FINAL REPORT

Volume 1 of 6
(Text and Tables 1-75)

STUDY TITLE

A COMBINED 28-DAY REPEATED DOSE ORAL TOXICITY STUDY WITH THE REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST OF PERFLUOROHEXANOIC ACID AND 1H, 1H, 2H, 2H-TRIDECAFLUORO-1-OCTANOL IN RATS, WITH RECOVERY

STUDY NUMBER

WIL-534001

DATA REQUIREMENT

OECD Guideline 422

STUDY DIRECTOR

Jeannie B. Kirkpatrick, MS

STUDY INITIATION DATE

5 January 2005

STUDY COMPLETION DATE

2 September 2005

PERFORMING LABORATORY

WIL Research Laboratories, LLC
1407 George Road
Ashland, OH 44805-9281

SPONSOR

AGC Chemical
Asahi Glass Company, Ltd.
10 Goikaigan, Ichihara-shi
Chiba 290-8566, JAPAN

COMPLIANCE STATEMENT

This study, designated WIL-534001, was conducted in compliance with the United States Environmental Protection Agency (EPA) Good Laboratory Practice Standards (40 CFR Part 160), 16 October 1989; the United States Environmental Protection Agency (EPA) Good Laboratory Practice Standards (40 CFR Part 792), 18 September 1989; the Organisation for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice [C(97) 186/Final], 26 November 1997; the standard operating procedures of WIL Research Laboratories, LLC and the protocol as approved by the sponsor. Certificates of Analysis were provided by the sponsor (presented in Appendix C); the characterization analyses were not conducted according to Good Laboratory Practices (GLP).

The study was conducted in accordance with the OECD Guideline for Testing of Chemicals, Guideline 422, Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test, 22 March 1996. Functional Observational Battery and motor activity observations were omitted from the study because the OECD Guideline 422 states that these observations may be omitted when the study is conducted as a preliminary study to a subchronic study. A 90-day study of PFHxA which includes Functional Observations has been initiated.

_____ _____

Jeannie B. Kirkpatrick, MS
Staff Toxicologist
Study Director

Date

TABLE OF CONTENTS

VOLUME 1 Page

VOLUME 1 (continued) Page

VOLUME 1 (continued)

Page

VOLUME 1 (continued)

Page

VOLUME 1 (continued)

Page

INDEX OF TABLES

Note: Some table titles have been shortened due to software spacing constraints.

VOLUME 1 (continued) Page

SUMMARY DATA

VOLUME 1 (continued) Page

VOLUME 1 (continued) Page

VOLUME 1 (continued) Page

VOLUME 1 (continued) Page

VOLUME 2

INDIVIDUAL DATA

VOLUME 2 (continued) Page

VOLUME 3

Page

VOLUME 4

VOLUME 4 (continued) Page

VOLUME 4 (continued) Page

INDEX OF APPENDICES

VOLUME 5

VOLUME 6

1. SUMMARY

1.1. OBJECTIVE

This study was designed to evaluate the potential toxic effects of perfluorohexanoic acid (hereafter referred to as PFHxA) or 1H, 1H, 2H, 2H-tridecafluoro-1-octanol (hereafter referred to as C6-2 alcohol) when administered to rats for 28 days and to evaluate the potential of PFHxA or C6-2 alcohol to affect male and female reproductive performance, such as gonadal function, mating behavior, conception, parturition and early postnatal development.

1.2. STUDY DESIGN

The test articles, PFHxA, in the vehicle, deionized water, or C6-2 alcohol, in the vehicle, corn oil, were administered orally by gavage once daily to 3 groups each (Groups 2-4 received PFHxA and Groups 6-8 received C6-2 alcohol) of Crl:CD®(SD)[1] rats. Dosage levels for PFHxA were 50, 150 and 450 mg/kg/day administered at a dosage volume of 10 mL/kg, and dosage levels for C6-2 alcohol were 25, 75 and 225 mg/kg/day administered at a dosage volume of 5 mL/kg. Due to excessive toxicity noted at 450 mg/kg/day PFHxA, the dosage level was lowered to 300 mg/kg/day on study day 4. Concurrent control groups (Groups 1 and 5) received the appropriate vehicle on a comparable regimen. Groups 1, 4, 5 and 8 each consisted of 15 animals/sex/group and Groups 2, 3, 6 and 7 each consisted of 10 animals/sex/group. Males received 14 daily doses prior to mating and were dosed throughout the mating period through the day prior to euthanasia for a total of 32-34 doses; males assigned to the recovery phase received a total of 35 doses. Females received 14 daily doses prior to pairing and were dosed through lactation day 3 for a total of 39-44 doses; females with no evidence of mating/that failed to deliver were dosed through the day prior to euthanasia (post-mating or post-cohabitation day 25) for a total of 39-52 doses. Females assigned to the recovery period (not used for mating) were dosed through the day of euthanasia of the first lactation females for a total of 40 doses. At the end of the treatment period, 5 rats/sex, if

[1] = Prior to January 1, 2005, this strain of rat was designated the Crl:CD®(SD)IGS BR rat.

possible, from Groups 1, 4, 5 and 8 were assigned to a 14-day (nondosing) recovery period.

All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights and food consumption were recorded at appropriate intervals. All reproductive phase F_0 females were allowed to deliver and rear their pups until lactation day 4. F_1 clinical observations and body weights were recorded on postnatal days (PND) 1 and 4. Pups were necropsied on PND 4. Clinical pathology evaluations (hematology and serum chemistry) were performed on 5 animals/sex/group during study week 4 (reproductive phase males) and on lactation day 4 (reproductive phase females), and on all remaining animals during study week 7 (recovery phase males and females). With the exception of 4 males in the 450/300 mg/kg/day group assigned to the recovery phase after assignment to the reproductive phase, males assigned to the reproductive phase were euthanized following a minimum of 28 doses, and females assigned to the reproductive phase were euthanized on lactation day 4. Animals assigned to the recovery phase were euthanized following completion of the 14-day recovery period. Complete necropsies were conducted on all animals, and selected organs were weighed. Selected tissues were examined microscopically from all animals in the control and high-dose groups, and all animals that were found dead or euthanized in extremis. In addition, target organs were examined microscopically in the PFHxA and C6-2 alcohol low and mid-dose groups and the recovery animals as follows. In the PFHxA groups, the liver (males and females, no recovery evaluation), kidneys (males and females), mandibular and mesenteric lymph nodes (males and females), thymus and spleen (males and females), glandular and non-glandular stomach (males and females), pancreas (males and females), sternal bone marrow and adrenal cortex (females only) were examined. In the C6-2 alcohol groups, the kidneys and liver (males and females), mandibular and mesenteric lymph nodes (males and females), pancreas, thymus and spleen (males and females), adrenal cortex and sternal bone marrow (females only), mammary gland, uterus and vagina (females only) were examined.

For toxicokinetic evaluation, blood samples were collected from an additional 3 rats/sex/group dosed at 50, 150 and 300 mg/kg/day PFHxA at 0 (pre-dose), 1, 2, 4, 8 and 24 hours after dose administration on study days 0 and 25 (26 total doses). Urine samples were also collected from all surviving toxicokinetic phase animals from 0-6, 6-12 and 12-24 hours following the last dose administration on study day 26 (27 total doses). All toxicokinetic phase animals were euthanized and discarded following the last urine collection on study day 27.

1.3. RESULTS

1.3.1. TEST ARTICLE PFHxA

Five of 15 males and 6/15 females in the 450/300 mg/kg/day group were found dead or euthanized in extremis prior to the scheduled necropsies. The early termination of 4 males and 4 females occurred within the first five days of dosing when the dose level was 450 mg/kg/day. The cause of death of these animals was considered test article related for 2 males and all four females and was due to papillary necrosis, gastric ulceration or a combination of both. Following the early mortality at 450 mg/kg/day, the dosage level for the high dose was lowered to 300 mg/kg/day for the rest of the dosing period. One male and 2 females were found dead during this dosing period. However, the cause of death for these animals was considered incidental for the male and of undetermined origin for the 2 females. Therefore, while the 450 mg/kg/day dose level was clearly above the maximum tolerated dose, it was undetermined whether the 300 mg/kg/day dose level had an effect on survivability.

A wide range of test article related clinical signs (rales, gasping, material/discharge around the urogenital/anogenital, mouth and/or nose and salivation prior to dosing) were noted in the 450/300 mg/kg/day group while test article related clinical signs in the 150 mg/kg/day group were limited to red material around the nose. Clinical signs were more numerous and more severe in the animals that died/were euthanized in extremis at 450 mg/kg/day.

The mortality and clinical signs noted at 450 mg/kg/day were also associated with marked body weight losses and lower food consumption in both sexes. The lowering of the high dose to 300 mg/kg/day was associated with a return of the food consumption to control levels. Body weights remained lower for the 450/300 mg/kg/day males until the end of dosing. The effect on male body weight, although considered adverse, was reversible. Body weight gains and food consumption were not affected in females at 300 mg/kg/day except transiently during the late gestation period. These parameters returned to control group levels during lactation and there were no test article-related effects on litter size and pup body weights.

Despite the evidence of overt toxicity at the 450/300 mg/kg/day dose level, reproductive performance, precoital interval and gestation length were unaffected by PFHxA administration at all dosage levels.

Test article related clinical pathological changes were present in males only. Hematological changes consisted of decreased MCH, MCHC and hemoglobin and increased reticulocyte counts at the high dose only while hemoglobin was decreased at all dose levels. Serum chemistry changes consisted of decreased globulin (with subsequent decreased total protein) and decreased cholesterol at 450/300 mg/kg/day. These clinical pathology findings were not considered adverse based on low magnitude and were reversible at 450/300 mg/kg/day.

Several target organs/tissues were identified following the administration of the test article: kidneys (papillary necrosis), stomach (ulceration/erosion), lymphoid organs (lymphoid necrosis and/or depletion of the T and B areas of the lymph nodes, spleen and thymus), adrenal cortex (hyperplasia in the zona fasciculata) and liver (hepatocellular hypertrophy). With the exception of the liver changes, changes in the other target organs/tissues were restricted to the 450/300 mg/kg/day dose level and were mostly present in the animals that died prematurely (before dosage reduction). These lesions were considered adverse based on their severity and/or their link to the premature death of the affected animals.

The renal changes were induced by both the 300 and 450 mg/kg/day dose levels as it was present in one female that was euthanized on schedule. The gastric changes of ulceration/erosion could be secondary to a local irritant effect related to the very low pH of the test article formulation at 450 mg/kg/day or be related to a systemic toxic effect of the test article as other target organs were identified at this dosage level as well. Although the pH of the dosing formulations at 300 mg/kg/day were also low, there was no evidence of gastric irritation after the dosage level was reduced to 300 mg/kg/day.

Lymphoid organ changes in the animals that died prematurely during dose administration at 450 mg/kg/day were severe and widespread. A small number of females that were euthanized on schedule had lesions in the thymus and/or mesenteric lymph node, which indicated that the 300 mg/kg/day dose level induced lymphoid organ changes despite the absence of marked toxicity and/or stress. None of the males that were euthanized on schedule demonstrated any test article related lymphoid changes. Recovery from lymphoid lesions could not be assessed in females due to mortality.

Changes in the liver consisted of enlargement (increased liver weights, centrilobular hypertrophy) and were present in a dose related manner at 150 and 450/300 mg/kg/day in the animals that were sacrificed at the end of the study. The liver enlargement was not adverse and considered adaptive to hepatic enzyme induction (Amacher et al., 1998). The liver changes showed recovery in the males (females not assessed).

The exposures to PFHxA increased in proportion to the dosage. Exposures were 2- to 4-fold higher for male rats than for female rats. Exposure tended to decrease upon repeated oral dosing. For male rats, after 26 days of dosing, approximately 90% of the daily oral dose of PFHxA was eliminated in urine within 24 hours of dosing. For female rats, urinary elimination following oral administration of 50, 150 and 300 mg/kg/day PFHxA was approximately 100%, 80% and 70% of dose, respectively. The half-life for PFHxA in serum and for urinary elimination was approximately 2 to 3 hours.

1.3.2. TEST ARTICLE C6-2 ALCOHOL

One of 15 males and 11/15 females in the 225 mg/kg/day C6-2 alcohol-treated group were found dead or euthanized in extremis. These deaths were considered test article related in all animals except for 1 female with an incidental finding of vaginal ulceration of moderate severity. The higher incidence of early termination in females could be related to an increased sensitivity for this sex due to the physiological changes in pregnancy. The early terminations of these animals were related to tubular lesions in the kidneys (1 male, 5 females), necrosis of the adrenal cortex (2 females) and/or bone marrow depletion (1 female). The deaths of 3 additional females with no significant pathological changes were considered test article related as well based on their occurrence at the high dose and the high incidence of test article related deaths in females at 225 mg/kg/day. Clinical findings for these animals that died or were euthanized early included emaciation, hypoactivity, unkempt appearance, body cool to touch and labored respiration. All test article-related clinical findings subsided following cessation of the C6-2 alcohol administration. There were no test article-related clinical observations in the 25 and 75 mg/kg/day group males and females.

Reproductive performance, precoital interval and gestation length were unaffected by C6-2 alcohol administration at all dosage levels. One female in the 225 mg/kg/day group that was euthanized on gestation day 24 had clinical signs consistent with dystocia (hypoactivity, body cool to touch, labored respiration and hunched posture); however these findings were most likely secondary to the systemic general toxicity of the test article based on generalized bone marrow depletion and renal changes. The evaluation of the litter data was confounded in the 225 mg/kg/day group by dam mortality. However, the high incidence of pup mortality and lower mean F_1 pup weights of the surviving litters present at 225 mg/kg/day were most likely test article-related.

Test article-related reduced body weight gains in males in the 75 and 225 mg/kg/day groups. Following the cessation of C6-2 alcohol administration, mean male body weight

gains in these 2 groups were similar to the control group. Body weight gains in females treated at 225 mg/kg/day were reduced only during the gestation and lactation periods.

Test article related clinical pathology findings affected the following serum chemistry parameters in the 225 mg/kg/day group only: increases in urea nitrogen and creatinine in females, decreases in sodium and chloride and increases in potassium in females, increases in bilirubin, AST and ALT in females, increases in total protein and globulin in females and increases in albumin in males and females. Increases in urea nitrogen and creatinine in females were considered related to the tubular changes noted in the kidneys. Decreases in sodium and chloride and increases in potassium in the 225 mg/kg/day females led to a Na^+ / K^+ ratio that was markedly reduced compared to the controls. The decreased chloride was considered an indirect effect of the test article. The increases in total bilirubin, AST and ALT were considered test article related in females at 225 mg/kg/day, albeit of unknown biological significance based on the lack of correlating hepatic changes.

Target organs/tissues identified following the administration of the test article at the 225 mg/kg/day dose level included: kidneys (tubular degeneration, tubular dilatation and tubular vacuolation), adrenal cortex (necrosis, acute inflammation and/or hyperplasia), bone marrow (myeloid depletion or generalized depletion), lymphoid organs (lymphoid depletion and/or necrosis), pancreas (decreased secretion and single cell necrosis) and small intestine (villous atrophy). In addition, liver enlargement was considered test article related at all dosage levels in males and at 75 and 225 mg/kg/day in females based on organ weights. Hepatic centrilobular hypertrophy identified in 225 mg/kg/day males confirmed the liver enlargement which was consistent with hepatic enzyme induction and was considered adaptive in nature (Amacher et al., 1998). There were no test article-related microscopic changes in the 25 and 75 mg/kg/day groups.

Test article related effects in the kidneys were present in males and females at 225 mg/kg/day and associated with pale kidneys and higher kidney weights at necropsy. Although the tubular changes were considered the cause of death in 1 male and

5 females, similar changes were also noted in males that were euthanized at the end of treatment. Following the recovery period, a higher than normal incidence of chronic progressive nephropathy in males at 225 mg/kg/day was considered an exacerbation of a spontaneous process by the test article and demonstrated incomplete reversibility.

Test article related changes in the adrenal cortex were mostly present in females at 225 mg/kg/day that died prematurely in gestation or during lactation. Bone marrow changes were considered test article related at 225 mg/kg/day. All but one of the affected animals were females. Villous atrophy of the jejunum and/or ileum was present in 2 females that were found dead or died prematurely at 225 mg/kg/day. Decreased secretion and single cell necrosis of the pancreas were considered test article related at 225 mg/kg/day, were more severe in females than in males and were completely reversible.

Test article related lymphoid depletion and/or necrosis were present in all the 225 mg/kg/day animals that died during the course of the study. The changes affected the mandibular lymph node, the mesenteric lymph node, the spleen and the thymus. In most cases, affected animals had similar changes in more than one lymphoid organ. The minimal lymphoid necrosis present in the thymus of all of the 225 mg/kg/day females examined at the end of the recovery period was considered test article related as well and indicative of a lack of complete reversibility.

1.4. CONCLUSIONS

1.4.1. TEST ARTICLE PFHXA

Based on the results of this study, systemic toxicity was evident at a dosage level of 450/300 mg/kg/day as evidenced by the following. Mortalities, changes in the clinical condition of the animals, effects on body weight, food consumption, hematology (lower mean hemoglobin levels, mean corpuscular hemoglobin levels and mean corpuscular hemoglobin concentration levels and higher mean reticuloycte counts), serum chemistry (lower mean serum globulin, total protein and cholesterol levels), macroscopic findings (consisting of dark red discoloration or dark red or yellow areas in the stomach), organ

weights (higher mean liver weights) and microscopic changes (affecting the kidneys, stomach, lymph nodes, spleen, thymus and liver). No reproductive or neonatal toxicity was observed at dosage levels of 50, 150 or 300 mg/kg/day PFHxA. Therefore, a dosage level of 300 mg/kg/day was considered to be the no-observed-effect level (NOEL) for reproductive and neonatal toxicity and the no-observed-adverse-effect level (NOAEL) for systemic toxicity was considered to be 150 mg/kg/day PFHxA.

1.4.2. TEST ARTICLE C6-2 ALCOHOL

Based on the results of this study, systemic toxicity was evident at a dosage level of 75 (effects on body weight and body weight gain only) and 225 mg/kg/day (as evidenced by the following). Mortalities, changes in the clinical condition of the animals, effects on body weight, food consumption, serum chemistry (higher mean albumin, total protein, globulin, potassium, urea nitrogen, creatinine, bilirubin, alanine aminotransferase and aspartate aminotransferase levels and lower mean sodium and chloride levels), macroscopic findings (consisting of pale kidneys: males and small spleen: females), organ weights (higher mean liver and kidney weights) and microscopic changes (kidneys, pancreas, sternal bone marrow and lymphoid tissues, liver, adrenal cortex, jejunum and ileum). Dystocia was noted at the 225 mg/kg/day dosage level. Of the surviving dams and litters that could be evaluated during lactation days 1-4, decreases in the mean numbers of pups born, postnatal survival and F_1 pup weights were noted at 225 mg/kg/day. Therefore, a dosage level of 75 mg/kg/day was considered to be the marginal lowest-observed-effect level (LOEL) for systemic toxicity and the no-observed-effect level (NOEL) for reproductive and neonatal toxicity. The NOEL for systemic toxicity was 25 mg/kg/day.

