CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

202514Orig1s000

PHARMACOLOGY REVIEW(S)

Comments on NDA 202514 tafluprost
From Abigail Jacobs, AD
Date: July 25, 2011

1. I concur that there are no outstanding pharm/tox issues and that pregnancy category C is appropriate.

2. I have made some other comments to the reviewer and supervisor and the reviewer will address them as appropriate

---

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ABIGAIL ABBY C C JACOBS
07/25/2011

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	202514
Supporting document/s:	Supporting Document No. 2; New NDA
Applicant's letter date:	January 7, 2011
CDER stamp date:	January 7, 2011
Product:	Tafluprost, MK-2452
Indication:	Treatment of elevated intra-ocular pressure in open angle glaucoma or ocular hypertension.
Applicant:	Merck Sharp & Dohme Corp.
Review Division:	Division of Anti-Infectives and Ophthalmology Products.
Reviewer:	James S. Wild, Ph.D.
Supervisor/Team Leader:	Wendelyn J. Schmidt, Ph.D.
Division Director:	Wiley A. Chambers, M.D.
Project Manager:	Constantine Markos

Template Version: September 1, 2010

Disclaimer202514

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 202514 are owned by Merck Sharp & Dohme Corp. or are data for which Merck Sharp & Dohme Corp. has obtained a written right of reference. Any information or data necessary for approval of NDA 202514 that Merck Sharp & Dohme Corp. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 202514.

TABLE OF CONTENTS

1 EXECUTIVE SUMMARY 9

1.1 INTRODUCTION 9
1.2 BRIEF DISCUSSION OF NONCLINICAL FINDINGS 9
1.3 RECOMMENDATIONS 12

2 DRUG INFORMATION 15

2.1 DRUG 15
2.2 RELEVANT INDS, NDAS, BLAS AND DMFS 15
2.3 DRUG FORMULATION 15
2.4 COMMENTS ON NOVEL EXCIPIENTS 16
2.5 COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN 16
2.6 PROPOSED CLINICAL POPULATION AND DOSING REGIMEN 17
2.7 REGULATORY BACKGROUND 17

3 STUDIES SUBMITTED 17

3.1 STUDIES REVIEWED 17
3.2 STUDIES NOT REVIEWED 22
3.3 PREVIOUS REVIEWS REFERENCED 23

4 PHARMACOLOGY 23

4.1 PRIMARY PHARMACOLOGY 23
4.2 SECONDARY PHARMACOLOGY 25
4.3 SAFETY PHARMACOLOGY 25

5 PHARMACOKINETICS/ADME/TOXICOKINETICS 34

5.1 PK/ADME 34

6 GENERAL TOXICOLOGY 69

6.1 SINGLE-DOSE TOXICITY 69
6.2 REPEAT-DOSE TOXICITY 71

7 GENETIC TOXICOLOGY 101

7.1 *IN VITRO* REVERSE MUTATION ASSAY IN BACTERIAL CELLS (AMES) 101
7.2 *IN VITRO* ASSAYS IN MAMMALIAN CELLS 103
7.3 *IN VIVO* CLASTOGENICITY ASSAY IN RODENT (MICRONUCLEUS ASSAY) 108
7.4 OTHER GENETIC TOXICITY STUDIES 110

8 CARCINOGENICITY 110

9 REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY 131

9.1 FERTILITY AND EARLY EMBRYONIC DEVELOPMENT 131
9.2 EMBRYONIC FETAL DEVELOPMENT 135
9.3 PRENATAL AND POSTNATAL DEVELOPMENT 150

10 SPECIAL TOXICOLOGY STUDIES 155

11 INTEGRATED SUMMARY AND SAFETY EVALUATION..... 156

12 APPENDIX/ATTACHMENTS..... 170

Table of Tables

Table 1: Safety Margin Assessment Based on Plasma Exposure Comparisons..... 13
Table 2: Safety Margin Assessment Based on Body Surface Area..... 13
Table 3: Tafluprost Ophthalmic Solution (0.0015%) Formulation..... 16
Table 4: Current Specifications of Tafluprost Drug Substance (Sponsor's Table)..... 17
Table 5: *In Vitro* Primary Pharmacology Studies with Tafluprost Acid (AFP-172)..... 24
Table 6: *In Vivo* Primary Pharmacology Studies with Tafluprost..... 24
Table 7: *In Vivo* Primary Pharmacology Studies with Tafluprost Metabolites or Tafluprost in Combination with Timolol..... 25
Table 8: Mean Difference Between AFP-172 and Vehicle-Treated Groups (0.5 Hz) (Sponsor's Table)..... 27
Table 9: Mean Difference Between AFP-172 and Vehicle-Treated Groups (1 Hz) (Sponsor's Table)..... 27
Table 10: Effect of *dl*-Sotalol hydrochloride on Action Potential Parameters (0.5 Hz) (Sponsor's Table)..... 28
Table 11: Effects of AFP-168, Lantanoprost, and $PGF_{2\alpha}$ on Respiratory and Cardiovascular Parameters in Dogs (Sponsor's Table)..... 31
Table 12: The Effects of AFP-172 and $PGF_{2\alpha}$ on the Spontaneous Motility, Maximum Tension, and 10 Minute Resting Tension of Uteri from Non-Pregnant Rats..... 33
Table 13: The Effects of AFP-172 and $PGF_{2\alpha}$ on Maximum Tension, and 10 Minute Resting Tension of Uteri from Non-Pregnant Rabbits..... 34
Table 14: Summary of Aqueous Humor Pharmacokinetic Parameters for AFP-172 Following Topical Ocular Administration of Preservative-Free Tafluprost (Test Products-1 and -2) or Tafluprost Containing Benzalkinium Chloride (Control for Test Products-1 and -2) to Male NZW Rabbits. (Sponsor's Table)..... 38
Table 15: The Tissues and Organs Prepared for Radioanalysis. (Sponsor's Table)..... 39
Table 16: Select Pharmacokinetic Parameters in Plasma After a Single Ocular dose of ^{3}H-AFP-168 to Male Monkeys. (Sponsor's Table)..... 40
Table 17: Study Design for Study No.: MRL PK015. (Sponsor's Table)..... 41
Table 18: Plasma Pharmacokinetic Parameters for Total Radioactivity Following Ocular or Intravenous Administration of 2 μg ^{3}H-AFP-168 to Cynomolgus Monkeys. (Sponsor's Table)..... 41
Table 19: Mean Tissue Concentrations of Radioactivity Following a Single Bilateral Ocular Administration of ^{3}H-AFP-168 to Male Rats. (Sponsor's Table)..... 44
Table 20: Mean Tissue Concentrations of Radioactivity Following a Single Bilateral Ocular Administration of ^{3}H-AFP-168 to female rats. (Sponsor's Table)..... 45
Table 21: Mean Concentrations of Total Radioactivity in Milk, Plasma, and Whole-Blood following Single Ocular Administrations of 0.005% ^{3}H-AFP-168 to Both Eyes of Lactating Maternal Rats. (Sponsor's Table)..... 47
Table 22: Select Milk, Plasma, and Whole-Blood Total Radioactivity Pharmacokinetic Parameters Following a Single Ocular Administration of 0.005% ^{3}H-AFP-168 Solution to Lactating Maternal Rats. (Sponsor's Table)..... 47
Table 23: Mean Total Radioactivity in Tissues Following a Single Ocular Administration of ^{3}H-AFP-168 to Pregnant Rats on Gestation Day 12. (Sponsor's Table)..... 48

Table 24: Mean Total Radioactivity in Tissues Following a Single Ocular Administration of ^{3}H-AFP-168 to Pregnant Rats on Gestation Day 18. (Sponsor's Table) 49
Table 25: Human Metabolites and the Corresponding Animal Equivalents. (Sponsor's Table) 52
Table 26: Metabolism of ^{3}H-AFP-168 by Cyopreserved Pooled Human Hepatocytes after 4 hours Incubation. (Sponsor's Table) 53
Table 27: Metabolites Identified in Rat, Monkey, and Human Hepatocytes After Incubation with Either ^{3}H-AFP-168 or AFP-172. (Sponsor's Table) 54
Table 28: Concentrations of Radioactivity and the Amount of Tritiated Water in Plasma at Sacrifice After the Day 2 Intravenous Administration of 100 μg/kg/day ^{3}H-AFP-168 to Male Rats Dosed Once Daily for Two Days. (Sponsor's Table) 56
Table 29: Percent of Radioactive Dose in Urine, Feces, Bile, Cage Wash, Cage Wipe, Bile Cannula, and Jacket Rinse, at Specified Intervals After a Single Intravenous Administration of ^{3}H-AFP-168 to Male Rats Dosed Once Daily for Two Days. (Sponsor's Table) 57
Table 30: Metabolites found in Plasma, Urine, and Bile in Male Rats Dosed Once Daily for Two Days with Intravenous 100 μg/kg/day ^{3}H-AFP-168. (Sponsor's Table) 58
Table 31: Study Design for MRL Study PK029. (Sponsor's Table) 59
Table 32: Mean Percent Radioactive dose in Urine, Feces, Cage Rinse, Cage Wash, and Cage Wipe at Specificed Intervals After Topical Ocular Administration of ^{3}H-AFP-168 to Male and Female Cynomolgus monkeys. (Sponsor's Table) 60
Table 33: Concentrations of Radioactivity in Blood, Plasma, and Specific Ocular and Systemic Tissues at Specified Times After Topical Ocular Administration of 10 μg/eye of ^{3}H-AFP-168. (Sponsor's Table) 61
Table 34: Percent of Radioactive Dose Recovered in Blood, Plasma, and Specific Ocular and Systemic Tissues at Specified Times After Topical Ocular Administration of 10 μg/eye of ^{3}H-AFP-168. (Sponsor's Table) 62
Table 35: AFP-168 Metabolites Following Topical Ocular Administration in Monkeys .. 62
Table 36: Metabolite profile for Urine and Feces Following Topical Ocular Dosing of ^{3}H-AFP-168 in Monkeys (Sponsor's Table) 64
Table 37: Mean Values for Liver Microsomal and Cytosolic Parameter Measurements for the AFP-168 High-Dose Group Expressed as a Percentage of the Corresponding Vehicle Control Group Mean (Sponsor's Table). 66
Table 38: Total Amounts of the Principal AFP-168 Metabolites Excreted in Bile and Urine 24 Hours After Dosing and in Feces 48 hours After Dosing in Bile Cannulated Male and Female Rats. (Sponsor's Table) 69
Table 39: Single-Dose Studies with Oral or Intravenous AFP-168. 70
Table 40: Studies Employing Multiple Topical Ocular Doses of AFP-168 in a Single Day. 70
Table 41: Non-Pivotal Repeated-Intravenous-Dose Toxicology Studies 71
Table 42: Non-Pivotal Repeated-Ocular-Dose Toxicology Studies 72
Table 43: Study Design for Study No.: MRL TT #01-5526. (Sponsor's Table) 74
Table 44: Mortality Results in the 26-Week Intravenous-Dose Rat Toxicology Study. (Sponsor's Table) 74
Table 45: Significant Changes in Group Mean Hematology Parameters 76
Table 46: Histopathology in Femoral and Sternum Bone Marrow. (Sponsor's Table) ... 78

