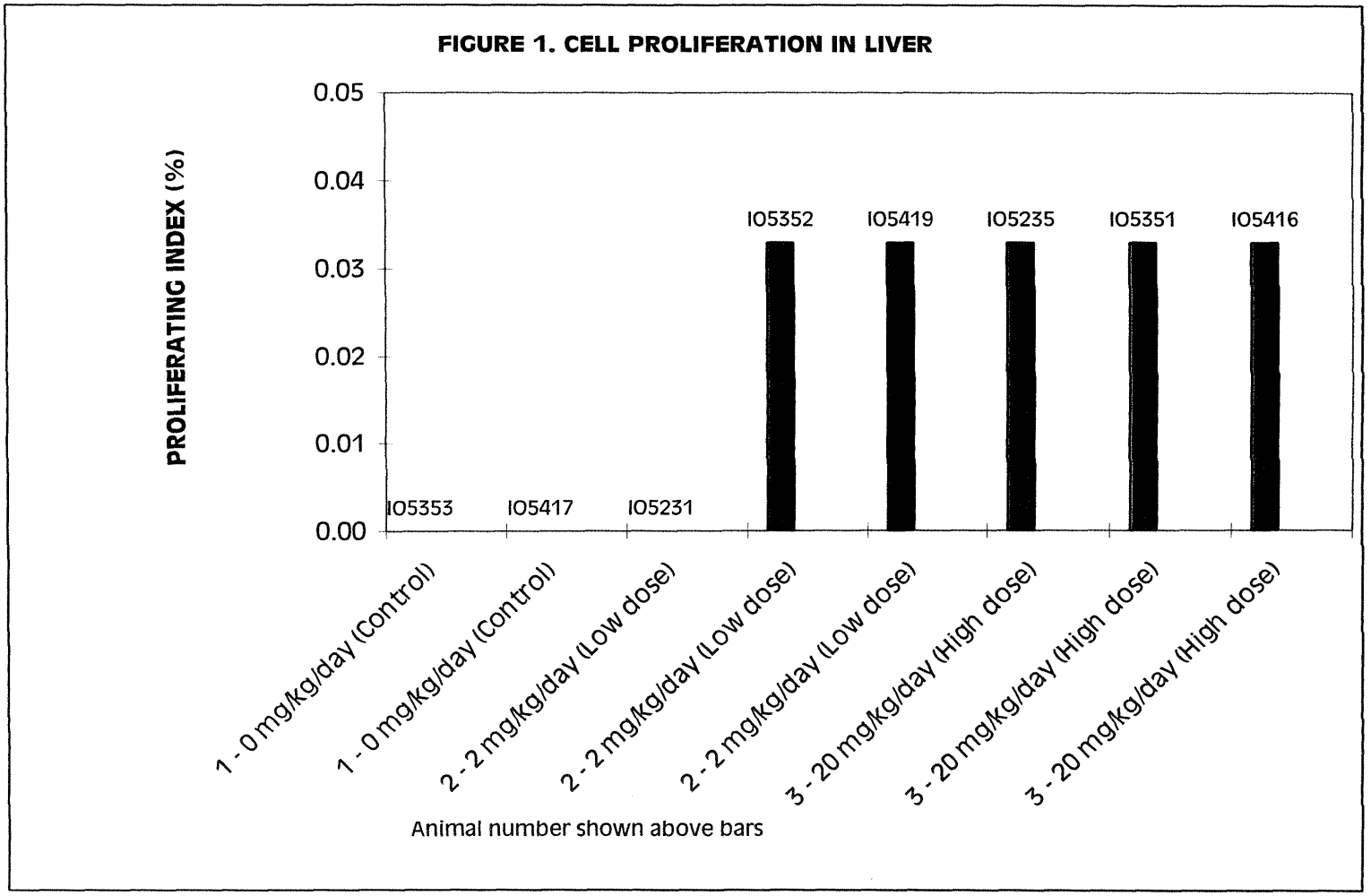
COVANCE STUDY NO. 6329-230

**FIGURE 1. CELL PROLIFERATION IN LIVER**



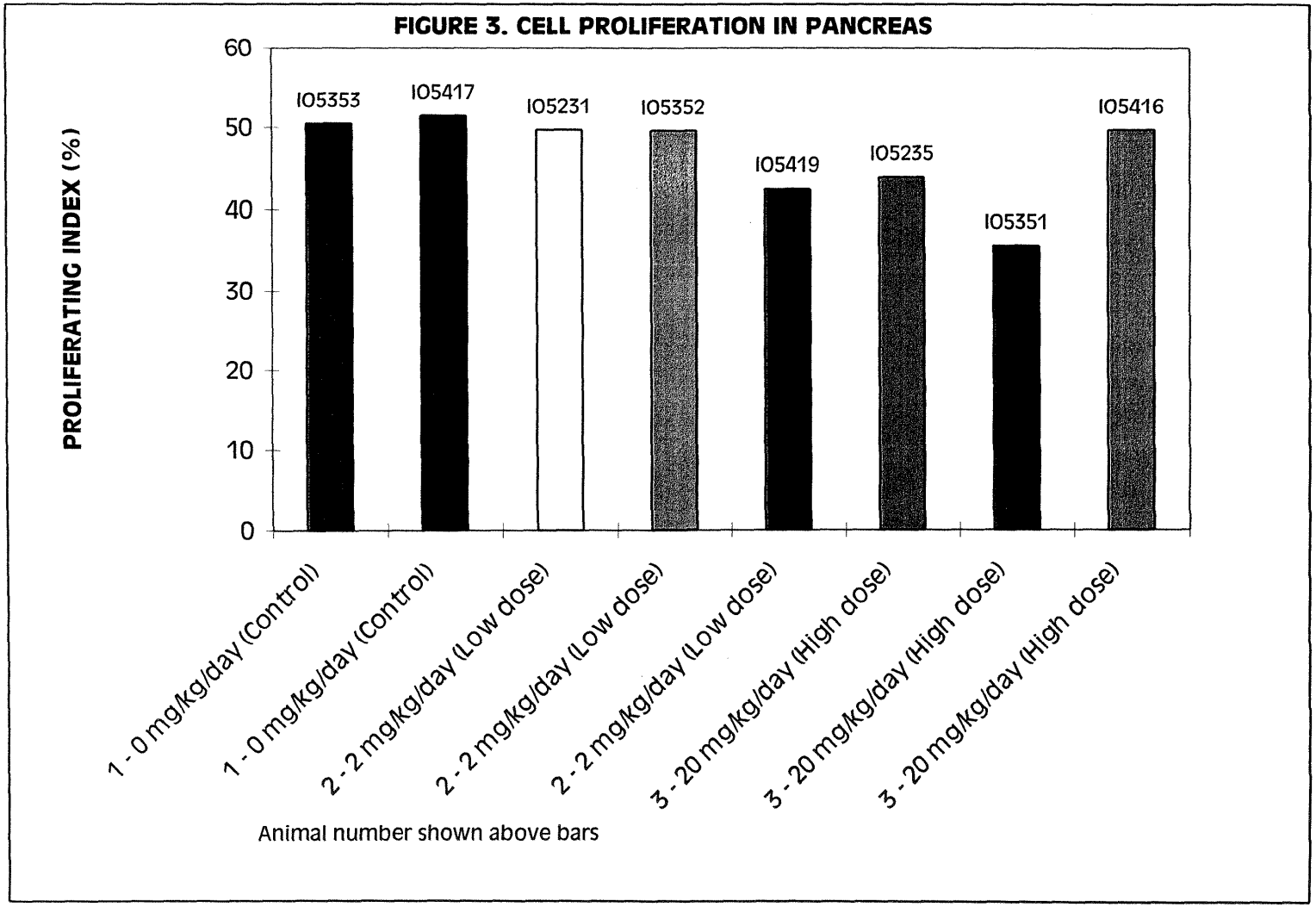
COVANCE STUDY NO. 6329-230

**FIGURE 1. CELL PROLIFERATION IN LIVER**



COVANCE STUDY NO. 6329-230

**FIGURE 3. CELL PROLIFERATION IN PANCREAS**



## IV. SIGNATURE PAGE

**4-WEEK CAPSULE TOXICITY STUDY WITH AMMONIUM PERFLUOROOCTONOATE (APFO) IN CYNOMOLGUS MONKEYS COVANCE STUDY NUMBER 6329-230**

Submitted by:

PAI Project Manager:

Sandra R. Eldridge, Ph.D. — Date: 8-2-01

PAI Project Pathologist:

Carolyn Moyer, D.V.M., Diplomate, A.C.V.P. — Date: 7/27/01

# V. QUALITY ASSURANCE STATEMENT

PATHOLOGY ASSOCIATES
A CHARLES RIVER COMPANY

## Cell Proliferation Report

## 4-Week Capsule Toxicity Study with Ammonium Perfluorooctonoate (APFO) in Cynomolgus Monkeys

Covance Study Number 6329-230

## QUALITY ASSURANCE STATEMENT

This cell proliferation project has been inspected and audited by the PAI Quality Assurance Unit (QAU) as required by the Good Laboratory Practice (GLP) regulations promulgated by the U.S. Environmental Protection Agency (EPA-TSCA). The cell proliferation report is an accurate reflection of the recorded data. The following table is a record of the inspections/audits performed and reported by the QAU.