2. INTRODUCTION

2.1. GENERAL STUDY INFORMATION

This report presents the data from "A Combined 28-Day Repeated Dose Oral Toxicity Study with the Reproduction/Developmental Toxicity Screening Test of Perfluorohexanoic Acid and 1H, 1H, 2H, 2H-Tridecafluoro-1-Octanol in Rats, with Recovery". Due to software spacing constraints, the study title is presented as "28 Day Dose w/ Repro Screening of PFHxA and C6-2 in Rats" on the report tables. Due to spacing constraints, the dose level for Group 4 was designated as 300 mg/kg PFHxA even though the animals in this group were initially administered 450 mg/kg/day during study days 0-3. For reporting purposes, this dosage level is referred to as 450/300 mg/kg PFHxA in the text. Dosage levels for this study were selected based on results of the 7-day and 5-day pilot studies (see Section 4.1.6.). Data for the 7-day and 5-day pilot studies are presented in Appendices A and B, respectively.

The following computer protocols were used for data collection during the study:

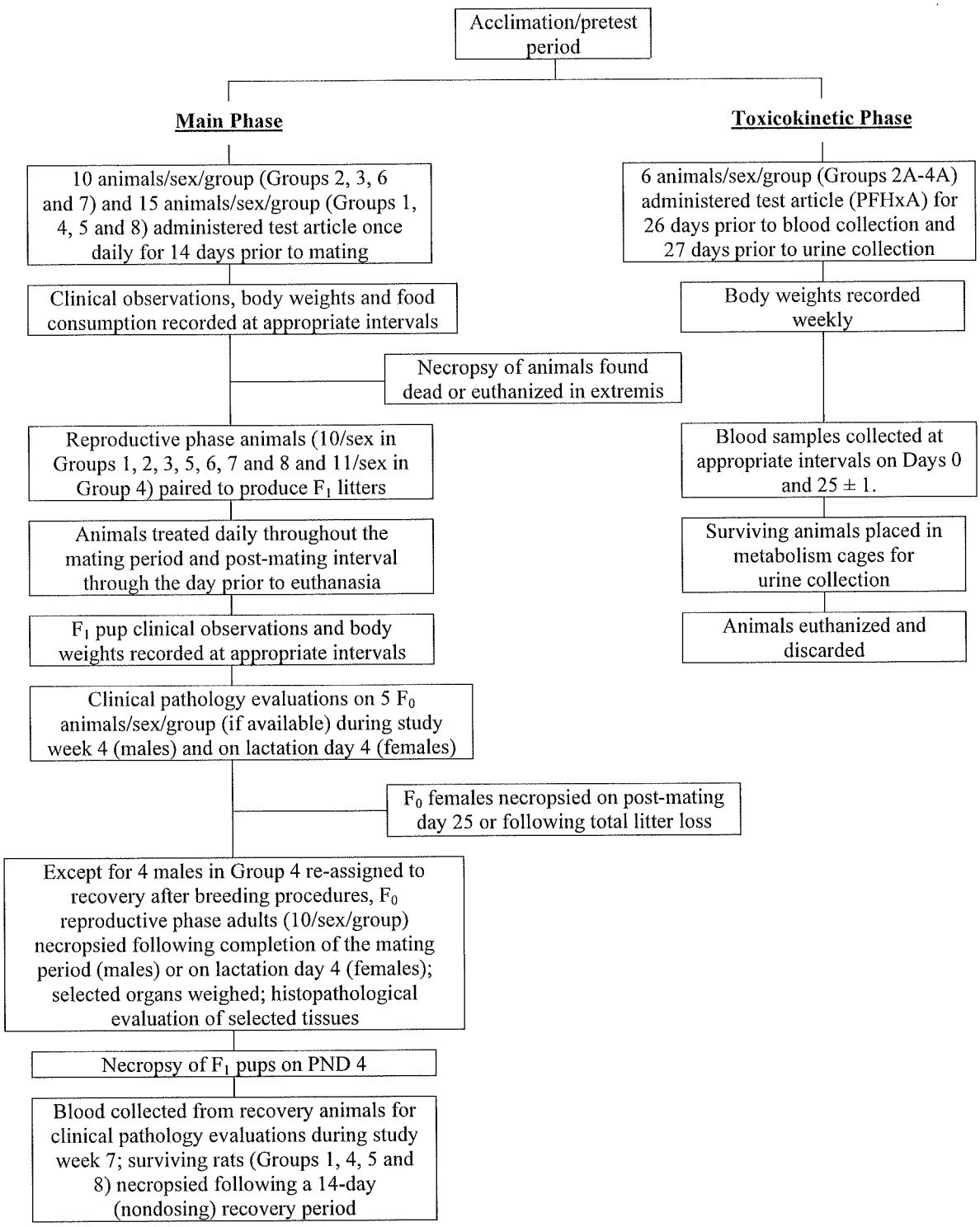
Computer Protocol	Type of Data Collected
WIL-534001N	7-day pilot study data
WIL-534001A	5-day pilot study data
WIL-534001M	Main study data (males)
WIL-534001F	Main study data (females)
WIL-534001Q	Pretest data (males)
WIL-534001S	Pretest data (females)
WIL-534001T	Toxicokinetic phase data (female no. 75763)
WIL-534001V	Toxicokinetic phase data

2.2. KEY STUDY DATES

Date(s)	Event(s)
4 January 2005	Experimental starting date (animal receipt - 7-day pilot study)
18 January 2005	Animal receipt (main study)
20 January 2005	Experimental start date (initiation of test article administration - 7-day pilot study)

Date(s)	Event(s)
3 February - 8 March 2005	Test article administration (males)
3 February - 26 March 2005	Test article administration (females)
8 February 2005	Receipt of toxicokinetic animals
16 February 2005	Initiation of mating period
24 February 2005	Initiation of test article administration (Toxicokinetic groups)
9 March 2005	Last male necropsy
19 March 2005	Last lactation day 4 necropsy
23 March 2005	Euthanasia (Toxicokinetic groups)
2 July 2005	Experimental completion/termination date (last bioanalytical stability analysis)

3. STUDY DESIGN



4. EXPERIMENTAL PROCEDURES - MATERIALS AND METHODS

4.1. TEST ARTICLE AND VEHICLE

4.1.1. TEST ARTICLE 1 IDENTIFICATION

The test article, perfluorohexanoic acid (PFHxA), was received from AGC Chemical via Miki & Co., LTD., Tokyo, Japan, on 4 January 2005, as follows:

Identification	Quantity Received	Physical Description
PFHxA Lot no. C15004301 CAS No. 307-24-4 [WIL log no. 6394A]	1 bottle Gross weight: 1403.7 g	Clear, colorless liquid

A Certificate of Analysis for the test article was provided by the sponsor and is presented in Appendix C. The purity of the test article was 98.50%; dose formulations were not adjusted for purity. The test article was stored at room temperature and was considered stable under this condition. A reserve sample of the test article (approximately 2 g) was collected on 17 January 2005, and stored in the Archives of WIL Research Laboratories, LLC.

4.1.2. VEHICLE 1 IDENTIFICATION

The vehicle used in preparation of the PFHxA formulations and for administration to control Group 1 was deionized water.

4.1.3. TEST ARTICLE 2 IDENTIFICATION

The test article, 1H, 1H, 2H, 2H-tridecafluoro-1-octanol (C6-2 alcohol), was received from AGC Chemical via Miki & Co., LTD., Tokyo, Japan, on 4 January 2005, as follows:

Identification	Quantity Received	Physical Description
C6-2 alcohol Lot no. re-AL-27,28 CAS No. 647-42-7 WIL log no. 6395A	1 bottle Gross weight: 1592.3 g	Clear, colorless liquid

A Certificate of Analysis for the test article was provided by the sponsor and is presented in Appendix C. The purity of the test article was 98.52%; dose formulations were not adjusted for purity. The test article was stored at room temperature and was considered stable under this condition. A reserve sample of the test article (approximately 1 g) was collected on 5 January 2005, and stored in the Archives of WIL Research Laboratories, LLC.

4.1.4. VEHICLE 2 IDENTIFICATION

The vehicle used in preparation of the C6-2 alcohol formulations and for administration to the control group during the 5-day pilot and main phase studies was Mazola® corn oil (exp. dates: 6 and 13 April 2006, distributed by ACH Food Companies, Inc., Memphis, Tennessee and purchased locally).

4.1.5. PREPARATION

Deionized water was used to prepare the test article formulations for Groups 2-4 and corn oil was used to prepare the test article formulations for Groups 6-8, a sufficient amount of deionized water, or corn oil, as appropriate, was dispensed in a labeled container. The respective vehicles were divided into aliquots for daily dispensation and stored under refrigeration.

The PFHxA and C6-2 alcohol formulations were weight/volume (test article/vehicle) mixtures. For the test article-treated groups, the appropriate amount of test article for each group was weighed into a tared, labeled storage container and an approximately 70% of the vehicle was added glass containers. The formulations were mixed at a

medium speed until uniform using a magnetic stirrer. A sufficient volume of the vehicle was added to each container to bring the formulations to the calibration mark. The PFHxA and C6-2 alcohol formulations were prepared weekly as single formulations for each dose level, divided into aliquots for daily dispensation and stored under refrigeration. The test article formulations were stirred continuously throughout the preparation, sampling and dose administration procedures as well as overnight prior to being dispensed for dosing. The test article formulations were visually inspected by the study director on 2 February 2005 and were found to be visibly homogeneous and acceptable for dosing.

4.1.6. ADMINISTRATION

The vehicle and test article formulations were administered orally by gastric intubation, via an appropriately sized flexible, polypropylene-shafted, silicone bulb-shaped tipped dosing cannula (Instech Solomon, Plymouth Meeting, Pennsylvania) once daily. Males received 14 daily doses prior to mating and were dosed throughout the mating period through the day prior to euthanasia for a total of 32-34 doses; males assigned to the recovery phase received a total of 35 doses. Females received 14 daily doses prior to pairing and were dosed through lactation day 3 for a total of 39-44 doses; females with no evidence of mating/that failed to deliver were dosed through the day prior to euthanasia (post-mating or post-cohabitation day 25) for a total of 39-52 doses. Females assigned to the recovery period (not used for mating) were dosed through the day of euthanasia of the first females scheduled for the natural delivery phase for a total of 40 doses. The toxicokinetic groups were dosed for a total of 26 doses prior to blood collection and 27 doses prior to urine collection. The dose volumes for the PFHxA and C6-2 alcohol groups (and their respective vehicle control groups) were 10 and 5 mL/kg, respectively. Individual dosages were based on the most recently recorded body weights to provide the correct mg/kg/day dose. All animals were dosed at approximately the same time each day.

The following table presents the study group assignment:

Main Phase (WIL-534001M and WIL-534001F)						
Group Number	Test Article	Dosage Level (mg/kg/day)	Dosage Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Animals[a]	
					Males	Females
1	Deionized Water	0	0	10	15[e]	15[e]
2	PFHxA	50	5	10	10	10
3	PFHxA	150	15	10	10	10
4	PFHxA	450/300[f]	45/30[f]	10	15[b,c]	15[b,d]
5	Corn Oil	0	0	5	15[e]	15[e]
6	C6-2 Alcohol	25	5	5	10	10
7	C6-2 Alcohol	75	15	5	10	10
8	C6-2 Alcohol	225	45	5	15[e]	15[e]

[a] = Reproductive parameters were evaluated for 10 rats/sex/group.
[b] = All Group 4 animals were used for breeding.
[c] = Four surviving males were used for the recovery phase.
[d] = No females in Group 4 were evaluated for recovery.
[e] = 5 rats/sex necropsied following a 14-day (nondosing) recovery period. Recovery animals were not evaluated for reproductive parameters.
[f] = The dosage level for Group 4 was lowered from 450 mg/kg/day to 300 mg/kg/day on study day 4 (following 4 doses).

Toxicokinetic Phase (WIL-534001V)						
Group Number	Test Article	Dosage Level (mg/kg/day)	Dosage Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Animals	
					Males	Females
2A	PFHxA	50	5	10	6	6
3A	PFHxA	150	15	10	6	6
4A	PFHxA	300	30	10	6	6

Dosage levels were selected based on the results of previous studies with PFHxA and C6-2 alcohol, including 5-day and 7-day pilot studies (see Appendices A and B). In the 7-day range-finding pilot study (Appendix A), 6 groups of Crl:CD®(SD) rats (3 animals/sex/group) received either PFHxA in deionized water or C6-2 alcohol in 0.5% methylcellulose (MC) with 0.1% Tween® 80 at dosage levels of 100, 300 and 1000 mg/kg/day. A seventh group of 3 animals/sex received PFHxA at 600 mg/kg/day.

The 1000 mg/kg/day PFHxA group was terminated following the first dose, due to mortality and excessive toxicity. Mortalities (2/3 males and females) were observed at a dosage level of 600 mg/kg/day PFHxA during study days 4 through 6. Animals in the 600 mg/kg/day PFHxA group also lost weight and consumed less food compared to the control group during study days 0-7.

Mortalities (2/3 males and 3/3 females) were observed at a dosage level of 1000 mg/kg/day C6-2 alcohol group during study days 4 through 6. Animals in the 1000 mg/kg/day C6-2 alcohol group also lost weight and consumed less food compared to the control group during study days 0-7. One female in the 300 mg/kg/day C6-2 alcohol group lost weight and only consumed 2.2 g/animal/day of food during study days 3-7.

Based on the results of the 7-day pilot study and due to analytical results indicating poor stability of the C6-2 alcohol formulations in the original vehicle, an additional 5-day pilot study was conducted (Appendix B) in which 2 groups of Crl:CD®(SD) rats (3 animals/sex/group) received C6-2 alcohol at dosage levels of 100 and 300 mg/kg/day with corn oil as the vehicle. In the 5-day study, all animals survived and clinical findings were limited to hair loss on the forelimbs for the males in the 100 mg/kg/day C6-2 alcohol group, single occurrences of red material around the nose and mouth and decreased defecation for females in the 300 mg/kg/day C6-2 alcohol group and clear material around the mouth in 1 and 2 males in the 100 and 300 mg/kg/day C6-2 alcohol groups. Mean cumulative body weight losses (6.3 g for males and 22.6 g for females) were observed in the 300 mg/kg/day C6-2 alcohol group.

The selected route of administration for this study was oral (gavage) because, historically, this route has been used extensively for studies of this nature. The animal model, the Crl:CD®(SD) rat, is recognized as appropriate for general and reproductive toxicity studies, and has been proven susceptible to the effects of reproductive toxicants. In addition, significant reproductive historical control data are available for the Crl:CD®(SD) rat.

4.1.7. SAMPLING AND ANALYSES

Prior to the initiation of dosing (2 February 2005), duplicate samples (1 mL each) for homogeneity determination were collected from the top, middle and bottom strata of each test article formulation and 1 sample (1 mL) was collected from the middle stratum of both vehicles. In addition, duplicate samples (1 mL each) from the top and bottom strata of each test article formulation and 1 sample (1 mL) from the middle stratum of both vehicles were collected following 9 days of refrigerated storage for resuspension homogeneity and stability determinations. Additional duplicate samples (1 mL) from the top and bottom strata of the PFHxA and C6-2 alcohol formulations were collected following 5 and 2 days, respectively, of refrigerated storage for homogeneity and stability determinations. Samples (1 mL each; duplicate for test article-treated groups) for concentration analysis were collected from the middle stratum of each dosing formulation (including the control groups) during the 1st, 2nd and 7th weeks of dose administration.

All analyses were conducted by the Analytical Chemistry Department, WIL Research Laboratories, LLC. The methodology and results of these analyses are presented in Appendix D. The PFHxA formulations were analytically confirmed to be homogenous, and formulations for Groups 2 (5 mg/mL) and 3 (15 mg/mL) were stable for 9 days while formulations for Group 4 (45 mg/mL) were stable for 5 days under refrigerated storage conditions. The C6-2 alcohol formulations were analytically confirmed to be homogeneous and were stable for 9 days under refrigerated storage conditions.

4.2. ANIMAL RECEIPT AND ACCLIMATION/PRETEST PERIOD

Twenty-five male and 25 female Crl:CD®(SD) rats were received on 4 January 2005, 120 male and 120 female Crl:CD®(SD) rats were received on 18 January 2005, and 22 male and 22 female Crl:CD®(SD) rats were received on 8 February 2005 from Charles River Laboratories, Inc., Raleigh, North Carolina; all rats were received in good health. Animals from the first, second and third shipments were used for the 7-day pilot study, main study phase and toxicokinetic phase, respectively. The animals were 56-62 days old upon receipt. Each rat was uniquely identified by a Monel® metal eartag displaying

the animal number. The animals were housed for a minimum acclimation period of 16 days prior to the first day of treatment. During the acclimation period, the animals were observed twice daily for mortality and general changes in appearance and behavior. Each animal was examined by a qualified technician on the day of receipt and weighed the day following each receipt.

Individual body weights were recorded and detailed physical examinations were performed periodically during the pretest period beginning 1 week prior to the initiation of test article administration. Food consumption data were also recorded for pretest animals prior to the initiation of dose administration. Pretest clinical observations are presented in Appendix E.

4.3. ANIMAL HOUSING

Following receipt and until pairing, all F_0 animals were housed individually in clean, stainless steel wire-mesh cages suspended above cage-board; animals not used for breeding remained individually housed. The cage-board was changed at least 3 times per week. The rats were paired for mating in the home cage of the male. Following positive evidence of mating, the males were housed in suspended wire-mesh cages until the scheduled necropsy, and the females were transferred to plastic maternity cages with nesting material, ground corncob bedding (Bed-O'Cobs®; The Andersons, Industrial Products Division, Maumee, Ohio). Females for which there was no evidence of mating were placed in plastic maternity cages with nesting material upon completion of a 14-day mating period. The nesting material is periodically analyzed by the manufacturer for contaminants. No contaminants were present in the bedding at concentrations sufficient to interfere with the outcome of the study. The results of these analyses are maintained at WIL Research Laboratories, LLC. The dams were housed in these cages until euthanasia on post-mating/post-cohabitation day 25 or lactation day 4. Animals were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996). The animal facilities at WIL Research Laboratories, LLC are

accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

4.4. DIET, DRINKING WATER AND MAINTENANCE

The basal diet used in this study, PMI Nutrition International, LLC, Certified Rodent LabDiet® 5002, is a certified feed with appropriate analyses performed by the manufacturer and provided to WIL Research Laboratories, LLC. Feeders were changed and sanitized at least once per week. Municipal water supplying the facility is sampled for contaminants according to standard operating procedures. No contaminants were present in animal feed or water at concentrations sufficient to interfere with the objectives of this study. Reverse osmosis-purified (on-site) drinking water, delivered by an automatic watering system, and the basal diet were provided ad libitum throughout the acclimation period and during the study.