Table 47: The Incidence and Severity of Spleen, Liver, and Femoral Bone Marrow Hematopoiesis. (Sponsor's Table) 79
Table 48: The Incidence and Severity of Kidney Corticomedullary Mineralization (Sponsor's Table). 79
Table 49: Toxicokinetic Parameters Associated with Intravenous Administration of AFP-168 to Rats for 26 Weeks. (Sponsor's Table). 80
Table 50: Study Design for Study No.: MRL TT #01-5530. (Sponsor's Table) 82
Table 51: Mean Heart Rates. (Sponsor's Table) 83
Table 52: Group Incidence of AFP-168-Related Histopathology Findings. (Sponsor's Table) 86
Table 53: C_{max} Values for Plasma AFP-172 Following 39 Weeks of AFP-168 Intravenous Dosing in Male and Female Dogs. (Sponsor's Table) 87
Table 54: AUC Plasma Exposure Values for AFP-172 Following 39 Weeks of AFP-168 Intravenous Dosing in Male and Female Dogs. (Sponsor's Table) 87
Table 55: T_{max} Values for Plasma AFP-172 Following 39 Weeks of AFP-168 Intravenous Dosing in Male and Female Dogs. (Sponsor's Table) 87
Table 56: Actual Formulation Concentrations. (Sponsor's Table) 88
Table 57: Study Design for Study No.: MRL TT #11-7800. (Sponsor's Table) 90
Table 58: Plasma Toxicokinetic Parameters for AFP-172 in Monkeys Following Topical Administration of DE-111 Ophthalmic Solution. 93
Table 59: Plasma Toxicokinetic Parameters for Timolol in Monkeys Following Topical Administration of DE-111 Ophthalmic Solution. 94
Table 60: Study Design for Study No.: MRL TT #01-5531 96
Table 61: Select Toxicokinetic Parameters Following Topical Ocular Administration of High-Dose AFP-168 to Monkeys for 52 Weeks (Sponsor's Table). 100
Table 62: Concentrations Selected for Cytogenicity Analysis for Experiments 1 and 2 (Sponsor's Table). 105
Table 63: Frequency of Cells With Polyploidy in the 3+17 hour Incubation Without S9. (Sponsor's Table) 107
Table 64: Summary of the Group Mean Frequencies of Micronucleated PCE for the 24 Hour Sample Timepoint. (Sponsor's Table) 109
Table 65: Summary of the Group Mean Frequencies of Micronucleated PCE for the 48 Hour Sample Timepoint. (Sponsor's Table) 110
Table 66: Spleen and Kidney Histopathology Associated with Daily Subcutaneous Administration of AFP-168 for 13 Weeks to Rats. (Sponsor's Table) 112
Table 67: Select Toxicokinetic Parameters for AFP-172 Following Subcutaneous Dosing to Rats for 13 Weeks. 112
Table 68: Select Plasma Toxicokinetic Parameters Associated with Daily Subcutaneous Dosing with AFP-168 for 13 Weeks in Mice. (Sponsor's Table) 114
Table 69: Rat Mortality Parameters for the Two year Rat Cancer Study 117
Table 70: Pituitary Tumors in Males and Females 121
Table 71: Female Mammary Gland and Uterine Neoplastic Lesions 122
Table 72: Non-Neoplastic Lesions in the Two-Year Rat Carcinogenesis Study. (Sponsor's Table) 122
Table 73: Schedule for Toxicokinetic Sampling in the Two-Year Rat Carcinogenesis Study. 123

Table 74: AFP-172 Toxicokinetic Parameters for Day 1, Week 13, and Week 26 in the Two Year Rat Carcinogenesis Study. (Sponsor's Table) 123
Table 75: Study Design for the 78-Week Mouse Carcinogenicity Study. (Sponsor's Table) 126
Table 76: Mortality in the 78-Week Mouse Carcinogenicity Study. (Sponsor's Table). 126
Table 77: Tumor Incidence in Male Mice 129
Table 78: Tumor Incidence in Female Mice 130
Table 79: Toxicokinetic Collection Schedule for the 78-Week Mouse Carcinogenicity Study 130
Table 80: Selected Toxicokinetic Values for Plasma AFP-172 Associated with Subcutaneous AFP-168 Dosing in the Mouse Carcinogenicity Study. (Sponsor's Table). 131
Table 81: Study Design for the Rat Fertility Study. (Sponsor's Table) 133
Table 82: Non-Pivotal Range-Finding Embryo-Fetal Development Studies 135
Table 83: Study Design for the Rat Embryo-Fetal Study. (Sponsor's Table)..... 137
Table 84: Toxicokinetics Parameters for AFP-172 Following AFP-168 Administration to Pregnant Rats. (Sponsor's Table) 138
Table 85: Actual Concentrations of the AFP-168 Formulations. (Sponsor's Table) 138
Table 86: Maternal Caesarean Data 140
Table 87: Rat Fetal Malformations and Variations 141
Table 88: Study Design for the First Rabbit Embryo-Fetal Study. (Sponsor's Table).. 142
Table 89: Actual Concentrations of the AFP-168 Dosing Solutions in the Rat Embryo-Fetal Study. (Sponsor's Table) 144
Table 90: Study Design for the Second Rabbit Embryo-Fetal Study. (Sponsor's Table) 147
Table 91: Incidence of Select Variations in the Second Rabbit Embryo-Fetal Study. . 150
Table 92: Non-Pivotal Range-Finding Prenatal and Postnatal Development Studies . 151
Table 93: Study Design for the Pre- and Post-Natal Development Study in Rats. (Sponsor's Table) 152
Table 94: Comparative Metabolism of Tafluprost in Rat, Dog, Monkey, and Human Hepatocytes. (Sponsor's Table) 160
Table 95: Safety Analysis for Tafluprost-Related Systemic Toxicity Based on the Intravenous Dose Toxicology Studies in Dogs 162
Table 96: Safety Analysis for Tafluprost-Related Systemic Toxicity Based on the Ocular Dose Toxicology Studies in Monkeys 163
Table 97: Safety Analysis for Tafluprost-Related Ocular Toxicity Based on the Ocular-Dose Toxicology Studies in Monkeys 164
Table 98: The AUC Exposure Ratio for AFP-172 Following Subcutaneous Dosing in Rats Versus Topical Ocular Dosing in Humans. 166
Table 99: The AUC Exposure Ratio for AFP-172 Following Subcutaneous Dosing in Mice Versus Topical Ocular Dosing in Humans. 167
Table 100: Hematology and Coagulation Parameter Table 170
Table 101: Clinical Chemistry Parameter Table 171
Table 102: Histopathology and Organ Weight Inventory Table 171

Table of Figures

Figure 1: Group Mean Body Weights for Male Rats. (Sponsor's Figure)..... 118
Figure 2: Group Mean Body Weights for Female Rats. (Sponsor's Figure) 118
Figure 3: Male Group Mean Body Weights. (Sponsor's Figure)..... 127
Figure 4: Female Group Mean Body Weights. (Sponsor's Figure)..... 128

1 Executive Summary

1.1 Introduction

Tafluprost ophthalmic solutions are intended for the treatment of elevated intraocular pressure in open angle glaucoma or ocular hypertension. Tafluprost acid (AFP-172), the tafluprost metabolite and the pharmacologically active agent, is a fluorinated analogue of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) with high affinity and selectivity for the FP prostanoid receptor. The presumed mechanism of tafluprost with regard to glaucoma is reduction of intraocular pressure by increasing uveoscleral outflow of aqueous humor. Further activity may be mediated by tafluprost-induced relaxation of the ciliary muscle and changes in ciliary muscle extracelluar matrix thus facilitating increased outflow from the aqueous humor.

Three other PGF_{2a} analogues are currently approved for the treatment of elevated intraocular pressure in patients with open angle glaucoma or ocular hypertension. These are Xalatan® (latanoprost), Travatan® (travoprost), and Lumigan® (bimatoprost). According to their product labels, all of the approved $PGF_{2\alpha}$ analogues are associated with specific ocular and eye adnexa adverse effects. These include increased pigmentation of the iris, periorbital tissue (eyelid), and eyelashes, and macular edema.

1.2 Brief Discussion of Nonclinical Findings

- Tafluprost (AFP-168) is a $PGF_{2\alpha}$ analogue intended for topical ocular administration. The active metabolite, tafluprost acid (AFP-172) is the pharmacologically active agent. In primary pharmacology studies, tafluprost acid (AFP-172) was shown to bind to the FP prostanoid receptor with subnanomolar affinity, and binding was shown to be substantially selective for this receptor.

- The primary safety signals in safety pharmacology studies were a low incidence of central nervous effects in mice and a dose-dependent increase in blood pressure, heart rate, and Qtc intervals in anesthetized dogs. However, the cardiovascular effects occurred only minimally in repeated-intravenous dose toxicology studies in dogs, and did not occur in repeated-ocular dose studies in monkeys. Because clinical exposures are expected to be on the order of 100 fold lower than the exposures associated with the high ocular doses in the monkey studies, cardiovascular toxicity is not expected to be a clinical concern.