| Date of Inspection | Phase Inspected | Date Findings Reported to PAI Management/Project Manager |
|---|---|---|
| 08/12/98 | Microtomy | 08/12/98 |
| 10/05/98 | Study Data and Supporting Documentation | 10/06/98 |
| 10/05/98 | Draft Cell Proliferation Report | 10/06/98 |
| 07/25/01 | Final Cell Proliferation Report | 07/25/01 |

Sharon E. Abel
Quality Assurance Officer

July 25, 2001
Date

# APPENDIX I

Covance Study Number 6329-230

## Individual Animal Histopathologic Findings

### 4-Week Capsule Toxicity Study with Ammonium Perfluorooctonate (APFO) in Cynomolgus Monkeys

### Tissues Examined: Liver, Testes, Pancreas

| Animal Number | Sex | Dose Group | Histologic Findings (Liver, Testes, Pancreas) |
|---|---|---|---|
| I05353 | M | 1-0 mg/kg/day (Control) | No Significant Findings |
| I05417 | M | 1-0 mg/kg/day (Control) | Some tissue distortion in testis, changes do not preclude evaluations |
| I05231 | M | 2-2 mg/kg/day (Low Dose) | No Significant Findings |
| I05352 | M | 2-2 mg/kg/day (Low Dose) | No Significant Findings |
| I05419 | M | 2-2 mg/kg/day (Low Dose) | No Significant Findings |
| I05235 | M | 3-20 mg/kg/day (High Dose) | No Significant Findings |
| I05351 | M | 3-20 mg/kg/day (High Dose) | No Significant Findings |
| I05416 | M | 3-20 mg/kg/day (High Dose) | Some tissue distortion in testis, changes do not preclude evaluations |