4.5. ENVIRONMENTAL CONDITIONS

All rats were housed throughout the acclimation period and during the study in an environmentally controlled room. The room temperature and humidity controls were set to maintain daily averages of 71°F ± 5°F (22°C ± 3°C) and 50% ± 20% relative humidity. Room temperature and relative humidity were monitored using the Metasys DDC Electronic Environmental control system and were recorded approximately hourly. These data are summarized in Appendix F. Actual mean daily temperature ranged from 70.7°F to 70.9°F (21.5°C to 21.6°C) and mean daily relative humidity ranged from 34.6% to 37.7% during the study. Light timers were calibrated to provide a 12-hour light (0600 hours to 1800 hours)/12-hour dark photoperiod. Air handling units were set to provide approximately 10 fresh air changes per hour. The 12-hour light/12-hour dark photoperiod was interrupted as necessary to allow for the performance of protocol-specified activities (e.g., urine collection, etc.).

4.6. ASSIGNMENT OF ANIMALS TO TREATMENT GROUPS

At the conclusion of the acclimation period, all available males and females were weighed and examined in detail for physical abnormalities. At the discretion of the study

director, each animal judged to be in good health and meeting acceptable body weight requirements (300 g to 500 g for males and 200 g to 300 g for females) was selected for use in the computerized randomization procedure. At that time, the individual body weights and corresponding animal identification numbers were entered into the WIL Toxicology Data Management System (WTDMS™). A printout containing the animal numbers, corresponding body weights and individual group assignments was generated using a computer randomization procedure that ensured homogeneity of group means and variances for body weight. The animals then were arranged into groups according to the printout. Individual body weights at randomization were within ± 20% of the mean for each sex.

The experimental design for the main phase (WIL-534001M and WIL-534001F) consisted of 3 PFHxA- and 3 C6-2 alcohol-treated groups (Groups 2-4 and 6-8, respectively) and a corresponding vehicle control group for each test article (Groups 1 and 5, respectively). Groups 1, 4, 5 and 8 consisted of 15 rats/sex/group and Groups 2, 3, 6 and 7 consisted of 10 rats/sex/group. The toxicokinetic phase (WIL-534001V) consisted of 3 PFHxA-treated groups (Groups 2A-4A) consisting of 6 rats/sex/group. At the initiation of dose administration (study day 0), the males and females for the main phase were approximately 10 weeks old; body weights ranged from 312 g to 406 g for the males and 211 g to 280 g for females. Body weights on study day 0 ranged from 361 g to 418 g for males and 241 g to 280 g for females in the toxicokinetic phase. Reproductive phase animals were approximately 12 weeks old when paired on study day 13; female body weights ranged from 218 g to 317 g on gestation day 0.

5. PARAMETERS EVALUATED

5.1. CLINICAL OBSERVATIONS (MAIN PHASE) AND SURVIVAL (BOTH PHASES)

All rats for the main and toxicokinetic phase animals were observed twice daily, once in the morning and once in the afternoon, for moribundity and mortality. Individual clinical observations for the main phase were recorded once or occasionally twice daily (prior to test article administration during the treatment period). Each male and female in the main phase was also observed for signs of toxicity 1-2 hours following dose administration daily. Detailed physical examinations were conducted weekly for the main phase animals beginning 1 week prior to test article administration to prior to the scheduled necropsy. All significant findings were recorded. Toxicokinetic phase data are presented in Appendix G.

5.2. BODY WEIGHTS (BOTH PHASES)

Individual male body weights were recorded weekly throughout the study and prior to the scheduled euthanasia. Individual female body weights were recorded weekly until evidence of copulation was observed. An additional body weight was recorded for the males and females on study day 4 after lowering the dosage level in Group 4 to 300 mg/kg/day PFHxA. Mean body weights and body weight changes are presented for each interval. In addition, cumulative mean body weight changes are presented for the pre-mating period (study days 0-13), for the entire treatment period (study days 0-32 for males selected for mating, study days 0-34 for males not selected for mating and study days 41-55 for females not selected for mating,) and for the recovery period (study days 32-49 and 34-55 males and females, respectively). Body weights during the recovery period were recorded weekly beginning on study day 34 and prior to euthanasia. Once evidence of mating was observed, female body weights were recorded on gestation days 0, 4, 7, 11, 14, 17 and 20 and on lactation days 1 and 4. Mean gestation body weights and corresponding mean body weight changes are presented for these intervals and for the overall gestation interval (days 0-20); mean lactation days weight changes are presented for lactation days 1-4. For individual data, the time periods that a given animal

was not weighed were designated as "NA" (Not Applicable). Individual body weights were recorded weekly, beginning 1 week prior to dose administration, for the toxicokinetic animals and presented in Appendix G.

5.3. FOOD CONSUMPTION (MAIN PHASE)

Individual food consumption was recorded on the corresponding weekly body weight days (study days 0, 7 and 13) until pairing and during the recovery period. Food consumption was recorded weekly for males not selected for mating throughout the treatment and recovery periods. Food intake was not recorded during the mating period. Once evidence of mating was observed, female food consumption was recorded on gestation days 0, 4, 7, 11, 14, 17 and 20 and on lactation days 1 and 4. Following mating, food consumption for females with no evidence of mating and for all males was measured on a weekly basis until the scheduled euthanasia. Food consumption was reported as g/animal/day and g/kg/day for the corresponding body weight change intervals.

When food consumption could not be determined for an animal during a given interval (due to a weighing error, food spillage, obvious erroneous value, etc.), group mean values were calculated for that interval using the available data. The time periods when food consumption values were unavailable for a given animal were designated as "NA" (Not Applicable) on the individual report tables.

5.4. BREEDING PROCEDURES (MAIN PHASE)

The animals were paired on a 1:1 basis within each treatment group (10/sex/group with the exception of 11/sex/group for Group 4) following 14 days of treatment for the males and females. A breeding record containing the male and female identification numbers and the start date of cohabitation was prepared. Each female was housed in the home cage of the male. Positive evidence of mating was confirmed by the presence of a vaginal copulatory plug or the presence of sperm following a vaginal lavage. Each mating pair was examined daily. The day when evidence of mating was identified was termed gestation day 0. If evidence of copulation was not detected after 14 days of

pairing, any females that had not shown evidence of mating were placed in plastic maternity cages containing nesting material.

For the purpose of calculating pre-coital intervals, rats paired over a 12-hour dark cycle were considered to have been paired for 1 day.

Mating, fertility and copulation/conception indices were calculated as follows:

$$\text{Male (Female) Mating Index (\%)} = \frac{\text{No. of Males (Females) with Evidence of Mating (or Confirmed Pregnancy)}}{\text{Total No. of Males (Females) Used for Mating}} \times 100$$

$$\text{Male Fertility Index (\%)} = \frac{\text{No. of Males Siring a Litter}}{\text{Total No. of Males Used for Mating}} \times 100$$

$$\text{Male Copulation Index (\%)} = \frac{\text{No. of Males Siring a Litter}}{\text{No. of Males with Evidence of Mating (or Females with Confirmed Pregnancy)}} \times 100$$

$$\text{Female Fertility Index (\%)} = \frac{\text{No. of Females with Confirmed Pregnancy}}{\text{Total No. of Females Used for Mating}} \times 100$$

$$\text{Female Conception Index (\%)} = \frac{\text{No. of Females with Confirmed Pregnancy}}{\text{No. of Females with Evidence of Mating (or Confirmed Pregnancy)}} \times 100$$

5.5. PARTURITION (MAIN PHASE)

All females were allowed to deliver naturally and rear their young to PND 4. During the period of expected parturition, the females were observed twice daily for initiation and completion of parturition and for signs of dystocia. On the day parturition was initiated, pups were sexed and examined for gross malformations, and the numbers of stillborn and live pups were recorded. Any changes or abnormalities in nesting and nursing behavior were recorded. Individual gestation length was calculated using the date delivery started.

5.6. CLINICAL PATHOLOGY (MAIN PHASE)

Blood samples for clinical pathology evaluations (hematology and serum chemistry) were collected from 5 animals/sex/group (if available) at the scheduled necropsies (study

week 4 for males and lactation day 4 for females) and from 1 female in the 225 mg/kg/day C6-2 alcohol group that was euthanized in extremis on gestation day 24 (presented on lactation day 4 individual data tables but not included in the group mean calculations). In addition, blood samples were collected from the surviving animals in the recovery phase during study week 7. The animals were not fasted overnight prior to blood collection. Blood for coagulation parameters was collected from the vena cava at the time of necropsy. Blood for all other parameters was collected from the retro-orbital sinus of isoflurane-anesthetized animals prior to necropsy. Blood was collected into tubes containing EDTA (hematology), sodium citrate (clotting determinations) or no anticoagulant (serum chemistry). Clinical pathology methods, procedures and references are presented in Appendix H. The following parameters were evaluated:

5.6.1. HEMATOLOGY

Total leukocyte count (White Cells)
Erythrocyte count (Red Cells)
Hemoglobin
Hematocrit
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular hemoglobin concentration (MCHC)
Platelet count (Platelet)
Prothrombin time (ProTime)
Activated partial thromboplastin time (APTT)

Reticulocyte count
  Percent (Reticulocyte)
  Absolute (Retic Absolute)
Differential leukocyte count -
  Percent and absolute
  -Neutrophil
  -Lymphocyte
  -Monocyte
  -Eosinophil
  -Basophil
  -Large unstained cell

( ) - Designates tabular abbreviation

5.6.2. SERUM CHEMISTRY

Albumin
Total protein
Globulin [by calculation]
Albumin/globulin ratio (A/G Ratio) [by calculation]
Total bilirubin (Total Bili)
Urea nitrogen
Creatinine
Alkaline phosphatase (AlkalinePhos'tse)
Total Cholesterol (Cholesterol)
Alanine aminotransferase (Alanine Transfer)
Aspartate aminotransferase (AspartatTransfer)
Glucose
Calcium
Chloride
Phosphorus
Potassium
Sodium

( ) - Designates tabular abbreviation

5.7. MACROSCOPIC EXAMINATIONS (MAIN PHASE)

5.7.1. UNSCHEDULED DEATHS

Animals that were found dead or euthanized (by carbon dioxide inhalation) in extremis were subjected to a gross necropsy, and tissues were retained as described in Section 5.7.2. The pregnancy status (by ammonium sulfide staining, if necessary; Salewski, 1964) was determined for any mated females that were found dead or euthanized in extremis on gestation day 6 or later. If evidence of macroscopic implantation was present, the number and location of implantation sites and corpora lutes were counted and recorded. Recognizable fetuses were examined externally and preserved in 10% neutral-buffered formalin. Uteri stained with ammonium sulfide were discarded and not saved for histopathologic examination.

5.7.2. SCHEDULED EUTHANASIA

All surviving adults selected for the reproductive and recovery phases were euthanized by carbon dioxide inhalation followed by exsanguination. Males were euthanized following completion of the mating period. Females that delivered were euthanized on lactation day 4; the number of former implantation sites and corpora lutea were recorded. Females that failed to deliver were euthanized on post-mating/post-cohabitation day 25. Uteri

with no macroscopic evidence of implantation were opened and subsequently placed in 10% ammonium sulfide solution for detection of early implantation loss (Salewski, 1964); these uteri were discarded following ammonium sulfide staining. Females with total litter loss were euthanized within 24 hours of litter loss; the number of former implantation sites and corpora lutea were recorded. Necropsy included examination of the external surface, all orifices and the cranial, thoracic, abdominal and pelvic cavities, including viscera. At the time of necropsy, the following tissues and organs were placed in 10% neutral-buffered formalin (except as noted):

Adrenal glands (2)
Aorta
Bone with marrow (sternebrae)
Bone marrow smear[a]
Brain (Cerebrum levels 1 and 2, Cerebellum with pons/medulla)
Coagulating glands
Eyes with optic nerve (2)[b]
Gastrointestinal tract
  Esophagus
  Stomach
  Duodenum
  Jejunum
  Ileum
  Cecum
  Colon
  Rectum
Heart
Kidneys (2)
Exorbital lacrimal glands (2)
Liver (sections of 2 lobes)
Lungs (including bronchi, fixed by inflation with fixative)
Lymph node (mesenteric and mandibular)
Mammary gland (females only)
Ovaries and oviducts (2)
Pancreas
Peripheral nerve (sciatic)
Pituitary gland
Prostate gland
Salivary gland [mandibular (2)]
Seminal vesicles (2)
Skeletal muscle (rectus femoris)
Skin
Spinal cord (cervical, thoracic and lumbar)
Spleen
Testes with epididymides[c] (2)
Thymus gland
Thyroids [with parathyroids, if present (2)]
Trachea
Urinary bladder
Uterus[d] with vagina
All gross lesions

[a] = Not taken from animals found dead; not placed in formalin. Examined only if scientifically warranted.
[b] = Placed in Davidson's solution.
[c] = Fixed in Bouin's solution.
[d] = Not retained if placed in ammonium sulfide solution.

5.7.3. ORGAN WEIGHTS

The following organs were weighed from all F_0 animals at the scheduled necropsies:

Adrenal glands	Ovaries with oviducts
Brain	Spleen
Epididymides[a]	Testes[a]
Heart	Thymus gland
Kidneys	Thyroids with parathyroids[b]
Liver	

[a] = These paired organs were weighed separately.
[b] = Fixed in 10% neutral-buffered formalin prior to weighing.

Except as noted, paired organs were weighed together. Absolute weights and organ to final body weight and organ to brain weight ratios were reported.

5.8. MICROSCOPIC EXAMINATIONS (MAIN PHASE)

After fixation, protocol-specified tissues were trimmed according to standard operating procedures and the protocol. Trimmed tissues were processed into paraffin blocks, sectioned at 4 to 8 microns, mounted on glass microscope slides and stained with hematoxylin and eosin, with the following exceptions. The testes and epididymides were embedded in the same block. In addition to the section stained with hematoxylin and eosin, a separate section was stained with PAS; only the testes were examined on the PAS-stained slide.

Microscopic examination was performed on all tissues listed in Section 5.7.2. from all animals in the control groups (Groups 1 and 5) and high-dose groups (450/300 mg/kg/day PFHxA and 225 mg/kg/day C6-2 alcohol, Groups 4 and 8, respectively) at the scheduled necropsies, and animals found dead or euthanized in extremis. In addition, the kidneys, liver, lymph nodes (mandibular and mesenteric), thymus gland, pancreas and spleen were examined from all males and females in the low- and mid-dose PFHxA- and C6-2 alcohol-treated groups from the primary necropsy and the control and high-dose dose PFHxA- and C6-2 alcohol-treated groups from the recovery necropsy (liver was not evaluated in the PFHxA recovery groups). The stomach (glandular and nonglandular

portions) was examined from the Group 1 control and the PFHxA-treated group animals from the primary (all groups) and recovery necropsies (control and high-dose groups). The adrenal cortex and bone marrow were examined from all females in the low- and mid-dose PFHxA- and C6-2 alcohol-treated groups from the primary necropsy and the control and high-dose dose PFHxA- and C6-2 alcohol-treated groups from the recovery necropsy. Mammary gland, uterus and vagina were examined from the Group 5 control and the C6-2 alcohol-treated groups females from the primary (all groups) and recovery necropsies (control and high-dose groups). Missing tissues were identified as not found at necropsy, lost at necropsy, lost during processing, not in plane of section or other reasons as appropriate. Microscopic examination was performed by Joelle D. Ibanes, DVM, DACVP, Senior Pathologist, WIL Research Laboratories, LLC.

5.9. F_1 Litter Parameters (Main Phase)

5.9.1. Litter Viability And Deaths

Each litter was examined daily for survival, and all deaths were recorded. All pups were individually identified by application of tattoo markings on the digits following completion of parturition. A daily record of litter size was maintained. Intact offspring dying euthanized were necropsied using a fresh dissection technique including the heart and major vessels (Stuckhardt and Poppe, 1984). Pups euthanized in extremis were euthanized by an intraperitoneal injection of sodium pentobarbital and necropsied. If a skeletal anomaly was suspected, the pup was processed for skeletal evaluation (Dawson, 1926) and examined. Tissues were preserved in 10% neutral-buffered formalin for possible future histopathologic examination only as deemed necessary by the gross findings. The carcass of each pup was then discarded. Cannibalized pups were discarded without necropsy.

5.9.2. Clinical Observations

Litters were examined daily for survival and any adverse changes in appearance or behavior. Each pup received a detailed physical examination on PND 1 and 4. Any abnormalities in nursing behavior were recorded.

5.9.3. BODY WEIGHTS

Pups were individually weighed on PND 1 and 4. Mean pup weights were presented by sex for each litter and by dose group.

5.9.4. SEX DETERMINATION

Pups were individually sexed on PND 0, 1 and 4.

5.9.5. CALCULATION OF LITTER PARAMETERS

Litter parameters were defined as follows:

$$\text{Mean Live Litter Size} = \frac{\text{Total No. of Viable Pups on PND 0}}{\text{No. of Litters with Viable Pups PND 0}}$$

$$\text{Postnatal Survival Between Birth and PND 0 or PND 4 (\% Per Litter)} = \frac{\Sigma\,(\text{Viable Pups Per Litter on PND 0 or PND 4}/\text{No. of Pups Born Per Litter})}{\text{No. of Litters Per Group}} \times 100$$

$$\text{Postnatal Survival for All Other Intervals (\% Per Litter)} = \frac{\Sigma\,(\text{Viable Pups Per Litter at End of Interval N}/\text{Viable Pups Per Litter at Start of Interval N})}{\text{No. of Litters Per Group}} \times 100$$

Where N = PND 0-1 and 1-4

5.9.6. SCHEDULED NECROPSY - PND 4

On PND 4, all pups were euthanized by an intraperitoneal injection of sodium pentobarbital and necropsied with emphasis on developmental morphology. Gross lesions were preserved in 10% neutral-buffered formalin for possible histopathologic examination.

5.10. TOXICOKINETIC EVALUATION

Blood samples from 3 rats/sex/group (Subgroups A and B) were obtained for determination of concentration of PFHxA in serum were obtained as follows:

	Prior to Dosing (0 hours)	Time After Dosing (hr)				
		1	2	4	8	24
Day 0	A	B	A	B	A	B
Day 25 ± 1*	A	B	A	B	A	B

A = Subgroup A; 3 rats/sex/group
B = Subgroup B; 3 rats/sex/group
* = Animals received 26 total doses.