- Preservative free tafluprost formulations and formulations containing 0.01% benzalkonium chloride demonstrated similar corneal penetration. Tafluprost was rapidly absorbed following topical ocular administration with a high bioavailability. Plasma $T_{1/2}$ for tafluprost acid following ocular and intravenous dosing of tafluprost in monkeys was on the order of 10 hours. Plasma C_{max} and AUC values for tafluprost acid increased in a roughly dose-proportional manner following ocular and intravenous dosing, and tafluprost acid did not accumulate in plasma following repeated-tafluprost administration by both routes.

- Topical ocular administration of ^{3}H-tafluprost in rats and monkeys resulted in widespread ocular distribution. Repeated dosing produced a similar ocular distribution pattern, and accumulation did not occur in any tissue other than the lens where concentrations increased approximately 50% after 21 days of dosing.

- Tafluprost acid was >90% bound to serum albumin from rat, rabbit, dog and humans. Tafluprost demonstrated extensive tissue distribution consistent with renal and hepatobiliary excretion and limited CNS distribution. Tissue distribution following repeated ocular dosing was similar to that following a single dose indicating an absence of systemic tissue accumulation. Tafluprost administered topically to the eye or intravenously was excreted primarily in urine and through hepatobiliary excretion with final deposition in feces.

- ^{3}H-Tafluprost or its metabolites transferred into milk in lactating rats, and crossed the placental barrier in pregnant rats. Milk C_{max} radioactivity levels were similar to those in plasma, and fetal exposure was approximately two thirds that of plasma exposure.

- In mice, rats, dogs, monkeys, and humans, tafluprost was shown to rapidly metabolize to its active metabolite, tafluprost acid. All of the putative metabolites occurring above 10% in human hepatocytes were also present in hepatocyte suspensions from rat and monkey. Tafluprost was shown to be metabolized by rabbit eye carboxyesterases, but not by any of 12 different recombinant human CYP-450 isozymes.

- Repeated-intravenous administration of tafluprost for up to 26 weeks in rats and up to 39 weeks in dogs produced species-specific patterns of systemic toxicity. In rats, toxicity included dose-related mortality, hyperostosis and myelofibrosis in bone marrow of the femur and sternum, increased hematopoesis in the spleen, liver, and male femoral bone marrow, and an increase in corticomedullary mineralization in the kidney of females. Dose-dependent changes in the hematological composition of the blood and bone marrow were also observed. Less systemic toxicity occurred in dogs. The most pronounced effects, were dose-dependent and transient clinical signs including salivation, emesis, loose feces, increased respiration, increased heart rate, and increased blood pressure. A prolonged QTc interval was also noted in a 28-day intravenous dose study in dogs at a tafluprost dose of 10 μg/kg/day. In the 39-week dog study, one high-dose (10 μg/kg/day) male experienced severe hepatotoxicity, and all high-dose males demonstrated a slight but significant elevation in serum ALT. Increased serum ALT activity was also noted in the 28-day dog study and the change was reversible after two weeks recovery. A NOAEL value could not be determined for the 26-week rat study, but the plasma C_{max} and AUC exposures associated with the NOAEL values in the dog studies were more than 30-fold higher than those associated with the C_{max} and AUC exposures obtained in humans after 8 days of

daily topical ocular dosing with the tafluprost dose intended for marketing (0.0015%).

- Tafluprost was also administered by the topical ocular route in several repeated-dose topical ocular studies in monkeys, and in all of these studies, no systemic toxicity was observed. The systemic exposure values for tafluprost acid in these studies consistently exceeded the expected clinical exposure by more than 100 fold. These data strongly suggest that clinical administration of tafluprost by the topical ocular route is unlikely to cause systemic toxicity.

- Ocular changes included iridial darkening, sunken eyelids, and blue-gray discoloration of the lower eyelid. However, all of the tafluprost-related ocular changes are consistent with ocular changes observed with other marketed $PGF_{2\alpha}$ analogues including Xalatan® (latanoprost), Lumigan® (bimatoprost), and Travatan ® (travoprost). These effects are considered to be mainly cosmetic, not associated with loss of function, and not toxicologically significant. Other, more serious ocular toxicities including pronounced inflammation or alterations in electroretinography were not observed with topical ocular administration of tafluprost at any of the administered doses. The NOAEL doses for the topical ocular monkey studies provided ocular human equivalent doses that were 67 fold higher than the clinical dose intended for marketing.

- Trafluprost was shown to be negative for genotoxicity in an *in vitro* bacterial reverse mutation assay, an *in vitro* chromosome aberration assay in Chinese Hamster lung cells, and an *in vivo* mouse bone marrow micronucleus assay.

- In both a 24-month rat carcinogenicity study and a 78-week mouse carcinogenicity study where trafluprost was administered subcutaneously, no unusual tumors or significantly increased tumor incidence suggestive of trafluprost-related carcinogenicity was observed.

- Tafluprost at the highest intravenous dose tested of ≤100 μg/kg/day did not affect fertility in male or female rats.

- In a rat embryo-fetal development study, intravenous trafluprost produced dose-dependent skeletal malformations in the vertebral column, and a greater number of lumbar and thoracic vertebrae. The NOAEL was considered to be 3 μg/kg/day for fetal toxicity and the high-dose of 30 μg/kg/day for maternal toxicity. In an initial embryo-fetal study in rabbits, the low dose of 0.03 μg/kg/day produced abdominal wall malformations and cranial and/or spinal malformations while higher doses produced abortions and significant post-implantation loss with few live fetuses. In a second study, the high dose of 0.01 μg/kg/day did not produce any adverse effects and was considered to be the NOAEL dose, but at this dose, plasma concentrations of tafluprost acid were consistently below the limit of detection.

- In a pre- post-natal development study in rats, tafluprost doses of ≥ 1 μg/kg/day resulted in poor nursing behavior for some F_0 females and decreased F_1 offspring viability. At a dose of 10 μg/kg/day, delayed pinna unfolding at 3 days of age for F_1 offspring as well as decreased body weights and increased F_1 newborn mortality were observed. However, no tafluprost-related effects on physical development, sensory function, genital development, or fertility were noted for the F_1 generation. In the F_2 generation, no significant differences in embryonic mortality or the number of corpora lutea, implantations, live F_2 embryos, or pre-implantation loss were noted for any of the tafluprost treatment groups.

1.3 Recommendations

1.3.1 Approvability

Approvable from a Pharmacology/Toxicology Perspective

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Table 1 below summarizes the NOAEL doses or the lowest doses causing toxicity in the rat fertility study, the rat and rabbit embryo-fetal studies, and the rat carcinogenicity study. Safety margins are determined for the C_{max} and AUC values associated with the animal doses relative to clinical values associated with daily bilateral topical ocular dosing of non-preserved tafluprost at the expected clinical dose (0.0015%). In Table 2, the human equivalent dose (HED) and the safety margin based on body surface area are shown for the rat pre-postnatal development study. The safety margin assessments support the intended labeling information for Sections 8.1 (Pregnancy) and 13.1 (Carcinogenesis, mutagenesis, impairment of fertility) of the product label.

Table 1: Safety Margin Assessment Based on Plasma Exposure Comparisons

Study Type/ Study Number	Species/ Route	NOAEL (µg/kg/day)	AFP-172 C_{max} (ng/ml)	AFP-172 AUC (ng x h/ml)	Safety Margin (based on human C_{max}) [d]	Safety Margin (based on human AUC) [e]
Fertility/ TT #01-5527	Rat/ IV	100	379.4 [a]	26.4 [a]	14371	3832
Embryo-fetal/ TT #01-5529	Rat/ IV	3	9.13	NA	346	----
Embryo-fetal/ TT #01-5528	Rabbit/ IV	0.03 [b]	0.14 [c]	NA	5.3	----
Embryo-fetal/ TT #02-5551	Rabbit/ IV	0.01	NA	NA	390	----
Carcinogenicity Study/ TT #03-5575	Rat/ SC	30	10.3	11.9	390	1727

NA: values not obtained or not measureable. IV = intravenous. SC = subcutaneous

[a] Toxicokinetics were not performed for Study No.: TT #01-5527. The C_{max} and AUC values were obtained for the 100 µg/kg/day dose in Study No.: TT #99-5548, a 28-day intravenous-dose study in rats.

[b] The lowest dose in the first rabbit embryo-fetal study that was still associated with toxicity.

[c] The only measureable plasma AFP-172 measurement (1 minute after dosing) at the 0.03 µg/kg/day dose.

[d] C_{max} in humans for AFP-172: C_{max} = 26.4 pg/ml for 0.0015% AFP-168/eye/day administered to both eyes. This value is based on the mean Day 1 and Day 8 C_{max} values in clinical study 77551 for the unpreserved tafluprost preparation.

[e] AUC in humans for AFP-172: $AUC_{0\text{-last}}$ = 6.89 pg x hr/ml for 0.0015% AFP-168/eye/day administered to both eyes. This value is based on the mean Day 1 and Day 8 AUC values in clinical study 77551 for the unpreserved tafluprost preparation.

Table 2: Safety Margin Assessment Based on Body Surface Area

Study Type/ Study Number	**Species/ Route**	**NOAEL (µg/kg/day)**	**HED [a] (µg/kg/day)**	**Safety Margin [b]**
Pre- postnatal development/ TT #04-5574	Rat/IV	0.3	0.048	3.2

[a] A conversion factor of 0.16 was used to derive the human equivalent dose (HED) for the rat NOAEL based on relative body surface area.

[b] The clinical daily dose is 0.0015% administered in 30 µl to one or both eyes = 0.90 µg/day which for an average 60 kg human = 0.015 µg/kg/day.

Based on the safety margin comparisons above, Section 8.1 Pregnancy and Section 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility should be rewritten as follows:

8.1 Pregnancy

Pregnancy Category C

There are no adequate and well-controlled studies in pregnant woman.