Each rat was sampled 3 times per collection day. At each time point, blood samples (target 0.5 mL) were collected from a retro-orbital sinus, following isoflurane anesthesia, into tubes without anticoagulant. Serum was separated in a refrigerated centrifuge and stored frozen (approximately -20°C) with minimal delay until preparation for analysis.

All surviving toxicokinetic phase rats were transferred into plastic metabolism cages for urine collection following collection of the last blood sample. Urine was collected on wet ice 0-6, 6-12 and 12-24 hours following dose administration on study day 26 (total of 27 doses). Urine samples were frozen (approximately -20°C) with minimal delay until preparation for analysis. Following the final urine collection, all animals were euthanized by carbon dioxide inhalation and discarded on study day 27.

Serum and urine samples were analyzed for PFHxA by the Analytical Chemistry Department at WIL Research Laboratories, LLC using a validated LC/MS/MS method. Pertinent data (C_{max}, AUC and elimination half-life) were determined as data permitted, following a single dose and repeated dose administration. The results of these analyses are presented in Appendix I; the interpretation of the serum data is presented in Appendix J.

Toxicokinetic phase animals that were found dead or euthanized in extremis (by carbon dioxide inhalation) after initiation of the dose administration period were examined macroscopically to determined the possible cause of death and discarded.

5.11. STATISTICAL ANALYSES

All statistical tests were performed using appropriate computing devices or programs. Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%, comparing each test article-treated group to the appropriate control group by sex; Groups 2-4 were compared to Group 1 and Groups 6-8 were compared to Group 5. Each mean was presented with the standard deviation (S.D.) and the number of animals (N) used to calculate the mean. Due to the different rounding conventions inherent in the types of software used, the means and standard deviations on the summary and individual tables may differ by ±1 in the last significant figure. Statistical analyses were not conducted if the number of animals was 2 or less. Data obtained from nongravid females were excluded from statistical analyses following the mating period. Where applicable, the litter was used as the experimental unit.

Parental mating, fertility, conception and copulation indices were analyzed using the Chi-square test with Yates' correction factor (Hollander and Wolfe, 1999). Mean parental body weights (weekly, gestation and lactation), body weight changes and food consumption, offspring body weights and body weight changes, gestation length, numbers of implantation sites, number of pups born, live litter size on PND 0, unaccounted-for sites, absolute and relative organ weights, clinical pathology values and pre-coital intervals values were subjected to a parametric one-way analysis of variance (ANOVA; Snedecor and Cochran, 1980) to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test (Dunnett, 1964) was used to compare the test article-treated groups to the control group. Mean litter proportions (percent per litter) of males at birth and postnatal survival were subjected to the Kruskal-Wallis nonparametric ANOVA (Kruskal and Wallis, 1952) to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunn's test (Dunn, 1964) was used to compare the test article-treated groups to the control group.

5.12. DATA RETENTION

The sponsor has title to all documentation records, raw data, specimens or other work product generated during the performance of the study. All work product generated by WIL Research Laboratories, LLC, including raw paper data and specimens, are retained in the Archives at WIL Research Laboratories, LLC, as specified in the study protocol.

Reserve samples of the test articles, pertinent electronic storage media and the original final report are retained in the Archives at WIL Research Laboratories, LLC in compliance with regulatory requirements.

6. RESULTS

6.1. TEST ARTICLE PFHxA

6.1.1. F_0 GENERATION

6.1.1.1. CLINICAL OBSERVATIONS AND SURVIVAL

Summary Data: Tables 1 through 11

Individual Data: Tables 76 through 84

Mortality (inlcuding animals euthanized in extremis) occurred with relationship to treatment only in the 450/300 mg/kg/day PFHxA group. The early deaths of 4 males and 4 females occurred during dosing of PFHxA at 450 mg/kg/day. Three males (nos. 74303, 74316 and 74408) were euthanized in extremis and 1 male (no. 74422) was found dead following 4 doses at 450 mg/kg/day. An additional male (no. 74388) was found dead on study day 19 after the dosage level was reduced to 300 mg/kg/day on study day 4. Lower body weight gains or body weight losses were observed for all these animals prior to death. Clinical findings noted for these males prior to death included body or limbs cool to touch, emaciation, labored respiration, gasping, rales, unkempt appearance, red material around the nose/mouth, hypoactivity, partial to complete eye closure, tremors, hunched posture, yellow material on the urogenital/anogenital area, salivation prior to dosing, clear nasal discharge and clear material around the mouth. The cause of moribundity for male no. 74316 was determined to be renal papillary necrosis and tubular vacuolation, and the cause of death for male no. 74422 was renal papillary necrosis and ulceration in the non-glandular stomach. Probable gavage error (lesions in the trachea, esophagus and lungs) was determined to be the cause of moribundity for male nos. 74408 and 74388 on study days 4 and 19, respectively. The cause of moribundity for male no. 74303 on study day 4 was undetermined.

Prior to the mating period, 3 females (nos. 74444, 74514 and 74466) were found dead, and 1 female (no. 74505) was euthanized in extremis following 3, 3, 4 and 4 doses of PFHxA at 450 mg/kg/day, respectively. The cause of death for female no. 74444 (study day 2) was determined to be renal papillary necrosis. The cause of death for female

nos. 74514 and 74466 (study days 3 and 4, respectively) was determined to be erosion/ulceration in the gastrointestinal tract. Prior to euthanasia on study day 4, body weight loss was observed for female no. 74505; the cause of moribundity for this female was erosion in the glandular stomach and renal papillary necrosis. After the dosage level was reduced to 300 mg/kg/day and after completion of the mating period, 2 females (nos. 74534 and 74511) were found dead on gestation days 10 and 22 (study days 25 and 38), respectively; the causes of death were undetermined for these females. Clinical findings noted for the females that died or were euthanized in extremis included hypoactivity, decreased defecation, labored respiration, gasping, rales, coolness, dehydration, tremors, emaciation, clear material on the ventral thoracic, ventral abdominal and urogenital areas and red material around the nose and mouth. All other animals dosed with PFHxA survived to the scheduled necropsies.

Test article-related clinical observations noted for the surviving 450/300 mg/kg/day group males included rales, gasping, clear material around the mouth, salivation prior to dose administration and yellow material on the urogenital and/or anogenital areas, beginning as early as 1-2 hours following the first dose administration (study day 0). Test article-related clinical findings of rales, salivation prior to dose administration, clear or red material around the mouth and yellow material on the urogenital and/or anogenital areas were observed in the surviving 450/300 mg/kg/day group females as early as 1-2 hours following the first dose administration (study day 0). These findings continued to be observed after the dosage level was lowered to 300 mg/kg/day. In addition, decreased defecation was noted for the 300 mg/kg/day group females and red material around the nose was observed in a dose-related manner in the 150 and 450/300 mg/kg/day group males beginning as early as study day 3. All test article-related clinical findings subsided following cessation of dose administration.

All other clinical findings for the PFHxA-treated groups were noted with similar incidence in the control group, were limited to single animals, were not noted in a

dose-related manner and/or were common findings for laboratory rats of this age and strain.

6.1.1.2. REPRODUCTIVE PERFORMANCE

Summary Data: Table 12

Individual Data: Table 85

Historical Control Data: Appendix K

No test article-related effects on F_0 reproductive performance were observed at any dosage level. Male and female mating indices were 100.0%, 90.0%, 100.0% and 100.0% in the control, 50, 150 and 450/300 mg/kg/day groups, respectively. Male and female fertility indices were 100.0%, 90.0%, 90.0% and 100.0% in the same respective groups. Male copulation and female conception indices were 100.0%, 100.0%, 90.0% and 100.0% in the control, 50, 150 and 450/300 mg/kg/day groups, respectively. No statistically significant differences were noted between the control and test article-treated groups. One male each in the 50 and 150 mg/kg/day groups did not sire a litter. One female in the 150 mg/kg/day group had evidence of mating but did not deliver.

The mean numbers of days between pairing and coitus in the test article-treated groups were similar to the control group value. None of these differences were statistically significant.

6.1.1.3. BODY WEIGHTS

Summary Data: Tables 13 through 20

Individual Data: Tables 86 through 93

6.1.1.3.1. MALES

Test article-related lower ($p<0.05$ or $p<0.01$) mean body weight gains were observed in the 450/300 mg/kg/day group males prior to mating (study days 0-13) and during the entire dosing period (study days 0-32 for mated males and 0-34 for unmated males) when compared to the control group. This difference was primarily due to a large (statistically significant, $p<0.01$) mean body weight loss observed from study days 0-4 when the

animals in this group received 4 doses of PFHxA at 450 mg/kg/day. After the dosage level was lowered to 300 mg/kg/day, body weight gain continued to be slightly reduced throughout the remainder of the dosing period. Mean body weights in the 450/300 mg/kg/day group were 10.3% lower than the control group on study day 4 and approximately 7.8% lower by study day 13 (after dosage levels were lowered to 300 mg/kg/day); the difference from the control group was statistically significantly ($p<0.01$) on study day 4. Mean body weights in the 450/300 mg/kg/day group were 7.5% lower compared to the control group on study day 32. Following cessation of test article administration (study days 34-49), statistically significantly ($p<0.01$) higher mean body weights gains were observed in the 450/300 mg/kg/day group males. By study day 49, the mean body weight for males in this group was comparable to that in the control group, indicative of recovery.

There were no test article-related effects on body weights for males in the 50 and 150 mg/kg/day PFHxA groups. In these groups, differences from the control group were slight, did not occur in a dose-related manner and/or were not statistically significant.

6.1.1.3.2. FEMALES

6.1.1.3.2.1. WEEKLY

A large body weight loss (statistically significant, $p<0.01$) was observed during study days 0-4 following 4 doses at 450 mg/kg/day. After the dosage level was lowered to 300 mg/kg/day, higher mean body weight gains were observed in the 450/300 mg/kg/day group females when compared to the control group (for study days 4-7 and 7-13); the difference from the control group was statistically significant ($p<0.05$) from study day 7-13. Mean body weights in the 450/300 mg/kg/day group were 7.6% lower than the control group on study day 4 and 3.9% lower by study day 7 (after dosage levels were lowered to 300 mg/kg/day); the difference from the control group was statistically significantly ($p<0.01$) on study day 4.

Evaluation of body weights during the recovery period in the 450/300 mg/kg/day group females was precluded by mortality.

There were no test article-related effects on body weight for females in the 50 and 150 mg/kg/day PFHxA groups, differences from the control group were slight, did not occur in a dose-related manner and/or were not statistically significant.

6.1.1.3.2.2. GESTATION

Slight reductions in mean maternal body weight gains were noted in the 450/300 mg/kg/day PFHxA group during gestation days 14-17 and 17-20 and when the entire gestation period (gestation days 0-20) was compared to the control group; only the difference from gestation days 17-20 was statistically significant ($p<0.01$). These reductions corresponded to the decreased food consumption observed in this group during this interval and were considered test article-related. Mean body weights in this group were similar to control group values during gestation days 0-17 but were 5.8% lower on gestation day 20. The slight reductions in mean maternal body weight and body weight gain late in gestation may have been attributed in part to the slightly reduced litter size in this group. Another possible cause for the body weight effects on gestation day 20 was that the dams were somewhat over-dosed late in gestation when dosage adjustments were most affected by the contribution of fetal weights to the weight of the dams.

Mean body weights and body weight gains in the 50 and 150 mg/kg/day PFHxA groups were unaffected by test article administration; differences from the control group did not occur in a dose-related manner and were not statistically significant.

6.1.1.3.2.3. LACTATION

Mean body weights and body weight gains were unaffected in the 50, 150 and 450/300 mg/kg/day PFHxA groups during lactation days 1-4. Differences from the control group were slight and not statistically significant.

6.1.1.4. FOOD CONSUMPTION

Summary Data: Tables 21 through 28

Individual Data: Tables 94 through 101

6.1.1.4.1. MALES

Test article-related statistically significantly lower mean food consumption (g/animal/day and g/kg/day) was observed in the 450/300 mg/kg/day group males during study days 0 to 7 compared to the control group. This reduction in food consumption correlates to the body weight loss observed in this group during study days 0-4 during which the animals received PFHxA at 450 mg/kg/day before the dosage level was lowered to 300 mg/kg/day. After the dosage level was lowered to 300 mg/kg/day and during the recovery period (study days 34-49), food consumption in this group was similar to the control group values. The only statistically significant ($p<0.05$ or $p<0.01$) differences from the control group were increased g/kg/day values observed in the 450/300 mg/kg/day group males.

There were no test article-related effects on food consumption in the 50 and 150 mg/kg/day PFHxA groups. Differences from the control group were slight and not statistically significant.

6.1.1.4.2. FEMALES

6.1.1.4.2.1. WEEKLY

Test article-related statistically significantly ($p<0.01$) lower mean food consumption (g/animal/day and g/kg/day) was observed in the 450/300 mg/kg/day group females during study days 0-7 compared to the control group. This reduction in food consumption correlates to the body weight loss observed in this group during study days 0-4 during which the animals received PFHxA at 450 mg/kg/day before the dosage level was lowered to 300 mg/kg/day. After the dosage level was lowered to 300 mg/kg/day, food consumption in this group was similar to the control group values.

There were no test article-related effects on food consumption in the 50 and 150 mg/kg/day groups. In these groups, differences from the control group were slight and were not statistically significant.

6.1.1.4.2.2. GESTATION

Mean maternal food consumption in the 450/300 mg/kg/day PFHxA group, evaluated as g/animal/day and g/kg/day, was decreased compared to the control group during gestation days 14-17 and 17-20. The differences during gestation days 14-17 (g/kg/day) and 17-20 (g/animal/day) were statistically significant ($p<0.05$ or $p<0.01$). This decrease corresponded to the decreased body weight gains observed in this group during gestation days 14-17 and 17-20 and was considered test article-related.

Mean food consumption was unaffected in the 50 and 150 mg/kg/day PFHxA groups during gestation; differences from the control group were slight. The only statistically significant ($p<0.05$ or $p<0.01$) lower food consumption was noted in the 50 mg/kg/day group during gestation days 0-4, 4-7 and 0-20 (g/animal/day and g/kg/day); however, the differences were not observed in a dose-related manner.

6.1.1.4.2.3. LACTATION

Mean food consumption was unaffected in the 50, 150 and 450/300 mg/kg/day PFHxA groups during lactation days 1-4. Differences from the control group were slight and not statistically significant.

6.1.1.5. CLINICAL PATHOLOGY

6.1.1.5.1. HEMATOLOGY

Summary Data: Tables 29, 30

Individual Data: Tables 102, 103, 104, 105

Test article-related effects of PFHxA on hematology were observed in males only and consisted of lower mean hemoglobin levels at all PFHxA dosage levels, and lower mean corpuscular hemoglobin (MCH) and lower mean corpuscular hemoglobin concentration (MCHC) levels and higher reticulocyte counts at 450/300 mg/kg/day.

Statistically significant ($p<0.05$ or $p<0.01$) lower mean hemoglobin, MCH and MCHC and statistically significant ($p<0.05$) higher absolute and percent reticulocyte counts were considered test article-related in males at 450/300 mg/kg/day at the study week 4 evaluation. In addition, lower mean hemoglobin levels were observed in 1/5 males (no. 74339) at 50 mg/kg/day and 2/5 males (nos. 74317 and 74326) at 150 mg/kg/day at the study week 4 evaluation. These lower hemoglobin levels were also considered test article-related as the values for these animals were lower than any of the hemoglobin values in the control group. These hematological changes are consistent with blood loss and/or iron deficiency (Brockus and Andreasen, 2003). Based on their magnitude, these hematological changes were not considered adverse and showed partial recovery in the males following the 2 week recovery period.

No other test article-related effects on hematology parameters were observed in the 50, 150 and 450/300 mg/kg/day groups.

6.1.1.5.2. SERUM CHEMISTRY

Summary Data: Tables 31, 32

Individual Data: Tables 106, 107, 108, 109

Test article-related effects of PFHxA on serum chemistry were observed only in the 450/300 mg/kg/day group males and consisted of lower mean globulin, total protein (secondary to lower globulin levels) and cholesterol levels.

Statistically significant ($p<0.05$ or $p<0.01$) lower mean total protein, globulin and cholesterol levels were observed in males at 450/300 mg/kg/day at study week 4 (end of dosing) and study week 7 (recovery) evaluations. Lower ($p<0.05$) mean globulin and cholesterol levels were also observed in the 150 mg/kg/day group males at study week 4. However, only the effects on mean total protein, globulin and cholesterol levels in the 450/300 mg/kg/day group at the study week 4 evaluation were considered test article-related. The statistically significant values observed in the 150 mg/kg/day group males and in the 450/300 mg/kg/day group males at the end of the recovery period (study

week 7 evaluation) were considered spurious and the result of biological variations, as individual values were similar to control group values. The statistically significant increase in the mean albumin/globulin ratio as well as the lower mean total protein in the 450/300 mg/kg/day group at study week 4 were most likely secondary to the lower mean globulin level at this interval.

Statistically significantly ($p<0.05$ or $p<0.01$) lower mean calcium levels were observed in males at 150 and 450/300 mg/kg/day at the study week 4 evaluation. However, the changes were minimal, and there were no concomitant changes affecting the other electrolytes measured. Therefore the lower mean calcium levels were considered incidental.

6.1.1.6. GESTATION LENGTH AND PARTURITION

Summary Data: Table 33

Individual Data: Table 110

Historical Control Data: Appendix K

Mean gestation lengths in the 50, 150 and 450/300 mg/kg/day PFHxA groups were similar to those in the control group. No statistically significant differences were noted. No signs of dystocia were noted in these groups.

6.1.1.7. ANATOMIC PATHOLOGY

6.1.1.7.1. MACROSCOPIC EXAMINATIONS

Summary Data: Tables 34 through 42

Individual Data: Tables 111 through 119

Test article-related macroscopic changes were restricted to the stomach in the 450/300 mg/kg/day group animals that died or were euthanized in extremis during the first 5 days of the study prior to the lowering of the dose level from 450 mg/kg/day to 300 mg/kg/day.

Test article-related changes in the stomach were noted in 2/4 males and 4/4 females at 450/300 mg/kg/day that were found dead or euthanized in extremis prior to the lowering of the dosage level and consisted of dark red discoloration or dark red or yellow areas and correlated to erosion and/or ulceration observed microscopically.

All other macroscopic changes were considered to be spontaneous and/or incidental in nature and unrelated to test article administration.

The mean number of implantation sites in the 450/300 mg/kg/day group (16.3 per dam) was lower than the control group (18.1 per dam). However, the difference was not statistically significant and was higher than the mean value in the WIL historical control data (15.5 per dam); therefore, this decrease was not considered test article-related. The mean number of unaccounted for sites in the 50, 150 and 450/300 mg/kg/day groups and the mean number of implantation sites in the 50 and 150 mg/kg/day groups were unaffected by test article administration; none of the differences were statistically significant.