In embryo-fetal development studies in rats and rabbits, tafluprost administered intravenously was teratogenic. Tafluprost caused increases in post-implantation losses in rats and rabbits and reductions in fetal body weights in rats. Tafluprost also increased the incidence of vertebral skeletal abnormalities in rats and the incidence of skull, brain, and spine malformations in rabbits. In rats, there were no adverse effects on embryo-fetal development at a dose of 3 μg/kg/day corresponding to maternal plasma levels of tafluprost acid that were approximately 343-times the maximum clinical exposure based on C_{max}. In rabbits, effects were seen at a tafluprost dose of 0.03 μg/kg/day corresponding to maternal plasma levels of tafluprost acid during organogenesis that were approximately 5 times higher than the clinical exposure based on C_{max}. At the no-effect dose in rabbits (0.01 μg/kg/day), maternal plasma levels of tafluprost acid were below the lower level of quantification (20 pg/ml).

In a pre- and postnatal development study in rats, increased mortality of newborns, decreased body weights and delayed pinna unfolding were observed in offspring. The no observed adverse effect level was a tafluprost dose of 0.3 μg/kg/day which is greater than 3 times the maximum recommended clinical dose based on body surface area comparison.

Although animal reproduction studies are not always predictive of human response, SAFLUTAN should not be used during pregnancy unless the potential benefit justifies the potential risk to the fetus.

Women of childbearing age/potential should have adequate contraceptive measures in place.

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Tafluprost was not carcinogenic when administered subcutaneously daily for 24 months at doses up to 30 μg/kg/day in rats (over 1600-times the maximum clinical exposure based on plasma AUC).

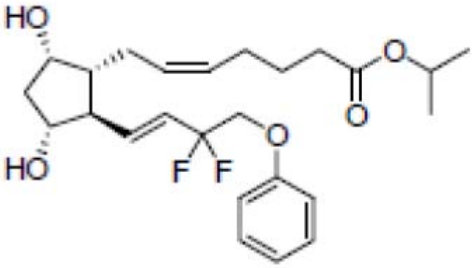
Tafluprost was not mutagenic or clastogenic in a battery of genetic toxicology studies, including an *in vitro* microbial mutagenesis assay, an *in vitro* chromosomal aberration assay in Chinese Hamster lung cells, and an *in vivo* mouse micronucleus assay in bone marrow.

In rats, no adverse effects on mating performance, fertility [redacted] (b) (4) were observed with intravenous dosing of tafluprost at a dose of 100 μg/kg/day (over 14000-times the maximum clinical exposure based on plasma C_{max} or over 3600-times based on plasma AUC).

2 Drug Information

2.1 Drug

CAS Registry Number
209860-87-7
Generic Name
Tafluprost
Code Name
AFP-168, MK-2452
Chemical Name
1-methylethyl (5Z)-7-{(1R, 2R, 3R, 5S)-2-[(1E)-3,3-difluoro-4-phenoxy- 1-butenyl]-3,5-dihydroxycyclopentyl}-5-heptenoate
Molecular Formula/Molecular Weight
$C_{25}H_{34}F_2O_5$/ 452.53
Structure or Biochemical Description



Pharmacologic Class
Prostaglandin analogue

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 62690

2.3 Drug Formulation

Each single-dose 1 ml eye drop solution of tafluprost ophthalmic solution (0.0015%) contains tafluprost and the excipients shown below in Table 3.

Table 3: Tafluprost Ophthalmic Solution (0.0015%) Formulation

Ingredients	Amount
Drug Substance	
Tafluprost	0.015 mg
Excipients	
glycerol	(b) (4)
Sodium dihyrogen phosphate Dihydrate (b) (4)	
Disodium edetate (b) (4)	
Polysorbate 80 (b) (4)	
Sodium hydroxide (pH adjustment)	
Hydrochloric acid (pH adjustment)	
Water for injection (b) (4)	

2.4 Comments on Novel Excipients

The excipients noted above in section 2.3, are all common excipients which according to the FDA Inactive Ingredient Search Database have been used at equal or higher concentrations in marketed ophthalmic products.

2.5 Comments on Impurities/Degradants of Concern

The identified and characterized impurities in tafluprost are (b) (4) . Other degradation products were also observed during stability studies, but these degradation products were not identified because they were not more than (b) (4) of the total drug substance. The maximum daily dose for tafluprost eye drops is (b) (4) which means that the identification and qualification threshold for impurities is 1.0% according to ICR guideline Q3B(R): "Impurities in New Drug Products." The current specifications for the tafluprost drug substance are shown below (Table 4).

Table 4: Current Specifications of Tafluprost Drug Substance (Sponsor's Table).

Test	Specification
Description	Colorless to light yellow viscous liquid
Identification/infared spectroscopy	Corresponds to reference spectrum
Identification/optical rotation	(b) (4)
Clarity of solution	
Related substances	
Residual solvents	
Water content	
Assay	
Enantiomeric impurity (in-house specification)	
Microbial limit	

2.6 Proposed Clinical Population and Dosing Regimen

Clinical population: Tafluprost is indicated for the reduction of elevated intraocular pressure in open-angle glaucoma or ocular hypertension in patients 18 years or older.

Dosing regimen: The recommended dose of 0.0015% tafluprost ophthalmic solution is one drop in the conjunctival sac of the the affected eye(s) once daily in the evening. Tafluprost ophthalmic solution can be used concomitantly with other topical ophthalmic drug products; however administration of different products should be at least 5 minutes apart.

2.7 Regulatory Background

NDA 202514 is the original NDA for tafluprost. This product was first submitted in IND 62690.

3 Studies Submitted

3.1 Studies Reviewed

Primary Pharmacodynamics

1. Analysis of Santen test compounds by competition for the binding of [^{3}H]PGF2a to membranes prepared from HEK cells stably expressing the human FP prostanoid receptor (Santen Report No.: SR2710; MRL Report PD001).
2. Receptor affinity of AFP-172 (Santen Report No.: 298GW04A; MRL Report PD002).
3. Ocular hypotensive effects of Xalatan® and AFP-168 ophthalmic solution in conscious ocular normotensive monkeys – dose dependent study (Santen Report No.: 049GS031; MRL Report PD003).
4. Ocular hypotensive effects of AFP-168 and Xalatan® in conscious laser-induced ocular hypertensive monkeys (Santen Report No.: 204GU141; MRL Report PD004).
5. Intraocular pressure-reducing effect of compound A in ocular normotensive Cynomolgus monkeys by repeated instillation (Santen Report No.: 2001MP241; MRL Report PD005).
6. Aqueous humor dynamics study for AFP-168 (Santen Report No.: 298GW03A; MRL Report PD006).
7. Ocular hypotensive effects of AFP-168 metabolites in conscious ocular normotensive monkeys (Santen Report No.: MP05168; MRL Report PD007).
8. Adjunctive ocular hypotensive effects of 0.0015% AFP-168 and 0.5% timolol maleate in ocular normotensive monkeys (Santen Report No.: 299GZ020; MRL Report PD008).

Secondary Pharmacodynamics

1. Effect of AFP-168 on optic nerve head blood flow in rabbits (Santen Study No.: 299GZ010; MRL Report PD009).

Safety Pharmacology

1. Effect of AFP-172 on HERG tail current recorded from stably transfected HEK293 cells (Santen Report No.: SR2710; MRL TT #03-5570).
2. Effects of AFP-172 on action potential parameters in dog isolated cardiac purkinje fibres ((b) (4) Study No.: DZLE1003, MRL TT #03-5571).
3. Safety pharmacology study of AFP-172: Effects on spontaneous motility in the isolated uterus of non-pregnant female rats (b) (4) Study No.: P030779; MRL TT #03-5572).
4. Safety pharmacology study of AFP-172: Effects on spontaneous motility in the isolated uterus of non-pregnant female rabbits ((b) (4) Study No.: P030780; MRL TT #03-5573).
5. AFP-168: Effects on general activity and behavior in the mouse following intravenous administration (b) (4) Report no.: 1241/009-D6146; MRL TT #99-5539).
6. Effects on general locomotor activity in the mouse following intravenous administration ((b) (4) Report No.; 1241/010-D6146; MRL TT #99-5540).

7. AFP-168: Cardiovascular and respiratory effects in the anaethetised dog following intravenous administration (b) (4) Report No.: 1241/007-D6146; MRL TT #99-5541).
8. Effects of AFP-168, Xalatan® and $PGF_{2\alpha}$ on the respiratory and cardiovascular systems in anesthetized dogs (b) (4) Study No.: MP99244, MRL TT #99-5542).

Pharmacokinetics

Absorption

1. ^{3}H-AFP-168: Pharmacokinetics, tissue distribution and excretion following administration of repeated ocular doses to male and female rats ((b) (4) Study No.: STN 030/003228: MRL Report PK010).
2. Corneal penetration of tafluprost into the rabbit aqueous humor following a topical instillation of preserved and preservative-free 0.0015% tafluprost eye drops (8 Page(s) of Draft Labeling have been Study #77501; MRL Report PK013). Withh ld i F ll b4 (CCI/TS)
3. Pharmacokinetics and tissue distribution of ^{3}H-AFP-168 following administration of a single ocular dose to Cynomolgus monkeys (b) (4) Project #7016-100; MRL Report PK014).
4. Pharmacokinetics and excretion of ^{3}H-AFP-168 following administration of a single ocular or intravenous dose to Cynomolgus monkeys ((b) (4) Project #7016-101; MRL Report PK015).

Distribution

1. Evaluation of the binding ratios of AFP-172 to rat, rabbit, dog, and human serum albumin by ultrafiltration method (Santen Pharmaceutical Co. Ltd Study No.: 319LW09A; MRL Report PK017).
2. ^{3}H-AFP-168: Systemic tissue distribution study in the rat using quantitative whole-body autoradiography following administration of a single ocular dose (b) (4) Study No.: STN 039/022091; MRL Report PK018).
3. ^{3}H-AFP-168: Milk secretion and placental transfer studies in female rats following administration of a single ocular dose, (b) (4) Study No.: STN 038/013724; MRL Report PK019).
4. ^{3}H-AFP-168: Systemic tissue distribution study in the rat using quantitative whole-body autoradiography following administration of a single intravenous dose ((b) (4) Study No.: STN 037/014191; MRL Report PK020).
5. Systemic tissue distribution of ^{3}H-AFP-168 by quantitative whole-body autoradiography in Cynomolgus monkeys after topical administration on the eyes (b) (4) Project No.: 7016-103; MRL Report PK021).