6.1.1.7.2. ORGAN WEIGHTS

Summary Data: Tables 43 through 60
Individual Data: Tables 120 through 137

Test article-related effects of PHFxA on organ weights were limited to higher liver weights in males at 150 and 450/300 mg/kg/day and in females at 450/300 mg/kg/day at the primary necropsy (study week 4 for males and lactation day 4 for females). The increase persisted to the study week 7 necropsy in the males.

Statistically significantly ($p<0.05$ or $p<0.01$) higher mean relative (to final body weight) liver weights were observed in the 150 and 450/300 mg/kg/day group males. Similarly, females at 450/300 mg/kg/day had statistically significantly ($p<0.05$ or $p<0.01$) higher mean absolute and relative (to final body weight) liver weights. The higher liver weights correlated to hepatocellular hypertrophy microscopically. At the end of the recovery period, mean relative (to final body weight and brain weight) liver weights remained

statistically significantly ($p<0.05$ or $p<0.01$) increased in the 450/300 mg/kg/day group males compared to control group. Reversibility could not be evaluated in females due to mortality.

A statistically significantly ($p<0.05$) lower mean absolute thymus weight was observed in the 450/300 mg/kg/day group males. However, individual thymus weights for males in this group were within the same range as those of the control group males and lacked any microscopic correlate. Therefore, the statistical significance was considered spurious and not test article-related.

6.1.1.7.3. MICROSCOPIC EXAMINATIONS

Summary Data: Tables 61 through 68
Individual Data: Tables 111 through 119

Test article-related effects of PFHxA were noted in the kidneys, stomach, lymph nodes, thymus and spleen of the 450/300 mg/kg/day males and females in the liver of the 150 and 450/300 mg/kg/day group males and females and in the adrenal cortex of the 450/300 mg/kg/day group females. Changes in the kidneys, stomach, adrenal cortex and lymphoid organs were almost entirely limited to animals that died or were euthanized early, while hepatic changes were observed only in animals that were euthanized at the end of the dosing period.

Five of 15 males and 6/15 females in the 450/300 mg/kg/day group were found dead or euthanized in extremis. The animal numbers and sexes, as well as the days and causes of death/moribundity are listed in the table below.

Animals found dead or euthanized in extremis (450/300 mg/kg/day)

Animal no./sex	Disposition	Day of death	Cause of death
74303/M	EE	4	Undetermined
74316/M	EE	4	Papillary necrosis and tubular vacuolation, kidneys
74408/M	EE	4	Probable gavage error
74422/M	FD	4	Papillary necrosis in kidney Ulceration in non-glandular stomach
74388/M	FD	19	Probable gavage error
74444/F	FD	2	Papillary necrosis, kidneys
74514/F	FD	3	Erosion/ulceration in glandular stomach and esophagus
74466/F	FD	4	Erosion/ulceration in glandular stomach and duodenum
74505/F	EE	4	Erosion in glandular stomach and papillary necrosis in kidney
74534/F	FD	25	Undetermined
74511/F	FD	38	Undetermined

EE = Euthanized in extremis
FD = Found dead

The deaths/moribundity of 2 males (nos. 74316 and 74422) and 4 females (nos. 74444, 74466, 74505 and 74514) were considered test article-related, as they were caused by renal papillary necrosis and/or gastric erosion/ulcerations induced by the PFHxA test article. These 6 animals received PFHxA at 450 mg/kg/day only. One male (no. 74303) and 2 females (nos. 74511 and 74534) in this group had undetermined causes of death. However, male no. 74303 and female no. 74511 had lymphoid necrosis in the lymph nodes, thymus and/or spleen; therefore, test article administration could not be ruled out as the cause of death/moribundity. The deaths of the 2 males (nos. 74388 and 74408) that died following probable gavage errors were considered incidental deaths due to findings in the lungs, trachea and esophagus and not directly related to test article toxicity.

Test article-related effects in the kidneys consisted of papillary necrosis in 2/11 males and 5/15 females in the 450/300 mg/kg/day group at the unscheduled or primary necropsies. Of all the affected animals, only 1 female was euthanized at the scheduled

necropsy (lactation day 4) at the end of the treatment period, while all others (2 males and 4 females) were found dead or euthanized in extremis within the first 5 days of dosing (prior to the decrease in dosage level) and received PFHxA at 450 mg/kg/day. Papillary necrosis was mild to severe in all cases, and was most often bilateral. Additional changes such as mineralization, tubular vacuolation and/or dilatation were present as well. These changes were considered secondary to the papillary necrosis and not direct test article-related effects. These changes were not observed in the 4 males in the 450/300 mg/kg/day group at the recovery necropsy.

Test article-related erosions and/or ulcerations were observed at 450/300 mg/kg/day in 3/5 males and 4/6 females that died or were euthanized in extremis. None of the animals that were examined at the scheduled necropsies (at the end of the treatment or the recovery periods) had similar changes. The findings primarily affected the glandular portion of the stomach; the non-glandular stomach or other portions of the intestinal tract (esophagus, duodenum) were rarely involved.

Test article-related changes in the adrenal cortex were observed only in females at 450 mg/kg/day and were restricted to the zona fasciculata. These changes consisted of mild bilateral hyperplasia in 2/4 females that died or were euthanized in extremis prior to the reduction in dosage level. None of the animals examined at the scheduled necropsy (at the end of the treatment period) had similar changes.

Test article-related lymphoid necrosis and/or depletion were mostly noted in the lymph nodes, spleen and thymus in males and females that died or were euthanized in extremis in the 450/300 mg/kg/day group (see table below). In most cases, affected animals had similar changes in more than 1 lymphoid organ.

Incidence of test article-related changes in the lymphoid organs (450/300 mg/kg/day)

Finding	Control		450/300 mg/kg/day	
	M	F	M	F
Unscheduled Deaths				
No. examined	**0**	**0**	**5**	**6**
Mandibular lymph node				
Necrosis	-	-	-	2/6
Depletion	-	-	3/5	2/6
Mesenteric lymph node				
Necrosis	-	-	2/5	4/6
Depletion	-	-	2/5	-
Spleen				
Necrosis	-	-	1/5	4/6
Depletion	-	-	3/5	4/6
Thymus				
Necrosis	-	-	4/5	5/5
Atrophy	-	-	3/5	4/6
Primary Necropsy				
No. examined	**10**	**10**	**6**	**9**
Mesenteric lymph node				
Necrosis	-	-	-	2/9
Thymus				
Necrosis	1/10	-	-	-
Atrophy	-	-	-	3/9

Lymphoid necrosis in the mesenteric and mandibular lymph nodes affected mostly the B areas of the lymph nodes (germinal centers of reactive follicles) with the T areas (medullary cords and paracortex) being involved less frequently. Lymphoid depletion in the lymph nodes was observed mostly in the cortical area with decreased cellularity and no discernible B follicles left. Lymphoid depletion in the spleen affected mostly the B cell areas with B follicles and marginal zones absent or barely discernible. Lymphoid necrosis characterized by cellular debris and tangible bodies affected both T and B cell areas. Lymphoid necrosis in the thymus was characterized by a starry sky pattern (in the least severe cases) to a diffuse distribution of cellular debris and tangible bodies with few normal lymphocytes left (in the most severe cases).

Hepatocellular hypertrophy was identified in animals treated with PFHxA at 150 and 450/300 mg/kg/day euthanized at the study week 4 or lactation day 4 primary necropsy (see table below for incidence and severity). Hepatocellular hypertrophy was associated with slightly increased liver weights. Taken together, these findings are consistent with hepatic enzyme induction and were considered adaptive in nature (Amacher et al., 1998). The effect was completely reversible in the males; however, recovery could not be assessed in the females due to mortality.

Incidence and severity of hepatocellular hypertrophy at 150 and 450/300 mg/kg/day (primary or lactation day 4 necropsy)

Grade	150 mg/kg/day		450/300 mg/kg/day	
	M	F	M	F
Minimal	2/10	2/9	4/6	3/9
Mild	0/10	0/9	2/6	0/9

All other microscopic changes were consistent with normal background lesions in clinically normal rats of the strain and ages used in this study, and were considered to be spontaneous and/or incidental in nature and unrelated to test article administration.

6.1.2. F_1 LITTER DATA

6.1.2.1. PND 0 LITTER DATA AND POSTNATAL SURVIVAL

Summary Data: Tables 69, 70

Individual Data: Tables 138, 139

Historical Control Data: Appendix K

The mean number of pups born and the live litter size in the 450/300 mg/kg/day group (15.2 and 14.8 per dam, respectively) was lower (not statistically significantly) compared to the control group (17.1 and 16.9 per dam, respectively). However, the differences from the control group were not statistically significant and the values in the 450/300 mg/kg/day group were higher than the mean values in the WIL historical control data for mean number of pups born and live litter size (14.5 and 14.2 per dam, respectively), and were not considered test article-related. The mean number of pups born and live litter size in the 50 and 150 mg/kg/day groups were unaffected by test

article administration. The percentage of males at birth in the 50, 150 and 450/300 mg/kg/day groups were similar to the control group values. Postnatal survival in the 50, 150 and 450/300 mg/kg/day groups was unaffected by maternal test article administration.

6.1.2.2. GENERAL PHYSICAL CONDITION AND MORTALITIES

Summary Data: Table 71

Individual Data: Table 140

The numbers of F_1 pups found dead and/or missing, as well as the general physical condition of all F_1 pups in the PFHxA groups were unaffected by maternal test article administration. Pups that were found dead numbered 6, 6, 7 and 4 in the control, 50, 150 and 450/300 mg/kg/day PFHxA groups, respectively. Three, 0, 5 and 0 pups in the same respective groups were missing and presumed to have been cannibalized.

6.1.2.3. OFFSPRING BODY WEIGHTS

Summary Data: Tables 72, 73

Individual Data: Tables 141, 142

Historical Control Data: Appendix K

Mean male and female pup body weights and body weight changes in the 50, 150 and 450/300 mg/kg/day PFHxA groups were unaffected by maternal test article administration. No statistically significant differences from the control group were noted.

6.1.2.4. NECROPSIES OF PUPS FOUND DEAD

Summary Data: Table 74

Individual Data: Table 143

The numbers of pups (litters) found dead during PND 0-4 numbered 6(4), 6(6), 7(3) and 4(3) in the control, 50, 150 and 450/300 mg/kg/day groups, respectively. Aside from the presence or absence of milk in the stomach, malformations consisted of microphthalmia

and mandibular micrognathia and a variation of 14th rudimentary rib(s) were noted for pup no. 74500-01 in the 450/300 mg/kg/day group.

6.1.2.5. SCHEDULED PUP NECROPSIES

Summary Data: Table 75

Individual Data: Table 144

No internal findings that could be attributed to F_0 parental test article administration were noted at the necropsy of pups euthanized on PND 4. Internal findings included a hemorrhagic ring around iris (unilateral) in pup nos. 74459-02, 74456-10 and 74423-14 in the 50, 150 and 450/300 mg/kg/day groups, respectively, and malpositioned right kidney and cystic kidney in pup no. 74472-16 in the 450/300 mg/kg/day group. No other internal findings were noted.

6.1.3. TOXICOKINETIC EVALUATION

Summary and Individual Data: Appendices I and J

Oral administration of PFHxA at dosages of 50, 150 and 300 mg/kg/day resulted in systemic exposure to PFHxA. The exposures to PFHxA increased in proportion to the dosage. Exposures were 2- to 4-fold higher for male rats than for female rats. Exposure tended to decrease upon repeated oral dosing. For male rats, after 26 days of dosing, approximately 90% of the daily oral dose of PFHxA was eliminated in urine within 24 hours of dosing. For female rats, urinary elimination following oral administration of 50, 150 and 300 mg/kg/day PFHxA was approximately 100%, 80% and 70% of dose, respectively. The half-life for PFHxA in serum and for urinary elimination was approximately 2 to 3 hours.

The toxicokinetic parameters for PFHxA are summarized in the following table:

TOXICOKINETIC RESULTS

	PFHxA Results					
Gender/ PFHxA (mg/kg/day)	AUC (ng•h/mL)*	C_{max} (ng/mL)	t_{max} (h)	Serum half-life (h)	Urinary Elimination (% of dose)	Urinary Elimination Half-life (h)
Males						
Day 0						
50	499396	138758	1	2.2	NE	NE
150	915755	275011	1	2.4	NE	NE
300	2533093	385230	1	2.5	NE	NE
Day 25**						
50	354083	90484	1	2.2	87.6	2.0
150	886828	239887	1	2.7	87.8	2.1
300	1637875	240727	1	2.8	87.0	2.9
Females						
Day 0						
50	118291	58863	1	2.6	NE	NE
150	337337	217592	1	2.2	NE	NE
300	1048392	356289	1	2.1	NE	NE
Day 25**						
50	92228	39200	1	2.7	102	1.9
150	345543	158653	1	2.4	80.6	2.2
300	887031	277334	1	2.3	72.4	3.0

*$AUC_{0-\infty}$ on Day 0 and AUC_{0-24} on Day 25.

**Day 26 for urine parameters.

NE = not evaluated.

6.2. TEST ARTICLE C6-2 ALCOHOL

6.2.1. F_0 GENERATION

6.2.1.1. CLINICAL OBSERVATIONS AND SURVIVAL

Summary Data: Tables 1 through 11

Individual Data: Tables 76 through 84

Early deaths and euthanasia in extremis (1 male and 11 females) occurred only in the 225 mg/kg/day group. Male no. 74330 in the 225 mg/kg/day group was euthanized in extremis on study day 20. A body weight loss of 116 g was observed for this animal prior to euthanasia (study days 13 to 20). Clinical sings of hypoactivity, rapid respiration, eyes partially closed, yellow and red material on the urogenital area, unkempt appearance, red material on the forelimbs, nose and mouth, emaciation and decreased defecation were noted for this male. The cause of moribundity for this male was determined to be tubular degeneration of the kidney. All other males in the C6-2 alcohol-treated groups survived to the scheduled necropsy.

Female no. 74536 was euthanized in extremis prior to the mating period on study day 7, and female no. 74499 (not selected for pairing) was found dead on study day 18. Both females were emaciated, hypoactive and had unkempt appearances on the day of or 1-3 days prior to moribundity/mortality. The cause of death could not be determined microscopically for female no. 74536, but tubular vacuolation of the kidney was determined to be the cause of death for female no. 74499.

Seven females in the 225 mg/kg/day group died during gestation. Female no. 74440 was found dead on gestation day 1 and no. 74492 was euthanized in extremis on gestation day 3; both females had evidence of mating but died prior to implantation. Clinical findings for these females included red and yellow material on various body surfaces for female no. 74440, and female no. 74492 was emaciated, hypoactive, had no defecation and had rapid respiration. The cause of death/moribundity for female no. 74492 was determined to be ulceration of the vagina; however, the cause of death could not be

determined microscopically for female no. 74440. Female nos. 74488 and 74453 were found dead on gestation days 7 and 8, respectively, and had clinical findings of hypoactivity on the day prior to death and unkempt appearance on the day of death, respectively. Both females were gravid with normally developing implantations and microscopically, the causes of death were necrosis of the adrenal cortex for female no. 74488 and tubular degeneration and vacuolation of the kidney for female no. 74453. Female no. 74437 was found dead on gestation day 18 and was observed to be emaciated during gestation days 15-17; this female had an entirely resorbed litter and tubular degeneration and dilatation of the kidneys observed microscopically was determined to be the cause of death. Female no. 74425 died on gestation day 22 and female no. 74432 was euthanized in extremis on gestation day 24; both females had retained fetuses in utero noted at the macroscopic examinations. Apart from yellow material on various body surfaces noted for female no. 74425 during study days 14 and 15, no other clinical findings were noted at the daily examinations for this female. Clinical findings noted for female no. 74432 included body cool to touch, hypoactivity, hunched posture, eyes closed and labored respiration on the day of euthanasia indicative of dystocia. The cause of death for female no. 74425 was not determined by microscopic examination, and the cause of death for female no. 74432 was determined microscopically to be generalized depletion of the bone marrow.

Two females in the 225 mg/kg/day C6-2 alcohol-treated group died during lactation. Female no. 74446 was euthanized in extremis on lactation day 1 and had a body cool to touch and diarrhea on that day and female no. 74430 was found dead on lactation day 4 with red material on various body surfaces noted prior to death. The cause of death was determined microscopically to be necrosis of the adrenal cortex or tubular degeneration and dilatation of the kidneys for female nos. 74446 and 74430, respectively. All other females administered C6-2 alcohol survived to the scheduled necropsies.

Other test article-related clinical observations included red material around the nose or mouth and yellow material around the urogenital area for the 225 mg/kg/day group males

and females and decreased defecation for the 225 mg/kg/day group females. Test article-related clinical signs of clear material around the nose or mouth 1-2 hours following dose administration were observed in the 225 mg/kg/day group males and females. All test article-related clinical findings subsided following cessation of dose administration.

All other clinical findings for the C6-2 alcohol-treated groups were noted with similar incidence in the control group, were limited to single animals, were not noted in a dose-related manner and/or were common findings for laboratory rats of this age and strain.

6.2.1.2. REPRODUCTIVE PERFORMANCE

Summary Data: Table 12

Individual Data: Table 85

Historical Control Data: Appendix K

No test article-related effects on F_0 reproductive performance were observed at any dosage level. Male and female mating and fertility indices were 100.0%, 100.0%, 90.0% and 100.0% in the control, 25, 75 and 225 mg/kg/day groups, respectively. Male copulation and female conception indices were 100.0% for all groups. No statistically significant differences were noted between the control and test article-treated groups. One male in the 75 mg/kg/day group did not sire a litter.

The mean numbers of days between pairing and coitus in the test article-treated groups were similar to the control group value. None of these differences were statistically significant.

6.2.1.3. BODY WEIGHTS

Summary Data: Tables 13 through 20

Individual Data: Tables 86 through 93

6.2.1.3.1. MALES

Test article-related slightly lower (not statistically significant) mean body weight gains were observed in the 75 and 225 mg/kg/day group males prior to mating (study days 0-13) when compared to the control group. However, the decreases were not of sufficient magnitude to affect mean body weight in these groups prior to the mating period. Mean body weight gains in these 2 groups were lower following mating (study days 13-20, 20-27 and 27-32 [statistically significant, $p<0.01$ during study days 20-27 and 27-32 in the 225 mg/kg/day group only]). The lower mean body weight gains in the 75 mg/kg/day group males were exacerbated by body weight losses for male no. 74336, the only male in this group that lost weight during these intervals. These decreases resulted in statistically significant ($p<0.05$ or $p<0.01$) lower mean body weight gains in the 75 and 225 mg/kg/day group males when the entire dosing period (study days 0-32 for mated males and 0-34 for unmated males) was evaluated when compared to the control group values. Mean body weights in the 225 mg/kg/day group males were 6.4% to 8.8% lower than the control group during study days 20-32; the difference on study day 32 was statistically significant ($p<0.05$). Mean body weights in the 75 mg/kg/day group were 5.8% lower (not statistically significant) than the control group on study day 32. Following cessation of test article administration (study days 34-49), mean body weight gains in the 225 mg/kg/day group males were higher (not statistically significant) than the control group. Mean body weights in the 225 mg/kg/day group recovery males were 9.4% to 11.6% lower than the control group during study days 34-49; the differences were statistically significant ($p<0.01$).