Metabolism

1. AFP-168: Comparative *in vitro* metabolism studies with rat, dog, Cynomolgus monkey and human hepatocytes ((b) (4) Study No.: 026/994667; MRL Report PK023).

2. *In vitro* metabolism of [^{3}H]-AFP-168 and AFP-172 by rat, monkey, and human hepatocytes ((b) (4) Study No.: 7016-124; MRL Report PK023).
3. Determination of esterase enzyme related to AFP-168 metabolism in corneal tissue of rabbits (Santen Pharmaceutical Co. Ltd., Study No.; 387LU081; MRL report PK024).
4. AFP-168: Effect on levels of hepatic cytochromes P450 and phase II drug metabolizing enzymes in male and female rats after intravenous administration for 26 weeks ((b) (4) Study No.: STN 051/014502; MRL Report PK025).
5. Metabolism of [^{3}H]-AFP-168 in rats following intravenous administration ((b) (4) Study No.: 7016-125; MRL Report PK027).
6. Metabolism of H-AFP-168 in Cynomolgus monkeys after topical administration on the eyes (b) (4) Study No.; 7016-104; MRL Report PK029).
7. Cytochrome P450 in vitro reaction phenotyping study of AFP-172 with human recombinant cytochrome P450s (Santen Pharmaceutical Co., Ltd., Study No.: 476LZ16A; MRL Report PK032).

Excretion

1. ^{3}H-AFP-168: Biliary excretion, enterohepatic circulation and metabolism in the rat following a single ocular administration (b) (4) Study No.: STN 036/014402; MRL Report PK031).

Toxicology

Single-Dose Toxicity

1. AFP-168: Single dose oral toxicity study in the rat (b) (4) Report No.: 1241/015-D6144; MRL TT #99-5543).
2. AFP-168: Single dose intravenous toxicity study in the rat (b) (4) Report No.: 1241/016-D6144; MRL TT #99-5544).
3. One-day ocular irritation study in rabbits treated with AFP-168 ophthalmic solution 10 times at 30 minute intervals (Santen Pharmaceutical Co.: Ltd. Study No.: 999401; MRL TT #99-5545).
4. Single-dose toxicity study of AFP-168 in dogs by intravenous administration (Santen Pharmaceutical Co., Ltd Study No.: 991504; MRL TT #99-5546).
5. One day ocular irritation study in the Cynomolgus monkey ((b) (4) Study No.: 1241-011; MRL TT #99-5547).

Repeat-Dose Toxicity

1. AFP-168: 28-day intravenous administration toxicity study in the rat with a 14 day treatment-free period (b) (4) Report No.: 1241/018-D6154; MRL TT #99-5548).
2. 4-week repeated-dose toxicity study of AFP-168 in dogs by intravenous administration (Santen Pharmaceutical Co., Ltd. Study No.: 992506; MRL TT #99-5550).

3. 28-day ocular toxicity study in Cynomolgus monkey (b) (4) Report No.: 1595-1241-012; MRL TT #99-5553).
4. AFP-168: 13-week ocular toxicity study in the Cynomolgus monkey with a 4-week recovery period ((b) (4) Report No.: 1681-1241-020; MRL TT #00-5537).
5. AFP-168: 26-week intravenous administration toxicity study in the rat ((b) (4) Study No.: 1241/031; MRL TT #01-5526).
6. AFP-168: 39-week intravenous administration toxicity study in the dog (b) (4) Study N0.: 1241/030; MRL TT #01-5530.
7. DE-111 ophthalmic solution 13-week ocular toxicity study in the Cynomolgus monkey (b) (4) Report #8225872; MRL TT #11-7800).
8. AFP-168: 52-week ocular toxicity study in the Cynomolgus monkey ((b) (4) Study No.: 1241-034; MRL TT #01-5531).

Genotoxicity

1. AFP-168: Reverse mutation in four histidine-requiring strains of *Salmonella typhimurium* and one tryptophan-requiring strain of *Escherichia coli* (b) (4) Report No.: 1241/5-D5140; MRL TT #99-5551).
2. AFP-168: Induction of chromosome aberrations in cultured Chinese Hamster lung *CHL) cells, ((b) (4) Report No.: 1241/6-D5140; MRL TT #99-5552).
3. AFP-168: Induction of micronuclei in the bone marrow of treated mice (b) (4) Report No.: 1241/23-D6172; MRL TT #00-5538).

Carcinogenicity

1. AFP-168: 13 week subcutaneous administration range-finding study in the rat ((b) (4) Study No.: 1241/039; MRL TT #03-5574).
2. 13-week subcutaneous administration range-finding study in the mouse (b) (4) Study No.: 1241/040; MRL TT #03-5576).
3. A carcinogenicity study of AFP-168 in rats via subcutaneous administration (b) (4) Study No.: MP03279; MRL TT #03-5575).
4. AFP-168: 78 week subcutaneous administration oncogencity study in the mouse ((b) (4) Study No.: 1241/047; MRL TT #04-5572).

Reproductive and Developmental Toxicity

1. AFP-168: Intravenous study of fertility and early embryonic development in the rat (Report No.: 1241/32-D6154; MRL TT #01-5527).
2. AFP-168: Intravenous range-finding study of embryo-foetal development in the rabbit ((b) (4) Report No.: 1241/26-D6154; MRL TT#01-5523).
3. AFP-168: Intravenous range-finding study of embryo-foetal development in the rat ((b) (4) Report No.: 1241/28-D6154; MRL TT #01-5525).
4. AFP-168: Intravenous study of embryo-foetal development in the rabbit (b) (4) report No.: 1241/29-D6154; MRL TT #01-5528).
5. AFP-168: Intravenous study of embryo-foetal development in the rat ((b) (4) Report # 1241/29-D6154; MRL TT #01-5529).

6. AFP-168: Intravenous study of embryo-foetal development in the rabbit – second study ((b) (4) Report # 1241/35-D6154; MRL TT #02-5551).
7. A dose range-finding study for effects of AFP-168 on pre- and postnatal development, including maternal function in rats via intravenous administration ((b) (4) Study No.: MP04098; MRL TT #04-5573).
8. A study for effects of AFP-168 on pre- and postnatal development, including maternal function, in rats via intravenous administration ((b) (4) Study No.: MP04099; MRL TT #04-5574).

Special Toxicology

1. Skin sensitization study of AFP-168 ophthalmic solution by adjuvant and patch test in the guinea pig (Santen Pharmaceutical Co., Ltd. Study No.: 996305; MRL TT #99-5549.

3.2 Studies Not Reviewed

1. Validation of an analytical procedure for the determination of AFP-168 and AFP-172 in mouse plasma (heparin) using automated solid phase extraction and liquid chromatography with tandem mass spectrometric detection 8 Page(s) . Report No.: 1241/041-D1145; MRL Report PK001).
2. Validation of an analytical procedure for determination of AFP-168 and AFP-172 in rat plasma (heparin) using solid phase extraction for sample preparation and liquid chromatography with mass spectrometric detection (8 Page(s) of Draft Labeling have been Withheld in Full Report No.: 1241/13-D0142; MRL Report PK002).
3. Validation of an analytical method for determination of AFP-168 and AFP-172 in rat plasma (Report (b) (4) Study No.: MP03278; MRL Report PK003).
4. Validation of an analytical procedure for the determination of AFP-168 and AFP-172 in rabbit plasma (heparin) using solid phase extraction and liquid chromatography with tandem mass spectrometric detection 8 Page(s) Ltd. Report No.; 1241/21-D0142; MRL Report PK004).
5. Validation of an analytical procedure for the determination of AFP-172 in rabbit plasma (heparin) using liquid-liquid extraction and liquid chromatography with tandem mass spectrometric detection (8 Page(s) of Draft Labeling . Report No.: 1241/038-D0142; MRL Report PK005). h b Withh ld i F ll
6. Validation of an analytical procedure for the determination of AFP-168 and AFP-172 in dog plasma (heparin) using solid phase extraction and liquid chromatography with tandem mass spectrometric detection (8 Page(s) Report No.: 1241/22-D0142; MRL PK006). of Draft
7. Validation of an analytical procedure for the determination of AFP-168 and AFP-172 in monkey plasma (heparin) using solid phase extraction for sample preparation and liquid chromatography with mass spectrometric detection (8 Page(s) of Draft Labeling . Report No.: 1241/14-D0142; MRL Report PK007).
8. ^{3}H-AFP-168: Pharmacokinetics, tissue distribution, metabolism and excretion following administration of a single ocular dose to male and female rats ((b) (4) Study No.: STN031/003215; MRL Report PK008).

9. ^{3}H-AFP-168: Pharmacokinetic and tissue distribution study in the rat following administration of a single ocular dose [redacted] Study No.: STN 025/994009; MRL Report PK009).
10. ^{3}H-AFP-168: Pharmacokinetic, tissue distribution and excretion study in the rat following administration of a single intravenous dose ([redacted] Study No. STN 027/994221; MRL Report No.: PK011).
11. H-AFP-168: Pharmacokinetics, tissue distribution and metabolism following administration of a single 100 μg/kg intravenous dose to male and female rats ([redacted] Study No.: 053/022780; MRL Report PK012).
12. Pharmacokinetics and tissue distribution of ^{3}H-AFP-168 after administration of a single ocular dose of 0.0005% to male Cynomolgus monkeys ([redacted]. Project No.: 7016-102; MRL Report PK016).
13. H-AFP-168: Metabolism in the rat after a single intravenous administration ([redacted] Study No.: STN 035/014195; MRL Report PK026).
14. ^{3}H-AFP-168: Characterization of phase I and phase II metabolites in biological samples from rat and Cynomolgus monkey in vivo studies and human in vitro studies [redacted] Study No.: STN 072/052006; MRL Report PK028).
15. Metabolism of [^{3}H]-AFP-168 in Cynomolgus monkeys following intravenous administration ([redacted] Study No.: 7016-126; MRL Report PK030).
16. AFP-168: Intravenous preliminary study in the non-pregnant rabbit ([redacted] Report No.: 1241/25-D6154; MRL TT #01-5524).