There were no test article-related effects on body weights in the 25 mg/kg/day C6-2 alcohol-treated males. Differences from the control group were slight and were not statistically significant.

6.2.1.3.2. FEMALES

6.2.1.3.2.1. WEEKLY

No C6-2 alcohol-related effects were noted on mean body weight gains, cumulative body weight changes and body weights in the 25, 75 and 225 mg/kg/day group females during the pre-mating period (study day 0-13) or during the dosing period for unmated females in the 225 mg/kg/day group (study days 0-41). The values in the test article-treated groups were generally similar to the control group values for the entire study. Mean body weight gains, cumulative body weight changes and body weights in the 225 mg/kg/day group females were similar to the control group during the recovery period (study days 41-55). None of the differences from the control group were statistically significant.

6.2.1.3.2.2. GESTATION

Test article-related reductions in mean maternal body weight gains were noted in the 225 mg/kg/day C6-2 alcohol group during gestation days 0-4, 4-7, 17-20 (statistically significant, $p<0.01$) and when the entire gestation period (gestation days 0-20) was evaluated. Mean body weights in this group were similar to the control group on gestation days 0 and 4 but were up to 7.4% lower (not statistically significant) than the control group during gestation days 7-20.

Mean body weights and body weight gains in the 25 and 75 mg/kg/day C6-2 alcohol groups were generally similar to those in the control group throughout gestation. The differences from the control group were not statistically significant and did not occur in a dose-related manner.

6.2.1.3.2.3. LACTATION

Evaluation of maternal body weights and body weight gains in the 225 mg/kg/day C6-2 alcohol group was confounded by mortality. However, the 2 surviving females in this group had a body weight loss or a reduced body weight gain compared to the control

group mean during lactation days 1-4. As a result, body weights on lactation day 4 for these 2 females were up to 25.5% and 10.0% less than the mean control group value.

Mean body weights and body weight gains were unaffected by test article administration in the 25 and 75 mg/kg/day C6-2 alcohol groups during lactation days 1-4. Differences from the control group were slight and not statistically significant.

6.2.1.4. FOOD CONSUMPTION

Summary Data: Tables 21 through 28

Individual Data: Tables 94 through 101

6.2.1.4.1. MALES

Food consumption, evaluated as g/animal/day and g/kg/day, in the 25, 75 and 225 mg/kg/day C6-2 alcohol group males was unaffected by the test article during the pre-mating period for all animals and dosing period for unmated animals. The values in the test article-treated groups were generally similar to the control group values for the entire study. The only statistically significant ($p<0.05$) change in food consumption was an increase in g/kg/day noted in the 225 mg/kg/day group compared to the control group during study days 34-41.

6.2.1.4.2. FEMALES

6.2.1.4.2.1. WEEKLY

Food consumption, evaluated as g/animal/day and g/kg/day, in the 25, 75 and 225 mg/kg/day C6-2 alcohol group females was unaffected by the test article during the pre-mating period for all animals and dosing period for unmated animals. The values in the test article-treated groups were generally similar to the control group values for the entire study. Food consumption in the 225 mg/kg/day group was similar to the control group values during the recovery period (study days 41-55). None of the differences from the control group were statistically significant.

6.2.1.4.2.2. GESTATION

Mean maternal food consumption, evaluated as g/animal/day and g/kg/day, in the 25, 75 and 225 mg/kg/day C6-2 alcohol groups was unaffected by test article administration. The only statistically significant ($p<0.05$) difference from the control group was an increased g/kg/day value during gestation days 7-11 in the 225 mg/kg/day group.

6.2.1.4.2.3. LACTATION

Evaluation of mean maternal food consumption in the 225 mg/kg/day C6-2 alcohol group was confounded by mortality. Of the 2 surviving females in this group, 1 female had reduced food consumption (1 g/animal) when compared to the control group mean value during lactation days 1-4. The food consumption values for the other surviving female in this group were similar to the mean value in the control group.

Mean maternal food consumption was unaffected by test article administration in the 25 and 75 mg/kg/day C6-2 alcohol groups during lactation days 1-4. Differences from the control group were slight and not statistically significant.

6.2.1.5. CLINICAL PATHOLOGY

6.2.1.5.1. HEMATOLOGY

Summary Data: Tables 29, 30

Individual Data: Tables 102, 103, 104, 105

There were no test article-related effects on hematological parameters. Occasionally, statistically significant ($p<0.05$ or $p<0.01$) differences were observed when the 225 mg/kg/day C6-2 alcohol-treated group males were compared to the control group. These statistically significant differences were of minimal magnitude and were observed at the recovery evaluation (study week 7). Furthermore, there were no anatomic alterations which correlated with any of the statistically significant differences. Therefore, these changes were most likely the result of normal biological variation.

6.2.1.5.2. SERUM CHEMISTRY

Summary Data: Tables 31, 32

Individual Data: Tables 106, 107, 108, 109

Test article-related effects of C6-2 alcohol on serum chemistry consisted of higher mean albumin levels in the 225 mg/kg/day group males and females and higher mean total protein, globulin, potassium, urea nitrogen, creatinine, bilirubin, alanine aminotransferases (ALT) and aspartate aminotransferases (AST) levels as well as a lower mean sodium and chloride levels in the 225 mg/kg/day group females. Female no. 74430 was found dead on lactation day 4 between blood collection for serum chemistry evaluation and the scheduled necropsy on the same day. Prior to being euthanized in extremis, a blood sample for serum chemistry evaluation was collected from female no. 74432 in the 225 mg/kg/day group on gestation day 24. The data for female no. 74432 were excluded from the group mean values due to the different physiological state of this animal at the time of blood collection (gestation day 24 rather than lactation day 4). C6-2 alcohol-affected values on serum chemistry parameters were similar in female nos. 74430 and 74432 in the 225 mg/kg/day group.

Higher mean urea nitrogen and creatinine levels were considered test article-related in females at 225 mg/kg/day at the lactation day 4 evaluation. These changes are most likely related to the renal changes identified microscopically (see Section 6.2.1.7.3.).

Higher mean total bilirubin was observed in females at 225 mg/kg/day at the lactation day 4 evaluation. In addition, 2 females (nos. 74430 on lactation day 4 and 74432 on gestation day 24) in the 225 mg/kg/day group had elevated ALT and AST values. The differences in ALT and AST were considered test article-related, albeit of unknown biological significance based on the lack of correlating morphological changes.

Increases in mean albumin levels in the 225 mg/kg/day group males (statistically significant, $p<0.05$) and females and mean total protein and globulin levels in the 225 mg/kg/day group females only were considered test article-related at the primary

necropsy and lactation day 4 evaluations. The changes were of minimal magnitude, reversible and were most likely secondary to dehydration (Evans and Duncan, 2003). Statistically significant changes in the mean albumin/globulin ratios were considered secondary to the changes in albumin and/or globulin.

Lower mean sodium and chloride levels and higher mean potassium levels were observed in the 225 mg/kg/day group females at the lactation day 4 evaluation. The concurrent higher mean potassium level and lower mean sodium level led to a Na^+ / K^+ ratio that was markedly lower in this group compared to the control group (17 vs 26). Low Na^+ / K^+ ratio is generally associated with hypoaldosteronism or renal disease. However in the absence of significant pathological changes in the kidneys and adrenal glands of the females that were euthanized at the scheduled necropsies, this lower ratio is most likely secondary to the poor health status of 2/3 animals during late-term pregnancy (Wright, 2003). Chloride levels often parallel sodium levels and this change was considered an indirect effect of the test article.

Occasionally, statistically significant ($p<0.05$ or $p<0.01$) differences were observed when test article-treated group males were compared to the control group. These statistically significant differences were of minimal magnitude, did not display a clear dose-response relationship, were not biologically relevant and/or were inconsistent between the sexes. Furthermore, there were no anatomic alterations which correlated with any of the statistically significant differences. Therefore, none of the statistically significant differences were considered to be test article-related, and were most likely the result of normal biological variation.

6.2.1.6. GESTATION LENGTH AND PARTURITION

Summary Data: Table 33

Individual Data: Table 110

Historical Control Data: Appendix K

Mean gestation lengths in the 25, 75 and 225 mg/kg/day C6-2 alcohol groups were similar to those in the control group. No statistically significant differences were noted, although the sample size in the 225 mg/kg/day was reduced (n=3) due to dam mortality. Two females in this group were found dead (no. 74425 on gestation day 22) or in moribund condition (no. 74432 on gestation day 24) around the time of expected parturition. Only female no. 74432 had clinical signs of dystocia (hypoactivity body cool to touch and labored respiration and hunched posture); however, both females had normally developing fetuses retained in utero.

No signs of dystocia were noted at dosage levels of 25 or 75 mg/kg/day C6-2 alcohol.

6.2.1.7. ANATOMIC PATHOLOGY

6.2.1.7.1. MACROSCOPIC EXAMINATIONS

Summary Data: Tables 34 through 42

Individual Data: Tables 111 through 119

Test article-related macroscopic changes consisting of pale kidneys and small spleen were observed in 1/15 males at the primary necropsy and 1/11 females found dead or euthanized in extremis, respectively, in the 225 mg/kg/day group. The macroscopic findings correlated microscopically to renal changes and lymphoid depletion that were considered test article-related (see Section 6.2.1.7.3.).

All other macroscopic changes were considered to be spontaneous and/or incidental in nature and unrelated to test article administration.

The mean numbers of implantation sites and unaccounted-for sites in the 25, 75 and 225 mg/kg/day C6-2 groups were similar to the control group values. The differences from the control group were slight and not statistically significant.

6.2.1.7.2. ORGAN WEIGHTS

Summary Data: Tables 43 through 60

Individual Data: Tables 120 through 137

Test article-related effects of C6-2 alcohol on organ weights consisted of higher liver weights in males at all dose levels and in females at 75 and 225 mg/kg/day and higher kidney weights in males and females at 225 mg/kg/day.

Statistically significantly ($p<0.05$ or $p<0.01$) higher mean absolute and relative (to brain weight) liver weights were observed in the 75 and 225 mg/kg/day C6-2 alcohol group males, and mean relative (to final body weight) liver weights were statistically significantly ($p<0.01$) higher at all dose levels in the males. Higher mean absolute and relative (to final body weight and brain weight) liver weights were observed in the 75 and 225 mg/kg/day group females. The differences were only statistically significant ($p<0.01$) in the 75 mg/kg/day group females, as only 1 female in the 225 mg/kg/day group survived to the scheduled necropsy at the end of the treatment period. However, this female had higher absolute and relative liver weights as well. The effect was considered test article-related and correlated to hepatocellular hypertrophy observed microscopically in the 225 mg/kg/day group males only. Similar effects on the liver were not observed at the recovery necropsy; therefore, the liver enlargement was completely reversible.

Statistically significant ($p<0.01$) higher mean relative (to final body weight) kidney weights were observed in the 225 mg/kg/day C6-2 alcohol group males at the primary necropsy. The effect was due primarily to 2 males (nos. 74312 and 74332) that had markedly high values. The higher kidney weights for these males corresponded to test article-induced tubular degeneration and tubular dilatation observed microscopically (see

Section 6.2.1.7.3.). Therefore, the higher mean kidney weights were considered test article-related. In addition, the single 225 mg/kg/day group female that survived to the lactation day 4 necropsy had elevated absolute and relative kidney weights. Although there were no significant renal changes in this animal, the effect was most likely test article-related.

No other test article-related effects on organ weights were observed in the C6-2 alcohol-treated males and females. Statistically significant ($p<0.05$ or $p<0.01$) changes in mean absolute and relative (to final body weight and brain weight) spleen weights were observed at the end of the recovery period in the 225 mg/kg/day C6-2 alcohol group males. Since a similar effect was not present at the end of the treatment period, these changes were considered incidental and not test article-related.

6.2.1.7.3. MICROSCOPIC EXAMINATIONS

Summary Data: Tables 61 through 68
Individual Data: Tables 111 through 119

Test article-related effects of C6-2 alcohol were observed in the kidneys, pancreas, sternal bone marrow and lymphoid tissues in the 225 mg/kg/day group males and females; in the liver in the 225 mg/kg/day group males; and in the adrenal cortex, jejunum and ileum in the 225 mg/kg/day females. Following the recovery period, test article-related changes in the kidneys in males and the thymus in the females were also observed in the 225 mg/kg/day group.

One of 15 males and 11/15 females were found dead or euthanized in extremis during the course of the study. The animal numbers, the day and the cause of death are listed in the table below.

Animals found dead or euthanized in extremis (225 mg/kg/day group)

Animal no./sex	Disposition	Day of death	Cause of death
74330/M	EE	20	Tubular degeneration, kidney
74425/F	FD	GD 22	Undetermined
74430/F	FD	LD 4	Tubular degeneration/dilatation, kidney
74432/F	EE	GD 24	Depletion, bone marrow
74437/F	FD	GD 18	Tubular degeneration/dilatation, kidney
74440/F	FD	GD 1	Undetermined
74446/F	EE	LD 1	Tubular degeneration, kidney Necrosis, adrenal cortex
74453/F	FD	GD 8	Tubular degeneration/vacuolation, kidney
74488/F	FD	GD 7	Necrosis, adrenal cortex
74492/F	EE	GD 3	Ulceration, vagina
74499/F	FD	18	Tubular degeneration/vacuolation, kidney
74536/F	EE	7	Undetermined

EE = Euthanized in extremis
FD = Found dead
GD = Gestation day
LD = Lactation day

The unscheduled euthanasia of female no. 74492 was considered incidental to treatment with C6-2 alcohol as the clinical status of the animal was most likely related to the spontaneous finding of vaginal ulceration. All the other deaths were considered test article-related, based on their occurrence at 225 mg/kg/day, whether they were due to test article-related changes (kidney, adrenal cortex or bone marrow) or were considered of undetermined origin.

Test article-related effects in the kidneys consisted of tubular degeneration, tubular dilatation and tubular vacuolation. Most of the affected animals had a combination of renal findings, which involved mostly the convoluted proximal tubules. Although the tubular changes were considered the cause of death/moribundity in several animals,

similar changes were also noted in males that were euthanized at the end of treatment. The incidence and severity of these changes are presented in the table below.

Incidence and severity of test article related renal tubular changes at 225 mg/kg/day (unscheduled deaths or primary necropsy)

	M	F
Degeneration		
Minimal	1/10	1/12
Mild	2/10	3/12
Moderate	1/10	3/12
Dilatation		
Minimal	1/10	1/12
Mild	1/10	3/12
Moderate	1/10	2/12
Vacuolation		
Minimal	0/10	4/12
Mild	0/10	2/12

Tubular degeneration consisted of basophilic to eosinophilic epithelial cells that often had unclear cellular borders and contained eosinophilic amorphous cytoplasmic material. Less frequently, there was evidence of necrosis or increased number of cells. Tubular dilatation was present in the cortex as well as the medulla and affected tubules were often lined by flattened basophilic cells. Tubular vacuolation affected mostly the collecting tubules and consisted of hydropic ballooning. In addition, moderate mineralization of the proximal convoluted tubules was present in 1/10 males in the 225 mg/kg/day group. The change was moderate in the male and involved the proximal convoluted tubules. It was most likely secondary to the tubular degeneration associated with necrosis noted in this animal and was not directly related to the test article. In addition, systemic mineralization in the stomach, heart and/or aorta were also observed in 1/10 males and 4/12 females in the 225 mg/kg/day group and was considered secondary to the uremic status of these animals (Hard and Khan, 2004) and not a direct effect of the test article. Following the recovery period, 3/5 males in the 225 mg/kg/day group had minimal to mild chronic progressive nephropathy. Although this change is common in older

Sprague Dawley rats, the relatively high incidence for this age group is most likely due to an exacerbation of a spontaneous process by the test article.

Test article-related changes in the adrenal cortex were observed only in females in the 225 mg/kg/day C6-2 alcohol group and were restricted to the zona fasciculata. The findings consisted of minimal to mild hyperplasia in 4/12 females (3 females that died or were euthanized in extremis during the study), minimal to severe necrosis in 6/12 females (all of which were unscheduled deaths) and minimal to moderate acute inflammation in 7/12 females (all unscheduled deaths). While the necrosis was often unilateral, the acute inflammation tended to be bilateral and associated with either the necrosis or the hyperplasia. Alternatively, unilateral inflammation was present when severe necrosis occurred only in the contralateral adrenal. There were no test article-related changes following the recovery period.

Test article-related changes were noted in the sternal bone marrow of the 225 mg/kg/day C6-2 alcohol group animals that died or were euthanized early. They consisted of myeloid hyperplasia with erythroid depletion in 5/11 females and the 1 male that were euthanized in extremis or generalized depletion with myelofibrosis in 2/11 females. The myeloid hyperplasia and the erythroid depletion were noted in the same animals and tended to occur earlier in the study.

Minimal to mild villous atrophy was observed in 2/11 females in the 225 mg/kg/day C6-2 alcohol group that were found dead or euthanized in extremis. The change was minimal to mild and was characterized by short and blunted villi lined by basophilic cells. Based on the occurrence at 225 mg/kg/day, the change was considered test article-related, albeit of an unknown significance.

Test article-related lymphoid depletion and/or necrosis were noted in all the 225 mg/kg/day C6-2 alcohol group animals that were found dead. The changes affected the mandibular lymph node, the mesenteric lymph node, the spleen and the thymus. In most cases, affected animals had similar changes in more than 1 lymphoid organ.

Lymphoid depletion changes were more common and/or severe than lymphoid necrosis. Thymus atrophy was often the most severe change in the lymphoid organs with barely any lymphocytes left. In addition, 3/3 females in the 225 mg/kg/day group at the study week 7 recovery necropsy had minimal lymphoid necrosis in the thymus that was considered test article-related.

Decreased secretion and single cell necrosis of the pancreas were considered test article-related at 225 mg/kg/day C6-2 alcohol (see incidence table below). The changes were more severe in females than in males based on incidence and grading (minimal to mild in males and minimal to severe in females). The necrosis was characterized by scattered acinar cells with autophagic vacuoles. The change was completely reversible.

Incidence of test article related pancreatic changes at 225 mg/kg/day
(There were 10 males and 12 females at primary necropsy or unscheduled deaths)

	225 mg/kg/day	
	M	F
Decreased secretion	4/10	10/12
Necrosis	1/10	7/12

Minimal to mild hepatocellular hypertrophy was noted in 3/10 males in the 225 mg/kg/day C6-2 alcohol group, either unscheduled death or at the primary necropsy. This change was associated with slightly increased liver weights (see Section 6.2.1.7.2.). Taken together, these findings are consistent with hepatic enzyme induction and are considered adaptive in nature (Amacher et al., 1998). The hepatic effects were completely reversible.

There was a dose-related decrease in the incidence of physiologic hyperplasia of the mammary glands in females treated with C6-2 alcohol. Although the low incidence at 225 mg/kg/day was, in large part, related to the stage of gestation at which the females died, only 7/10 females in the 75 mg/kg/day group had physiologic hyperplasia despite a 100% gestation and delivery rate. However, pup growth and survival in the 75 mg/kg/day group were comparable to the control group. Therefore, the change in physiologic hyperplasia was considered of no physiologic significance.

A decreased number of females with implantation sites in the uterus and an increased incidence of mucification in the vagina were noted in the 225 mg/kg/day C6-2 alcohol group. These changes affected unscheduled death females and could be related to the different gestation statuses at the time of death and may not be a direct effect of the test article.

All other microscopic findings were consistent with normal background lesions in clinically normal rats of the strain and ages used in this study, and were considered to be spontaneous and/or incidental in nature and unrelated to test article administration.

6.2.2. F_1 Litter Data

6.2.2.1. PND 0 Litter Data And Postnatal Survival

Summary Data: Tables 69, 70

Individual Data: Tables 138, 139

Historical Control Data: Appendix K

Evaluation of PND 0 litter data and postnatal survival was confounded in the 225 mg/kg/day C6-2 alcohol group by dam mortality; only 3 females in this group survived to lactation. There was a high incidence of pup mortality in 2 of these 3 litters; 17 of 18 pups in litter no. 74446 were found dead on PND 0 or 1 and 18 of 18 pups in litter no. 74430 were found dead between PND 0 and 4. All pups in litter no. 74467 survived to the scheduled euthanasia on PND 4. As a result of the pup mortality in this group, the mean live litter size on PND 0 in this group (13.3 pups per dam) was slightly reduced compared to the control group (15.5 pups per dam) and the WIL historical control data (14.2 pups per dam). Decreases were also noted for postnatal survival from birth to PND 0, PND 0-1, PND 1-4 and birth to PND 4 (87.0%, 67.5%, 50.0% and 50.0% per litter, respectively) when this group was compared to the control group (96.4%, 100.0%, 98.1% and 94.6% per litter, respectively) and the mean values in the WIL historical control data (98.0%, 99.0%, 99.0% and 96.1% per litter, respectively).

The mean number of pups born and the live litter size in the 25 and 75 mg/kg/day C6-2 alcohol groups and the percentage of males at birth in all C6-2 alcohol groups were similar to the control group values. Postnatal survival in the 25 and 75 mg/kg/day C6-2 alcohol groups was unaffected by maternal test article administration. Postnatal survival from birth to PND 4 in the 25 mg/kg/day group was slightly lower than the control group value due to dam no. 74462 with a litter of 1 pup that died on PND 1.

6.2.2.2. General Physical Condition And Mortalities

Summary Data: Table 71

Individual Data: Table 140

Pups (litters) that were found dead or euthanized in extremis between PND 0 and 4 numbered 8(4), 4(3), 2(2) and 35(2) in the control, 25, 75 and 225 mg/kg/day C6-2 alcohol groups, respectively. One, 4, 6 and 0 pups in the same respective groups were missing and presumed to have been cannibalized. The litter of dam no. 74446 in the 225 mg/kg/day group was sent to necropsy due to the death of the dam. The general physical condition of the F_1 pups was unaffected by F_0 parental test article administration.

6.2.2.3. Offspring Body Weights

Summary Data: Tables 72, 73

Individual Data: Tables 141, 142

Historical Control Data: Appendix K

Evaluation of offspring body weights in the 225 mg/kg/day C6-2 alcohol group was confounded by dam and pup mortality; only 3 females delivered, and a single litter survived past PND 1. Of the surviving litters in the 225 mg/kg/day group, PND 1 and 4 body weight gains and body weights were lower than the mean control group values.

Mean F_1 male and female pup body weights and body weight changes in the 25 and 75 mg/kg/day C6-2 alcohol groups were unaffected by F_0 parental test article administration. No statistically significant differences from the control group were noted.

6.2.2.4. NECROPSIES OF PUPS FOUND DEAD

Summary Data: Table 74

Individual Data: Table 143

The numbers of pups (litters) found dead or euthanized in extremis during PND 0-4 numbered 8(4), 4(3), 2(2) and 35(2) in the control, 25, 75 and 225 mg/kg/day C6-2 alcohol groups, respectively. Aside from the presence or absence of milk in the stomach, variations consisting of renal papilla(e) not fully developed, distended intestine and/or distended urinary bladder were noted for pup nos. 74430-10 and 74430-12 in the 225 mg/kg/day group.

6.2.2.5. SCHEDULED PUP NECROPSIES (PND 4)

Summary Data: Table 75

Individual Data: Table 144

No internal findings that could be attributed to F_0 parental test article administration were noted at the necropsy of pups euthanized on PND 4. Aside from the absence of milk noted in pup no. 74446-16 in the 225 mg/kg/day group that was euthanized on LD 1 due to death of dam, no internal findings were noted.

7. DISCUSSION

This study was designed to evaluate the potential toxic effects of perfluorohexanoic acid (hereafter referred to as PFHxA) or 1H, 1H, 2H, 2H-tridecafluoro-1-octanol (hereafter referred to as C6-2 alcohol) when administered to rats for 28 days and to evaluate the potential of PFHxA or C6-2 alcohol to affect male and female reproductive performance, such as gonadal function, mating behavior, conception, parturition and early postnatal development.

7.1. TEST ARTICLE PFHXA

Five of 15 males and 6/15 females in the 450/300 mg/kg/day group were found dead or euthanized in extremis prior to the scheduled necropsies. The early terminations of 4 males and 4 females occurred within the first five days of dosing when the dose level was 450 mg/kg/day. The cause of death of these animals was considered test article-related for 2 males and all four females and was due to papillary necrosis, gastric ulceration or a combination of both. The cause of death of the remaining 2 males that were euthanized in extremis on day 4 of the study was undetermined in one male based on the lack of significant morphological changes upon microscopic examination and incidental in the other male based on lesions consistent with a gavage error. Because of the early mortality at 450 mg/kg/day, the dosage level for the high dose was lowered to 300 mg/kg/day for the rest of the dosing period. One male and 2 females were found dead during this dosing period. However, the cause of death for these animals was considered incidental for the male based on microscopic findings consistent with a gavage error, and of undetermined origin for the 2 females as they did not exhibit any significant clinical signs and/or microscopic findings that could definitively link their death to the test article. Therefore, while the 450 mg/kg/day dose level was clearly above the maximum tolerated dose, effects on survival at the 300 mg/kg/day dose level were uncertain.

A wide range of test article-related clinical signs were noted in the 450/300 mg/kg/day group while test article-related clinical signs in the 150 mg/kg/day group were limited to

red material around the nose (the incidence for this finding was higher at the 450/300 mg/kg/day dose level). Clinical signs were more numerous and more severe in the animals that died or were euthanized in extremis at 450 mg/kg/day (coolness of body/limbs, emaciation, unkempt appearance, hypoactivity, partial to complete eye closure, tremors, hunched posture). These observations were consistent with a high degree of systemic toxicity and were considered the clinical correlates to the severe morphological changes noted in these animals. Additional less severe clinical signs were present at 450 mg/kg/day and 300 mg/kg/day regardless of the disposition status of the animals (unscheduled and scheduled termination). These clinical signs consisted of rales, gasping, material/discharge around the urogenital/anogenital areas, mouth and/or nose and salivation prior to dosing. These findings are evidence of non-specific mild systemic toxicity caused by the test article as the affected animals had little to no clinical or anatomic pathology changes. One of these clinical observations was red material around the nose and was noted in a dose-related manner at 150 mg/kg/day and 450/300 mg/kg/day. This is a common finding in laboratory animals and is generally considered of no toxicological significance in the absence of other findings.

The mortality and clinical signs noted at 450 mg/kg/day were also associated with marked body weight losses and lower food consumption. The lowering of the high dose to 300 mg/kg/day was associated with a return of the food consumption to control levels in males and females. However, body weights remained lower for the 300 mg/kg/day males through the dosing period. The effect on male body weight, although considered adverse, was reversible as the affected animals had higher body weight gains compared to the control groups during the recovery period. Administration of 300 mg/kg/day of the test article did not have a significant effect on the body weight gains and/or food consumption in females, except transiently during the late gestation period when both body weight gains and food consumption were reduced compared to the control groups. The significance of this change is unclear as the body weight gains and food consumption levels returned to control values during lactation and there were no test article-related effects on litter size and pup body weights.

Despite the evidence of overt toxicity at the 450/300 mg/kg/day dosage level, reproductive performance, precoital interval and gestation length were unaffected by PFHxA administration at all dosage levels.

Test article-related clinical pathological changes were present in males only. Based on their magnitude the hematological and serum chemistry findings were not considered adverse and were reversible at 450/300 mg/kg/day. Hematological changes consisted of decreased MCH and MCHC and increased reticulocyte counts at the high dose only while hemoglobin was decreased at all dose levels. These changes are generally associated with blood loss/iron deficiency (Brockus and Andreasen, 2003). Serum chemistry changes consisted of decreased globulin (with subsequent decreased total protein) and decreased cholesterol at 450/300 mg/kg/day.

Several target organs/tissues were identified following the administration of the test article: kidneys, stomach, lymphoid organs, adrenal cortex and liver. Changes in the kidneys, stomach, adrenal cortex and lymphoid organs were restricted to the 450/300 mg/kg/day dose level and were mostly present in the animals that died prematurely before dosage reduction. These lesions were considered adverse based on their severity and/or their link to the premature death of the affected animals.

The renal changes consisted of mild to severe papillary necrosis. The change was present in 2 males and 5 females at the high dose and was considered the cause of death for the 2 males and 2 of these females that died or were euthanized in extremis within the first 5 days of the study. However, both the 300 and 450 mg/kg/day dose levels induced this change as it was present in one female that was euthanized on schedule. The test article induced gastric changes of ulceration/erosion at the 450 mg/kg/day dose level only in 3 males and 4 females that died or were euthanized in extremis within the first 5 days of dosing. The change was identified grossly at necropsy in several of the affected animals and was considered the cause of death in 1 of these 3 males and 3 of these 4 females. The glandular mucosa was the primary target for this finding with the non-glandular portion of the stomach and the duodenum or the esophagus being rarely involved as well.

This effect could be secondary to a local irritant effect related to the very low pH of the test article formulation at 450 mg/kg/day. However the pH of the dosing formulations at 300 mg/kg/day were also low and there was no evidence of gastric irritation after the dose level reduction. Therefore, the ulcerations could be related to a systemic toxic effect of the test article as other target organs were identified at this dose level as well.

The lymphoid organ changes affected the lymph nodes, spleen and thymus and consisted of lymphoid necrosis and/or depletion of the T and B areas. Lesions in the animals that died prematurely were severe and widespread. Whether they were a contributing factor to the death or were secondary to the poor clinical status of the affected animals during the dose administration of 450 mg/kg/day can not be ascertained. A small number of females that were euthanized on schedule had milder lesions in the thymus and/or mesenteric lymph node, which indicated that the 300 mg/kg/day dose level induced lymphoid organ changes despite the absence of marked toxicity and/or stress (affected females had normal body weight gain and food consumption, no effects on reproductive parameters). None of the males that were euthanized on schedule demonstrated any test article-related lymphoid changes. The statistically significant decrease in thymus weight in the high dose males at the primary necropsy was considered spurious in the absence of any microscopic changes in these animals and could have been related to the reduced body weights at this dose level as adipose tissue is often sampled along with the thymus at necropsy.

Changes in the liver consisted of enlargement (increased liver weights, centrilobular hypertrophy) and were present in a dose related manner at 150 and 450/300 mg/kg/day in the animals that were sacrificed at the end of the study. The liver enlargement was not adverse and considered adaptive to hepatic enzyme induction (Amacher et al., 1998).

7.2. TEST ARTICLE C6-2 ALCOHOL

One of 15 males and 11/15 females in the 225 mg/kg/day C6-2 alcohol-treated group were found dead or euthanized in extremis. These deaths were considered test article-related in all animals except for one female with an incidental finding of moderate

vaginal ulceration. The higher incidence of early termination in females could be related to an increased sensitivity for this sex due to the physiological changes in pregnancy. The early terminations of these animals were related to tubular lesions in the kidneys (1 male, 5 females), necrosis of the adrenal cortex (2 females) and/or bone marrow depletion (1 female). In addition, 3 females had no significant pathological changes that could explain the early death/euthanasia; however these deaths were considered test article-related as well based on their occurrence at the high dose and the high incidence of test article-related deaths in females at 225 mg/kg/day. Clinical findings for these animals that died or were euthanized early included emaciation, hypoactivity, unkempt appearance, body cool to touch and labored respiration. These observations were consistent with a high degree of systemic toxicity and were considered the clinical correlates to the morphological changes noted in these animals. Less severe clinical signs were observed in the 225 mg/kg/day animals that survived to the scheduled necropsies. They included material/discharge around the urogenital/anogenital, mouth and/or nose for both sexes and decreased defecation for females. These findings are evidence of non-specific mild systemic effect of the test article as the affected animals had little to no clinical or anatomic pathology changes. All test article-related clinical findings subsided following cessation of the C6-2 alcohol administration. There were no test article-related clinical observations in the 25 and 75 mg/kg/day group males and females.

Reproductive performance, precoital interval and gestation length were unaffected by C6-2 alcohol administration at all dosage levels. One female in the 225 mg/kg/day group that was euthanized on gestation day 24 had clinical signs consistent with dystocia (hypoactivity, body cool to touch, labored respiration and hunched posture); however these findings were most likely secondary to the systemic general toxicity of the test article based on the generalized bone marrow depletion and renal changes identified in this female. The evaluation of the litter data was confounded in the 225 mg/kg/day group by dam mortality. However, the high incidence of pup mortality and the lower mean F_1 male and female pup weights of the surviving litters present at 225 mg/kg/day were most likely test article-related.

Test article administration reduced body weight gains in males in the 75 and 225 mg/kg/day groups for both males selected and not selected for mating. Reduced body weight gains did not become statistically significant until after mating was initiated (study day 13). In the absence of any changes in the food consumption for these groups compared to the control group, the effect on body weight gain was considered a direct effect of the systemic toxicity of the test article. Following the cessation of C6-2 alcohol administration, mean male body weight gains in these 2 groups were similar to the control group. The test article reduced body weight gains in females treated at 225 mg/kg/day only during the gestation period. As with the males, there were no changes in food consumption compared to the control group during that period and the reduced body weight gains were considered to be directly related to the systemic toxicity of the test article. During the lactation period, body weight loss with no effect on food consumption or reduced body weight gain with concomitant lower food consumption were noted in the 2 females of the 225 mg/kg/day group that remained in the study for evaluation. Although the number of animals was limited, based on the findings during the previous reproductive phase, this effect on body weights was also considered related to the test article.

Test article-related clinical pathology findings affected the following serum chemistry parameters in the 225 mg/kg/day group only: increases in urea nitrogen and creatinine in females, decreases in sodium and chloride and increases in potassium in females, increases in bilirubin, AST and ALT in females, increases in total protein and globulin in females and increases in albumin in males and females. As with the effect on survivability, heightened sensitivity to the test article due to the physiological changes of gestation could explain why females were more severely affected than males. Increases in urea nitrogen and creatinine in females were considered related to the tubular changes (degeneration, vacuolation and/or dilatation) noted in the kidneys at 225 mg/kg/day. Decreases in sodium and chloride and increases in potassium in the 225 mg/kg/day females led to a Na^+ / K^+ ratio that was markedly reduced compared to the control group values. Low Na^+ / K^+ ratios have been related to poor health during late-term pregnancy

(Wright, 2003) and as such this effect could be secondary to the systemic toxicity of the test article. Chloride levels often parallel sodium levels and the decreased chloride was considered an indirect effect of the test article. The increases in total bilirubin, AST and ALT were considered test article-related in females at 225 mg/kg/day, albeit of unknown biological significance based on the lack of correlating hepatic changes. Minimal increases in total protein and globulin in females and increases in albumin in males and females were most likely secondary to dehydration (Evans and Duncan, 2003) induced by the test article.

Several target organs/tissues were identified following the administration of the test article at the 225 mg/kg/day dose level: kidneys, adrenal cortex, bone marrow, lymphoid organs, pancreas and small intestine. In addition, liver enlargement was considered test article-related at all dose levels in males and at 75 and 225 mg/kg/day in females based on organ weights. Hepatic centrilobular hypertrophy identified in the 225 mg/kg/day males confirmed the liver enlargement which was consistent with hepatic enzyme induction and was considered adaptive in nature (Amacher et al., 1998).

Test article-related effects in the kidneys of tubular degeneration, tubular dilatation and tubular vacuolation were present in males and females at 225 mg/kg/day. These microscopic findings were associated with pale kidneys and higher kidney weights at necropsy. Most affected animals had a combination of renal findings which involved mostly the convoluted proximal tubules. Although the tubular changes were considered the cause of death in 1 male and 5 females, similar changes were also noted in males that were euthanized at the end of treatment. Systemic mineralization in the stomach, heart and/or aorta in 1/10 males and 4/12 females at 225 mg/kg/day confirmed the uremic status of these animals (Hard and Khan, 2004) and the adverse nature of the renal changes. Following the recovery period, a higher than normal incidence of chronic progressive nephropathy in males at 225 mg/kg/day was considered an exacerbation of a spontaneous process by the test article and demonstrated incomplete reversibility.

Test article-related changes in the adrenal cortex were restricted to the zona fasciculata and were mostly present in females that died prematurely at 225 mg/kg/day (one female with hyperplasia was euthanized on schedule, all the other affected animals died prematurely). The changes consisted of necrosis, acute inflammation and/or hyperplasia. Various bone marrow changes were considered test article-related at 225 mg/kg/day: myeloid hyperplasia with erythroid depletion or generalized depletion. All but 1 of the affected animals were females. The myeloid hyperplasia and the erythroid depletion were noted in the same animals and occurred in animals with earlier deaths/euthanasia. Villous atrophy of the jejunum and/or ileum was present in 2 females that were found dead or died prematurely at 225 mg/kg/day. Based on the occurrence at the high dose, the change was considered test article-related, albeit of an unknown significance. Decreased secretion and single cell necrosis of the pancreas were considered test article-related at 225 mg/kg/day. The changes were more severe in females than in males based on incidence and grading (minimal to mild in males, minimal to severe in females) and was completely reversible. In the absence of a marked reduction in food consumption in the affected animals, this finding was considered a direct effect of the test article.