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

The primary pharmacology studies are summarized below in Table 5, Table 6, and Table 7. In a variety of primary pharmacology studies, tafluprost was tested for its receptor specificity (MRL Report Nos.: PD001, PD002), and its ocular hypotensive effects or the effects of its metabolites were tested in ocular normotensive and/or ocular hypertensive monkeys (MRL Report Nos.: PD003, PD004, PD007). The effects of tafluprost on ocular humor dynamics was explored (MRL Report Nos.: PD006) and the potential for additive or synergistic lowering of IOP by timolol and tafluprost in combination was tested (MRL Report Nos.: PD008).

Table 5: *In Vitro* Primary Pharmacology Studies with Tafluprost Acid (AFP-172)

Study Number/ Study type	Test system	Results
MRL Report No.: PD001/ *In vitro* assessment of affinity for prostaglandin FP receptors	HEK cells expressing human prostanoid FP receptor	AFP-172 bound to the FP prostanoid receptor with a Ki of 0.40 nM and an EC_{50} value of 0.53 nM.
MRL Report No.: PD002/ Investigation of ocular hypotensive effects of a single instillation in ocular normotensive monkeys	Human recombinant receptors, human platelet receptors, and guinea pig and rat tissue receptors	The IC_{50} for binding of AFP-172 was greater than 1 μM for a large panel of receptors with a single exception being the guinea pig prostanoid EP_3 receptor where the IC_{50} value was 67 nM.

Table 6: *In Vivo* Primary Pharmacology Studies with Tafluprost

Study Number/ Study type	Test system	Results
MRL Report No.: PD003/ Investigation of ocular hypotensive effects after a single instillation in ocular normotensive animals	Conscious Cynomolgus monkeys	Topical application of AFP-168 to one eye of each normal Cynomolgus monkey significantly lowered IOP at concentrations of 0.0005% and 0.0025% but not 0.00002% or 0.0001%. In comparison Latanoprost® produced significant reduction in IOP at a concentration of 0.005%.
MRL Report No.: PD004/ Investigation of ocular hypotensive effects after a single instillation in ocular hypertensive animals	Conscious Cynomolgus monkeys	A single topical application of AFP-168 to one eye of Cynomolgus monkeys with laser-induced ocular hypertension significantly lowered IOP at concentrations of 0.0005% and 0.0025% but not 0.00002% or 0.0001%.
MRL Report No.: PD005/ Investigation of ocular hypotensive effects after repeated ocular instillations in ocular normotensive animals	Conscious Cynomolgus monkeys	After five days of daily ocular instillations of AFP-168 to the left eyes of Cynomolgus monkeys, an AFP-168 concentration of 0.001% produced a significant reduction in IOP immediately prior to instillation on Days 5 and 6. AFP-168 concentrations of 0.0025% and 0.005% produced significant reductions in IOP from Days 3 to 6.
MRL Report No.: PD006/ Aqueous humor dynamics study	Cynomolgus monkey	1. Aqueous humor formation determined with a fluorophotometry method was slightly but not significantly increased following topical ocular doses of 0.005% tafluprost. This dose was also associated with a significant reduction in IOP. In a separate radioisotope dilution experiment, the same topical dose of tafluprost slightly but significantly increased aqueous humor production. 2. Topical ocular administration of 0.005% tafluprost significantly increased uveoscleral outflow and significantly increased total outflow facility.

Table 7: *In Vivo* Primary Pharmacology Studies with Tafluprost Metabolites or Tafluprost in Combination with Timolol

Study Number/ Study type	Test system	Results
MRL Report No.: PD007/ Investigation of ocular hypotensive effects of tafluprost metabolites in ocular normotensive animals	Conscious Cynomolgus monkeys	1. Tafluprost acid (AFP-172) reduced right eye IOP with a maximal effect 6 hours after administration into the anterior chamber of the right eyes of Cynomolgus monkeys. 2. Administration of three other metabolites of tafluprost, 1,2-dinor tafluprost acid, and the acid and lactone forms of 1,2,3,4-tetranor tafluprost acid did not reduce IOP.
MRL Report No.: PD008/ Investigation of ocular hypotensive effects by a single concomitant use of tafluprost and timolol in ocular normotensive monkeys	Conscious Cynomolgus monkeys	1. Individual topical ocular administration of 0.5% timolol or 0.0015% tafluprost significantly reduced IOP. 2. The maximal reduction of IOP following concomitant administration of both agents was significantly greater than the maximal reduction in IOP produced by either individual agent.

4.2 Secondary Pharmacology

A secondary pharmacology study was conducted exploring the effects of tafluprost on optic nerve head blood flow in rabbits (Study Report No.: PD009). Rabbits were treated for four weeks with 0.0015% tafluprost and 0.005% latanoprost (Xalatan®) once daily and optic nerve head blood flow was measured with the laser speckle method. Both compounds increased blood flow approximately 15% on Days 14 and 28 of dosing, and the effect was somewhat stronger with tafluprost.

4.3 Safety Pharmacology

Tafluprost (AFP-168) or its active metabolite (tafluprost acid, AFP-172) were extensively assessed in safety pharmacology studies designed to assess central nervous system, cardiovascular, and respiratory system function. All of the safety pharmacology studies are reviewed below. *In vitro* examinations of tafluprost on cardiovascular function included a hERG potassium channel study (MRL TT #03-5570), and a study examining the effects of tafluprost on action potential parameters in dog isolated purkinje fibers (MRL TT #03-5571). The *in vivo* effects of tafluprost on cardiovascular and respiratory function were assessed in two studies in anesthetized dogs (MRL TT #99-5541 and MRL TT #99-5542). Two studies were performed in mice to assess central nervous effects of tafluprost, one study addressed general activity and behavior (MRL TT #99-5539), and the other addressed general locomotor activity (MRL TT #99-5540). Tafluprost is a $PGF_{2\alpha}$ analogue, and $PGF_{2\alpha}$ is known to increase the force of uterine contractions. Consequently, tafluprost was assessed for its ability to affect uterine contractions in non-pregnant rats (MRL TT#03-5572) and rabbits (MRL TT #03-5573).

Study Title: Effect of AFP-172 on HERG tail current recorded from stably transfected HEK293 cells (Santen Report No.: SR2710; MRL TT No.: 03-5570).

Methods

This study was conducted in a GLP-compliant manner and a quality assurance statement was provided in the study report. A single concentration of AFP-172 (100 ng/ml) in a DMSO vehicle, as well as vehicle and reference solutions were applied to HEK293 cells stably transfected with hERG cDNA for 15 minutes exposure periods. Patch clamp recordings of hERG potassium tail currents in 4 cells for each treatment group were conducted in order to examine the effect of AFP-172, the vehicle (0.1% DMSO 0.1%), and the positive control reference substance (100 nM E-4031).

Results
Concentration analysis of the AFP-172 perfusion samples indicated that with one exception, samples were within 10% of the 100 ng/ml target concentration. The one exception was 30% higher than 100 ng/ml AFP-172. Application of 100 ng/ml AFP-172 did not produce a significantly greater decrease of hERG potassium tail currents compared to the application of vehicle (0.1% DMSO) alone. The vehicle reduced the tail current by 20.6% compared to steady state tail current recordings in perfusion solution alone, and 100 ng/ml AFP-172 decreased tail current recordings by a similar 25.6%. In contrast, 100 nM E-4031 inhibited hERG tail currents by 92.1%.

Study Title: Effects of AFP-172 on action potential parameters in dog isolated cardiac purkinje fibres ((b) (4) Study No.: DZLE1003, MRL TT No.: 03-5571).

Methods
This study was conducted in a GLP-compliant manner and a quality assurance statement was provided in the study report. For this study, cardiac purkinje fibres were obtained from 6 Beagle dogs, and three concentrations of AFP-172 (1, 10, and 100 ng/ml) were examined for their ability to alter purkinge fibre action potentials following electrical stimulation at frequencies of 0.5, 1, and 3 Hz. The following action potential parameters were assessed: action potential duration at 60% and 90% repolarization (APD_{60} and APD_{90}), maximum rate of depolarization (MRD), upstroke amplitude (UA) and resting membrane potential (RMP). Four purkinge fibres were examined for each of the treatment groups with one set of fibres used for vehicle assessments side by side with another set of fibres used to assess each concentration of AFP-172 applied from lowest to highest with washout perfusions between each treatment. Following the last AFP-172-treatment measurements the fibres used to assess AFP-172 effects were exposed to 50 μM *dl*-sotalol hydrochloride, a positive control agent. Measurements were conducted after approximately 30 minutes perfusion with vehicle, each concentration of AFP-172, and the positive control agent.

Results
Compared to the purkinje fibres perfused with vehicle, AFP-172 at 1, 10, and 100 ng/ml did not significantly alter the resting potential, maximum rate of depolarization, upstroke amplitude or action potential duration in a consistent manner at the 0.5 or 1 Hz stimulation frequencies (Table 8 and Table 9). In contrast, consistent with its known activity, 50 μM *dl*-sotalol hydrochloride produced an inverse frequency-dependent

prolongation of the action potential duration at both the 0.5 and 1 Hz stimulation frequencies (Table 10; 0.5 Hz only).

Table 8: Mean Difference Between AFP-172 and Vehicle-Treated Groups (0.5 Hz) (Sponsor's Table).

Action Potential Parameter	[AFP-172]/[DMSO] Mean difference between groups (± standard deviation)‡		
	1 ng/ml/0.1%	10 ng/ml/0.1%	100 ng/ml/0.1%
RMP (mV)	-1.1 ± 3.9	-0.7 ± 4.1	1.6 ± 2.4
UA (mV)	2.9 ± 6.2	1.0 ± 6.2	-0.5 ± 3.7
MRD (%)	12.0 ± 13.8	9.4 ± 11.1	-0.3 ± 10.6
APD_{60} (%)	2.3 ± 2.4	0.3 ± 7.2	1.9 ± 6.1
APD_{90} (%)	3.9 ± 3.8	2.4 ± 4.8	3.2 ± 5.1

***** P < 0.05 (unpaired, 2-tailed Student's t-test).

‡ Mean differences are relative to the vehicle control group.

Table 9: Mean Difference Between AFP-172 and Vehicle-Treated Groups (1 Hz) (Sponsor's Table).