Test article-related lymphoid depletion and/or necrosis were present in all the 225 mg/kg/day animals that died during the course of the study. The changes affected the mandibular lymph node, the mesenteric lymph node, the spleen and the thymus. In most cases, affected animals had similar changes in more than one lymphoid organ. Lymphoid depletion occurrences were more prevalent and/or severe than lymphoid necrosis occurrences. Thymus atrophy was often the most severe change in the lymphoid organs with few lymphocytes remaining. The minimal lymphoid necrosis present in the thymus of all of the 225 mg/kg/day females examined at the end of the recovery period was considered test article-related as well and indicative of a lack of complete reversibility.

8. CONCLUSIONS

8.1. TEST ARTICLE PFHxA

Based on the results of this study, systemic toxicity was manifested at a dosage level of 450/300 mg/kg/day as evidenced by the following. Mortalities, changes in the clinical condition of the animals, effects on body weight, food consumption, hematology (lower mean hemoglobin levels, mean corpuscular hemoglobin levels and mean corpuscular hemoglobin concentration levels and higher mean reticuloycte counts), serum chemistry (lower mean serum globulin, total protein and cholesterol levels), macroscopic findings (consisting of dark red discoloration or dark red or yellow areas in the stomach), organ weights (higher mean liver weights) and microscopic changes (affecting the kidneys, stomach, lymph nodes, spleen, thymus and liver. No reproductive or neonatal toxicity was observed at dosage levels of 50, 150 or 300 mg/kg/day PFHxA. Therefore, a dosage level of 300 mg/kg/day was considered to be the no-observed-effect level (NOEL) for reproductive and neonatal toxicity and the no-observed-adverse-effect level (NOAEL) for systemic toxicity was considered to be 150 mg/kg/day PFHxA.

8.2. TEST ARTICLE C6-2 ALCOHOL

Based on the results of this study, systemic toxicity was manifested at a dosage level of 75 (effects on body weight and body weight gain only) and 225 mg/kg/day (as evidenced by the following). Mortalities, changes in the clinical condition of the animals, effects on body weight, food consumption, serum chemistry (higher mean albumin, total protein, globulin, potassium, urea nitrogen, creatinine, bilirubin, alanine aminotransferase and aspartate aminotransferase levels and lower mean sodium and chloride levels), macroscopic findings (consisting of pale kidneys: males and small spleen: females), organ weights (higher mean liver and kidney weights) and microscopic changes (kidneys, pancreas, sternal bone marrow and lymphoid tissues, liver, adrenal cortex, jejunum and ileum). Dystocia was noted at the 225 mg/kg/day dosage level. Of the surviving dams and litters that could be evaluated during lactation days 1-4, decreases in the mean numbers of pups born, postnatal survival and F_1 pup weights were noted at 225 mg/kg/day. Therefore, a dosage level of 75 mg/kg/day was considered to be the

marginal lowest-observed-effect level (LOEL) for systemic toxicity and the no-observed-effect level (NOEL) for the reproductive and neonatal toxicity. The NOEL for systemic toxicity was 25 mg/kg/day.

9. KEY STUDY PERSONNEL AND REPORT SUBMISSION

Report Submitted By:

Jeannie B. Kirkpatrick, MS Staff Toxicologist Study Director	Date

Pathologist of Record:

Joelle D. Ibanes, DVM, DACVP Senior Pathologist	Date

Report Prepared By:

Renee B. Twining, BS Study Analyst	Date

Report Reviewed By:

Christopher P. Chengelis, PhD, DABT Director, Toxicology	Date
John F. Knapp, BS Staff Toxicologist, Developmental, Reproductive and Neurotoxicology	Date
Mark D. Nemec, BS, DABT Director, Developmental and Reproductive Toxicology	Date
Julie Sherman, BS, RAC Group Supervisor, Study Analysis and Reports	Date

KEY STUDY PERSONNEL AND REPORT SUBMISSION (CONTINUED)

Donald G. Stump, PhD, DABT Associate Director, Developmental and Reproductive Toxicology	Date
Evelyn Tanchevski, BS Group Supervisor, Study Analysis and Reports	Date
Robert A. Wally, BS, RAC Acting Manager, Reporting and Regulatory Technical Services	Date

Study Personnel:

Susan C. Haley, BS	Manager, Clinical Pathology
Sally A. Keets, AS	Senior Operations Manager, Vivarium
Carol A. Kopp, BS, LAT	Manager, Gross Pathology and Developmental Toxicology Laboratory
Theresa M. Rafeld	Group Supervisor, Formulations Laboratory
Michael A. Safron, AS, HT (ASCP)	Manager, Histology
Daniel W. Sved, PhD	Director, Metabolism and Analytical Chemistry
Bennett J. Varsho, BS, LATG	Operations Manager, Developmental, Reproductive and Neurotoxicology

10. QUALITY ASSURANCE UNIT STATEMENT

10.1. PHASES INSPECTED

Date(s) of Inspection(s)	Phase Inspected	Date(s) Findings Reported to Study Director	Date(s) Findings Reported to Management	Auditor(s)
17-Jan-2005	Test Article Preparation	18-Jan-2005	16-Feb-2005	K.Dobbs
19-Jan-2005	Test Article Preparation	19-Jan-2005	16-Feb-2005	J.Tooman K.Dobbs
20-Jan-2005	Test Article Administration	20-Jan-2005	16-Feb-2005	K.Dobbs
02-Feb-2005	Test Article Preparation	02-Feb-2005	25-Mar-2005	K.Dobbs
03-Feb-2005	Test Article Administration,	03-Feb-2005	25-Mar-2005	S.Solomon
11-Feb-2005	Test Article Administration	11-Feb-2005	25-Mar-2005	K.Dobbs
23-Feb-2005	Test Article Administration	23-Feb-2005	25-Mar-2005	K.Dobbs
28-Feb-2005	Animal Care and Equipment	01-Mar-2005	23-Apr-2005	K.Dobbs
07-Mar-2005	Blood Collection Analysis	07-Mar-2005	23-Apr-2005	A.Deppe K.Dobbs
07-Mar-2005	Necropsy	07-Mar-2005	23-Apr-2005	T.DeVan Booth K.Searl
10-Mar-2005	Parturition	10-Mar-2005	23-Apr-2005	K.Dobbs
18-Mar-2005	Post Dose Observations	18-Mar-2005	23-Apr-2005	K.Dobbs
30-Mar-2005	Trimming of Tissues	30-Mar-2005	23-Apr-2005	S.Solomon J.Tooman
31-May-2005, 08-Jun-2005, 09-Jun-2005, 16-Jun-2005	Study Records (I-2)	27-Jun-2005	25-Jul-2005	S.Solomon L.Goodrich
09-Jun-2005, 20-Jun-2005	Study Records (N-1)	20-Jun-2005	25-Jul-2005	S.Solomon L.Goodrich
09-Jun-2005, 20-Jun-2005	Study Records (N-2)	20-Jun-2005	25-Jul-2005	S.Solomon L.Goodrich
08-Jun-2005, 20-Jun-2005	Study Records (C1-C3)	20-Jun-2005	25-Jul-2005	S.Solomon L.Goodrich

WIL-534001
AGC Chemical

PFHxA and C6-2 Alcohol

Date(s) of Inspection(s)	Phase Inspected	Date(s) Findings Reported to Study Director	Date(s) Findings Reported to Management	Auditor(s)
15-Jun-2005, 21-Jun-2005	Study Records (Rx-1)	23-Jun-2005	25-Jul-2005	L.Goodrich C.ONeill
03-Jun-2005, 08-Jun-2005, 14-Jun-2005, 27-Jun-2005	Study Records (I-1)	27-Jun-2005	25-Jul-2005	S.Solomon L.Goodrich
08-Jun-2005, 09-Jun-2005, 15-Jun-2005, 27-Jun-2005	Study Records (I-3)	27-Jun-2005	25-Jul-2005	S.Solomon L.Goodrich
08-Jun-2005, 09-Jun-2005, 20-Jun-2005, 27-Jun-2005	Study Records (I-4)	27-Jun-2005	25-Jul-2005	S.Solomon L.Goodrich
08-Jun-2005, 09-Jun-2005, 24-Jun-2005, 27-Jun-2005	Study Records (I-5)	27-Jun-2005	25-Jul-2005	S.Solomon L.Goodrich
12-Jul-2005, 18-Jul-2005	Study Records (H-1)	18-Jul-2005	25-Aug-2005	L.Goodrich S.Solomon
15-Jul-2005, 18-Jul-2005	Study Records (H-2)	18-Jul-2005	25-Aug-2005	L.Goodrich S.Solomon
12-Jul-2005, 18-Jul-2005	Study Records (P-1)	18-Jul-2005	25-Aug-2005	L.Goodrich S.Solomon
18-Jul-2005, 19-Jul-2005	Study Records (A-10 through A-15, Bioanalytical Data)	19-Jul-2005	25-Aug-2005	J.House
26-Jul-2005, 27-Jul-2005, 28-Jul-2005	Study Records (A-1 to A-9)	28-Jul-2005	25-Aug-2005	E.Crawford
25-Jul-2005, 26-Jul-2005, 27-Jul-2005, 28-Jul-2005, 30-Jul-2005, 02-Aug-2005	Draft Report (w/o AC, Bioanalytical, and TK appendices)	02-Aug-2005	02-Sep-2005	H.Osborn S.Solomon
31-Jul-2005	Draft AC Appendix - PFHxA	01-Aug-2005	02-Sep-2005	E.Crawford
31-Jul-2005	Draft AC Appendix - C6-2AL	01-Aug-2005	02-Sep-2005	E.Crawford

Date(s) of Inspection(s)	Phase Inspected	Date(s) Findings Reported to Study Director	Date(s) Findings Reported to Management	Auditor(s)
02-Aug-2005	Study Records (A-16)	02-Aug-2005	02-Sep-2005	J.House
03-Aug-2005, 04-Aug-2005	Draft Report, Bioanalytical Appendix	04-Aug-2005	02-Sep-2005	J.House
11-Aug-2005	Draft Report (Toxicokinetic Appendix)	11-Aug-2005	02-Sep-2005	J.House

This study was inspected in accordance with the U.S. EPA Good Laboratory Practice Standards (40 CFR Parts 160 and 792), the OECD Principles of Good Laboratory Practice, the standard operating procedures of WIL Research Laboratories, LLC and the sponsor's protocol and protocol amendments with the following exception. The data located in Appendix C (Certificates of Analysis) were the responsibility of the sponsor. Quality Assurance findings, derived from the inspections during the conduct of the study and from the inspections of the raw data and draft report, are documented and have been reported to the study director. A status report is submitted to management monthly.

This report accurately reflects the data generated during the study. The methods and procedures used in the study were those specified in the protocol, its amendments and the standard operating procedures of WIL Research Laboratories, LLC.

The raw data, the retention sample(s), if applicable, and the final report will be stored in the Archives at WIL Research Laboratories, LLC or another location specified by the sponsor.

10.2. APPROVAL

This study was inspected according to the criteria discussed in Section 10.1.

Report Audited By:

Elizabeth S. Crawford, BS Compliance Specialist	Date
Janet M. House, BS, RQAP-GLP Compliance Specialist	Date
Stacy A. Solomon, BS Associate Compliance Specialist	Date

Report Audited and Released By:

Heather L. Osborn, BS, RQAP-GLP Manager, Quality Assurance	Date

11. REFERENCES

Amacher, D.E.; Schomaker, S.J.; Burkhardt, J.A. The relationship among microsomal enzyme induction, liver weight and histological change in rat toxicology studies. *Food and Chemical Toxicology* **1998**, *36(9-10)*, 831-839.

Brockus, C.W.; Andreasen, C.B. Erythrocytes. In *Duncan and Prasse's Veterinary Laboratory Medicine: clinical pathology*. 4th ed.; Latimer, K.S., Mahaffey, E.A., Prasse, K.W., Eds.; Iowa State University Press, Ames, IA, **2003**; pp 3-45.

Dawson, A.B. A note on the staining of the skeleton of cleared specimens with Alizarin Red S. *Stain Technology* **1926**, *1*, 123-124.

Dunn, O.J. Multiple comparisons using rank sums. *Technometrics* **1964**, *6(3)*, 241-252.

Dunnett, C.W. New tables for multiple comparisons with a control. *Biometrics* **1964**, *20*, 482-491.

Evans, E.W.; Duncan, J.R. Proteins, lipids and carbohydrates. In *Duncan and Prasse's Veterinary Laboratory Medicine: clinical pathology*. 4th ed.; Latimer, K.S., Mahaffey, E.A., Prasse, K.W., Eds.; Iowa State University Press, Ames, IA, **2003**; pp 162-192

Hard, G.C.; Khan, K.N. A contemporary overview of chronic progressive nephropathy in the laboratory rat, and its significance for human risk assessment. *Toxicologic Pathology* **2004**, *32*, 171-180.

Hollander, M.; Wolfe, D.A. *Nonparametric Statistical Methods*, 2nd ed.; Hollander, M., Wolfe, D.A., Eds.; John Wiley and Sons, Inc.: New York, NY, **1999**; p 468.

Kruskal, W.H.; Wallis, W.A. Use of ranks in one-criterion variance analysis. *Journal of the American Statistical Association* **1952**, *47*, 583-621.

National Research Council. *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, Commission on Life Sciences; National Academy Press: Washington, DC, **1996**.

Salewski, E. Färbemethode zum makroskopischen Nachweis von Implantationsstellen am Uterus der Ratte. [Staining method for a macroscopic test for implantation sites in the uterus of the rat]. *Naunyn - Schmiedebergs Archiv für Experimentelle Pathologie und Pharmakologie* **1964**, *247*, 367.

REFERENCES (CONTINUED)

Snedecor, G.W.; Cochran, W.G. One Way Classifications; Analysis of Variance. In *Statistical Methods*, 7th ed.; The Iowa State University Press: Ames, IA, **1980**; pp 215-237.

Stuckhardt, J.L.; Poppe, S.M. Fresh visceral examination of rat and rabbit fetuses used in teratogenicity testing. *Teratogenesis, Carcinogenesis and Mutagenesis* **1984**, *4*, 181-188.

Wright, G.J. Water, electrolytes and acid base. *Duncan and Prasse's Veterinary Laboratory Medicine: clinical pathology*. 4th ed.; Latimer, K.S., Mahaffey, E.A., Prasse, K.W., Eds.; Iowa State University Press, Ames, IA, **2003**; pp 162-192

12. DEVIATIONS FROM THE PROTOCOL

This study was conducted in accordance with the protocol and protocol amendments, except for the following.

- **Protocol Section 6.2** states that environmental controls were to be set to maintain an average daily relative humidity of 50% ± 20%. On 20 January 2005 (study day 0 for the 7-day pilot study), mean relative humidity was 29.3%.
- **Protocol Section 5.5** states that at the start of the in-life phase the body weight range for males would be 300-500 g and females would be 200-300 g. However, on 27 January 2005, male no. 70767 weighed 528 g and female no. 70787 weighed 305 g.
- **Protocol Section 7.7.2** states that re-suspension homogeneity and stability of the formulations were to be assessed on 4 and 11 February 2005. The PFHxA formulations samples for re-suspension homogeneity and stability assessment were collected on 4 February 2005, however, the analyses were outside of SOP-specified range (± 10% of the target dose concentration). The PFHxA formulations were re-sampled on 7 February 2005.
- **Protocol Section 7.7.2** specified that 9-day stability assessments of the PFHxA and C6-2 alcohol formulations were to be performed during the 7-day pilot study. However, a 12-day re-suspension homogeneity/stability assessment was performed on the PFHxA formulations. For the C6-2 alcohol formulations a 6-day stability assessment was conducted and the formulations were not stable so the 12-day assessment was not performed and the vehicle was changed to corn oil.
- **Protocol Section 7.7.2** specified that stability was to be assessed for the 60 mg/mL PFHxA formulation. Stability was not assessed for the 60 mg/mL formulation, however; the 100 mg/mL formulation was stable for 12 days.
- **Protocol Section 7.7.2** states that stability assessments were to be assessed over a minimum of 10 days for the main study. The PFHxA formulations were sampled on day 9 instead of day 10.
- **Protocol Section 8.2** states that clinical observations would be performed 1-2 hours following dose administration. On 6 February 2005 (study day 3) the 1-2 hour observations were performed twice (approximately 30 minutes apart). Both observations for study day 3 are presented in the tables.
- **Protocol Section 8.3.1** specified that body weights were to be recorded prior to euthanasia. However, body weights were not collected for all animals in the toxicokinetic groups prior to euthanasia.

- **Protocol Section 8.5.2** lists the serum chemistry parameters that were to be evaluated. On 24 March 2005 (study week 7), triglycerides were analyzed in addition to those parameters noted in the protocol. The trigylceride values are as follows:

Group 1 males	mg/dL	Group 5 males	mg/dL
74401	80.0	74393	132.4
74407	128.8	74397	98.4
74409	61.9	74398	92.7
74413	110.8	74400	98.4
74420	131.3	74406	181.4

Group 4 males	mg/dL	Group 8 males	mg/dL
74362	118.4	74377	85.2
74367	134.2	74380	114.8
74386	69.1	74403	70.2
74392	114.9	74405	104.4
		74415	147.7

- **Protocol Section 8.6.4** states that the F_1 pups were to be sexed on PND 1 and 4. However, the pups were sexed on PND 0 in addition to PND 1 and 4.

- **Protocol Section 9.3.1** states that pups euthanized in extremis were to be euthanized by an intrathoracic injection (prior to 14 March 2005) of sodium pentobarbital. However, the documentation of euthanasia of pup no. 74436-01 (75 mg/kg/day C6-2 alcohol group) recorded on 11 March 2005 indicated that cardiac puncture was the method of sodium pentobarbital injection. It cannot be determined which method of euthanasia was used for this pup.

- **Protocol Section 9.3.2** states that a gross examination was to be performed on F_1 pups sent to necropsy. However, documentation that pup no. 74503-08 (C6-2 control group) was examined on PND 4 can not be located.

- **Protocol Section 9.4.1** states that 2 exorbital lacrimal glands were to be retained from each animal at necropsy. Only 1 lacrimal gland was retained from female no. 74529 (25 mg/kg/day C6-2 alcohol group) at necropsy.

These deviations did not negatively impact the quality or integrity of the data nor the outcome of the study.