Action Potential Parameter	[AFP-172]/[DMSO] Mean difference between groups (± standard deviation)‡		
	1 ng/ml/0.1%	10 ng/ml/0.1%	100 ng/ml/0.1%
RMP (mV)	-0.6 ± 2.9	-0.3 ± 2.7	0.5 ± 1.6
UA (mV)	1.1 ± 5.1	0.0 ± 3.7	-0.4 ± 4.4
MRD (%)	5.0 ± 8.2	0.3 ± 7.3	-3.9 ± 15.5
APD_{60} (%)	2.9 ± 3.5	2.1 ± 5.6	4.5 ± 4.1
APD_{90} (%)	3.4 ± 3.4	3.7 ± 5.4	5.6 ± 4.0*

***** P < 0.05 (unpaired, 2-tailed Student's t-test).

‡ Mean differences are relative to the vehicle control group.

Table 10: Effect of *dl*-Sotalol hydrochloride on Action Potential Parameters (0.5 Hz) (Sponsor's Table).

Action Potential Parameter	Vehicle	50 µM Sotalol
RMP (mV)	-85.8 ± 3.2	-86.5 ± 1.7
UA (mV)	117.4 ± 5.7	115.6 ± 2.9
MRD (V/s)	474.5 ± 81.7	483.1 ± 98.4
APD_{60} (ms)	307.5 ± 52.1	469.0 ± 75.0*
APD_{90} (ms)	385.0 ± 55.5	569.2 ± 82.8*

* P < 0.05 (unpaired, 2-tailed Student's t-test).

Study Title: AFP-168: Cardiovascular and respiratory effects in the anaethetised dog following intravenous administration ((b) (4) Report No.: 1241/007-D6146; MRL TT No.: 99-5541).

Methods

This study was conducted in a GLP-compliant manner, and a quality assurance statement was included in the study report. Two groups of four Beagle dogs (2/sex/group) received a single intravenous dose of vehicle (0.9% sodium chloride) or AFP-168 (0.003, 0.01, 0.1, and 1 µg/kg). The AFP-168-treated dogs were dosed according to an ascending dose paradigm. Respiratory parameters (peak inspiratory and expiratory flow, tidal volume, minute volume, and rate of respiration) and cardiovascular parameters (heart rate, systolic, diastolic and mean arterial blood pressure, left ventricular pressure and it derivative dP/dt_{max}, mean femoral blood flow and femoral resistance, RR, QRS, PR, ST, QT and QTc intervals, and R, P, and T wave heights) were measured 10 and 20 minutes before dosing and 2, 10, and 20 minutes after dosing for all groups and also at 30 and 40 minutes after dosing for the highest AFP-168 dose.

Results

Respiratory Parameters: The vehicle doses and the 0.003 and 0.01 µg/kg doses of AFP-168 did not alter any of the respiratory parameters compared to pre-dose measurements. The higher doses, 0.1 and 1 µg/kg AFP-168, stimulated a dose-dependent increase in the rate of respiration as well as other parameters. The 0.1 µg/kg dose caused an increase in respiration which was transient lasting approximately 20 minutes after dosing. The 1 µg/kg dose stimulated a greater increase in the respiration rate which was accompanied by increases in minute volume and peak inspiratory flow (PIF) and by decreased peak expiratory flow (PEF) and tidal volume (TV). With the exception of PIF, these changes returned to near baseline 40 minutes after dosing.

Cardiovascular parameters: The vehicle doses and the 0.003 and 0.01 μg/kg doses of AFP-168 did not alter any of the cardiovascular parameters compared to pre-dose measurements. Following administration of the 0.1 and 1 μg/kg AFP-168 doses, mean arterial pressure, heart rate, and dP/dt_{max} increased in a dose-dependent manner. The effects returned to baseline values 20 minutes after the 0.1 mg/kg dose, but were only partially recovered 40 minutes after the 1 μg/kg dose. The changes were only significant in the high-dose (1 μg/kg) group. Femoral blood flow was significantly but transiently decreased only in the high-dose group along with increased femoral artery resistance.

ECG waveforms were affected by the 0.1 and 1 μg/kg AFP-168 doses. The 0.1 μg/kg dose transiently stimulated a decreased RR interval and an increased QT_c interval with the changes lasting approximately 20 minutes. The same parameters were changed to a greater degree by the 1 μg/kg dose as well as an increase in the QT Interval. Values partially returned to baseline 40 minutes after dosing. Other intervals demonstrated a trend toward a dose-dependent decrease including the PR interval, T-wave height, and ST interval. However, the only significant changes were the RR interval, T wave height, and QT_c interval for the high dose (1 μg/kg) group compared to vehicle control animals. Even the significant changes were considered to be within normal limits by the Sponsor and the Sponsor did not consider any of the ECG changes to be abnormalities. While the changes may not have reached significantly abnormal levels they do indicate a dose-dependent potential for AFP-168 to alter ECG parameters.

Study Title: Effects of AFP-168, Xalatan® and $PGF_{2\alpha}$ on the respiratory and cardiovascular systems in anesthetized dogs (b) (4) Study No.: MP99244, MRL TT NO.: 99-5542).

Methods

Saline, AFP-168 at dose levels of 0.01, 0.1, 1, 10 μg/kg, and latanoprost (Xalatan®) and $PGF_{2\alpha}$ each at dose levels of 0.1, 1, 10 and 100 μg/kg were administered intravenously to anesthetized dogs (4 dogs per treatment) according to an ascending dose paradigm. A saline control group was provided, and vehicle controls were provided for every treatment. Respiratory rate, heart rate, blood pressures (systolic, diastolic, and mean blood pressures) and electrocardiograms (R, P and T wave amplitudes, R-R, QT, and QTc intervals, and QRS, PR, and ST durations) were evaluated predose and at 2, 10, 20, and 30 minutes after dosing.

Results

The respiratory and cardiovascular effects of AFP-168 appeared to be related to its pharmacological effect as a FP prostanoid receptor agonist. In accord with its greater affinity of the FP prostanoid receptor, AFP-168 was generally more potent than latanoprost or $PGF_{2\alpha}$ in stimulating adverse respiratory and cardiovascular effects.

As summarized in Table 11, no treatment-related effects on any of the respiratory or cardiovascular parameters were evident at AFP-168 concentrations of 0.01 μg/kg, lantanoprost concentrations of 0.1 μg/kg, or $PGF_{2\alpha}$ concentrations of 1 μg/kg. Respiratory rate was increased at AFP-168, latanoprost, and $PGF_{2\alpha}$ concentrations of ≥

0.1, 1, and 10 μg/kg respectively. Similarly, blood pressure was increased at concentrations of ≥ 1 μg/kg AFP-168 and ≥ 10 μg/kg $PGF_{2\alpha}$, and T wave amplitude was decreased at concentrations of ≥ 1 μg/kg AFP-168 or latanoprost, and 10 μg/kg or greater in the $PGF_{2\alpha}$ group.

Other changes were of less certain relationship to treatment due to an absence of a dose-response. In the AFP-168 group these changes included: shortened PR duration at 0.01 μg/kg and sporadic shortening of ST duration, and QT interval at 0.1 μg/kg but not at higher doses. Also the QTc interval was shortened at 0.1 μg/kg, prolonged at 1 μg/kg and unchanged at 10 μg/kg. In the latanoprost treatment group, T wave amplitude was increased at 0.1 μg/kg but decreased at higher concentrations and PR duration and Qtc interval were sporadically increased at 10 μg/kg, but not at 100 μg/kg. Treatment with $PGF_{2\alpha}$ resulted in increased T wave amplitude and decreased QT interval at 0.1 μg/kg, but not at higher doses, and sporadic increased in PR duration at 10 but not at 100 μg/kg.

Other changes were considered to be of uncertain relationship due to sporadic and inconsistent occurrence at the measurement timepoints or the small magnitude of the changes relative to concurrent changes in the vehicle control groups. In the AFP-168 group these changes included: increased heart rate, shortened R-R interval, and increased P wave amplitude. In the latanoprost group, these changes included: decreased R wave amplitude and increased P wave amplitude at 100 μg/kg and increased T wave amplitude at 0.1 μg/kg. In the $PGF_{2\alpha}$ group, these changes included: increased P wave amplitude, prolonged QRS duration, shortened PR duration, and prolonged QTc interval at 100 μg/kg and decreased R wave amplitude at 10 and 100 μg/kg.

Table 11: Effects of AFP-168, Lantanoprost, and $PGF_{2\alpha}$ on Respiratory and Cardiovascular Parameters in Dogs (Sponsor's Table).

Substance	AFP-168					Latanoprost[1)]					$PGF_{2\alpha}$				
Dose level (μg/kg)	0	0.01	0.1	1	10	0	0.1	1	10	100	0	0.1	1	10	100
Table 1 respiratory rate	—	—	↑	↑	↑	—	—	↑	↑	↑	↓	—	—	↑	↑
Table 2 heart rate	—	—	↑	↑	↑	—	—	—	—	—	—	↑	—	↑	—
Table 3 systolic b.p.	—	—	—	↑	↑	↑	↑	—	↑	↑	—	↑	—	↑	↑
Table 4 diastolic b.p.	—	—	—	↑	↑	—	↑	—	↑	↑	—	—	—	↑	↑
Table 5 mean b.p.	—	—	—	↑	↑	—	↑	—	↑	↑	—	—	—	↑	↑
Table 6 R wave amplitude	—	—	—	—	—	—	—	—	—	↓	—	—	—	↓	↓
Table 7 P wave amplitude	—	—	—	↑	↑	—	—	—	—	↑	—	—	—	—	↑
Table 8 T wave amplitude	—	—	—	↓	—	—	↑	↓	↓	↓	—	↑	—	↓	↓
Table 9 R-R interval	—	—	↓	↓	↓	—	—	—	—	—	—	↓	—	↓	—
Table 10 QRS duration	—	—	—	—	—	—	—	—	—	—	—	—	—	—	↑
Table 11 PR duration	—	↓	—	—	—	—	—	—	↑	—	—	—	—	↑	↓
Table 12 ST duration	—	—	↓	—	—	↑	—	—	—	—	—	—	—	—	—
Table 13 QT interval	—	—	↓	—	—	↑	—	—	—	—	—	↓	—	—	—
Table 14 QTc interval	—	—	↓	↑	—	—	—	—	↑	—	—	—	—	—	↑

↑ : Significant increase ↓ : Significant decrease — : No changes

[1)] Animals were treated with Xalatan® or its dilutions.

Study Title: AFP-168: Effects on general activity and behavior in the mouse following intravenous administration ([redacted] (b) (4) Report No.: 1241/009-D6146; MRL TT No.: 99-5539).

Methods

This study was conducted in a GLP-compliant manner and a quality assurance statement was included in the study report. Five groups of male CD-1 (ICR) mice (6 per group) received a single intravenous dose of vehicle (0.9% saline), AFP-168 (10, 30 and 100 μg/kg) or diazepam (5 mg/kg). A blinded observer performed Irwin observations pre-dose and at approximately 5, 15, 30, 60, 90, and 120 minutes after dosing. General observation of each animal was also performed the following day.

Results

The AFP-168 10 and 30 μg/kg doses did not significantly alter any physiological or behavioral Irwin parameters compared to the vehicle control mice. Also with one exception, mice receiving 100 μg/kg of AFP-168 did not demonstrate changes in any Irwin parameters. Within 5 minutes after dosing, the one affected mouse receiving high-dose AFP-168 exhibited a moderately ataxic gait, slightly decreased spatial locomotor activity and limb tone, and markedly decreased pupilary constriction in response to light. Also, piloerection, startle response, toe pinch response, corneal and pinna reflexes were increased. The majority of signs had decreased by 15 minutes after dosing, but slight ataxia and slight decrease in transfer arousal and spatial locomotion were

observed 60 minutes after dosing, and slightly decreased limb tone and failure to complete the wire maneuver occurred 90 and 120 minutes after dosing. The positive control, diazepam, as expected produced a number of changes in Irwin parameters which appeared within 5 minutes of dosing and decreased in intensity and frequency with increasing time after dosing. All animals appeared normal the day after dosing and no animals died in the study.

Study Title: Effects on general locomotor activity in the mouse following intravenous administration (b) (4) Report No.; 1241/010-D6146; MRL TT No.: 99-5540).

Methods
This study was conducted in a GLP-compliant manner, and a quality assurance statement was included in the study report. Five groups of 10 male CD-1 (ICR) mice received a single intravenous dose of vehicle (saline), AFP-168 (10, 30, and 100 μg/kg) or the positive control agent, diazepam (5 mg/kg). Individual locomotor activity (mean number of breaks and ambulations) was assessed every 15 minutes for two hours after dosing.

Results
Animals treated with AFP-168 at 10, 30, or 100 μg/kg did not exhibit significantly decreased locomotor activity compared to vehicle control animals at any observation time-point. Diazepam significantly decreased locomotor activity compared to vehicle control animals during the first 15 minutes after dosing.

Study Title: Safety pharmacology study of AFP-172: Effects on spontaneous motility in the isolated uterus of non-pregnant female rats ((b) (4) Study No.: P030779; MRL TT No.: 03-5572).

Methods
The vehicle control solution (0.1% DMSO), AFP-172 in 6 concentrations (log doses from 1×10^{-7} to 1×10^{-12}) and 4 concentrations of $PGF_{2\alpha}$ (log concentrations from 1×10^{-7} to 1×10^{-10} g/ml) were tested in isolated uteri from non-pregnant female Crj: CD(SD)IGS rats. Each concentration of test compound was tested in 5 uterine specimens. Following exposure to the test solutions, uterine spontaneous contractions were recorded for more than 20 minutes. The frequency of spontaneous contractions, maximum tension, and 10 minute resting tension were measured before and after each treatment.

Results
Relative to the effects of the vehicle, AFP-172 increased the maximum tension, resting tension, and frequency of spontaneous contractions of isolated rat uteri at concentrations of $\geq 10^{-9}$ for each parameter. By way of comparison, $PGF_{2\alpha}$ increased the maximum tension, resting tension, and frequency of spontaneous contractions at concentrations of $\geq 10^{-8}$ g/L for each parameter. These results indicate that with regard to the maximum tension, resting tension, and frequency of spontaneous contractions of

rat uteri, AFP-172 was roughly 10 times as potent as $PGF_{2\alpha}$. The results are summarized in Table 12 below.

Table 12: The Effects of AFP-172 and $PGF_{2\alpha}$ on the Spontaneous Motility, Maximum Tension, and 10 Minute Resting Tension of Uteri from Non-Pregnant Rats.

Group	Dose Level (g/ml)	Spontaneous Contraction Frequency		Maximum Tension		10 Minute Resting Tension (g)	
		Percent of pretreatment frequency[a]	Percent change from vehicle	Percent of pretreatment tension[a]	Percent change from vehicle	Increase from pretreatment value (g)[a]	change from vehicle (g)
Vehicle Control	----	105.7 ± 7.8	----	99.4 ± 3.5	----	0.03 ± 0.04	----
AFP-172	1×10^{-12}	92.1 ± 7.4	-13.6	101.3 ± 3.6	1.9	0.08 ± 0.07	0.05
	1×10^{-11}	111.3 ± 12.1	5.6	98.2 ± 3.5	- 1.2	0.08 ± 0.08	0.05
	1×10^{-10}	118.6 ± 36.4	12.9	106.4 ± 4.4	7.0	0.45 ± 0.22	0.42
	1×10^{-9}	231.3 ± 117.2*	125.6	116.6 ± 4.5*	17.2	2.45 ± 0.98*	2.42
	1×10^{-8}	189.5 ± 59.8	83.8	116.7 ± 5.9*	17.3	6.29 ± 2.07**	6.26
	1×10^{-7}	172.5 ± 53.9	66.8	121.3 ± 5.9**	21.9	7.21 ± 0.61**	7.18
$PGF_{2\alpha}$	1×10^{-10}	103.3 ± 7.5	-2.4	101.1 ± 0.6	2.5	0.01 ± 0.05	-0.02
	1×10^{-9}	135.7 ± 27.1	30.0	106.3 ± 3.1	6.9	0.40 ± 0.31	0.37
	1×10^{-8}	159.5 ± 39.4*	53.8	113.6 ± 8.3**	14.2	0.78 ± 0.21*	0.75
	1×10^{-7}	151.2 ± 33.0	45.5	118.0 ± 8.7**	18.6	2.91 ± 1.33**	2.88

[a] Values expressed as mean ± standard deviation. *: $p \leq 0.05$; **: $p \leq 0.01$ compared to vehicle control.

Study Title: Safety pharmacology study of AFP-172: Effects on spontaneous motility in the isolated uterus of non-pregnant female rabbits (b) (4) Study No.: P030780; MRL TT #03-5573).

Methods

The study report indicates that the study was conducted according to the GLP standards of Japan, but no quality assurance statement was provided. The vehicle control solution (0.1% DMSO), AFP-172 in 5 concentrations (log concentrations from 1×10^{-7} to 1×10^{-11} g/ml) and 4 concentrations (log concentrations from 1×10^{-7} to 1×10^{-10} g/ml) of $PGF_{2\alpha}$ were tested in isolated uteri from non-pregnant female kbs: NZW rabbits. Each concentration of test compound was tested in 5 uterine specimens. Following exposure to the test solutions uterine spontaneous contractions were recorded for more than 20 minutes. The maximum tension of the spontaneous contractions, and the 10 minute resting tension were measured before and after treatment.

Results

AFP-172 increased the maximum tension of spontaneous contractions in isolated rabbit uteri at concentrations ≥ 10^{-9} g/ml as did $PGF_{2\alpha}$. AFP-172 at concentrations up to 10^{-7} g/ml had little effect on uterine resting tension in contrast to $PGF_{2\alpha}$ which increased

resting tension by 0.54 g at a concentration of 10^{-7} g/ml. The results are summarized below in Table 13.

Table 13: The Effects of AFP-172 and $PGF_{2\alpha}$ on Maximum Tension, and 10 Minute Resting Tension of Uteri from Non-Pregnant Rabbits.

Group	Dose Level (g/ml)	Maximum Tension		10 Minute Resting Tension (g)	
		Percent of pretreatment Tension[a]	Percent change from vehicle	Increase from pretreatment value (g)[a]	change from vehicle (g)
Vehicle Control	----	99.7 ± 3.6	----	0.00 ± 0.11	----
AFP-172	1×10^{-11}	98.5 ± 5.3	-1.2	-0.06 ± 0.06	-0.06
	1×10^{-10}	98.3 ± 6.6	-1.4	-0.02 ± 0.05	-0.02
	1×10^{-9}	115.8 ± 18.3	16.1	-0.05 ± 0.11	-0.05
	1×10^{-8}	126.7 ± 18.0	27	0.13 ± 0.16	0.13
	1×10^{-7}	158.8 ± 42.5*	59.1	0.19 ± 0.46	0.19
$PGF_{2\alpha}$	1×10^{-10}	99.5 ± 5.8	-0.2	-0.04 ± 0.10	-0.04
	1×10^{-9}	112.6 ± 14.5	12.9	-0.04 ± 0.10	-0.04
	1×10^{-8}	138.5 ± 30.7	38.8	0.18 ± 0.17	0.18
	1×10^{-7}	181.7 ± 28.5**	82	0.54 ± 0.62	0.54

[a] Values expressed as mean ± standard deviation. *: $p \leq 0.05$; **: $p \leq 0.01$ compared to vehicle control.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Analytical Methods and Validation Reports

The Sponsor submitted study reports describing validated LC-MS/MS assays for the detection of tafluprost and tafluprost acid in plasma from mice (MRL Report No.: PK001) rats (MRL Report Nos.: PK002, PK003), rabbits (MRL Report Nos.: PK004 and PK005), dogs (MRL Report No. PK006), and monkeys (MRL Report No.: PK007). The tafluprost assay concentration ranges for mice, rats, rabbits, dogs and monkeys were 1-200 ng/ml, 4-400 ng/ml, 0.4-40 ng/ml, 0.4 to 30 ng/ml, and 0.4-40 ng/ml respectively. For tafluprost acid, the assay ranges were 0.5-100 ng/ml, 1-200 ng/ml, 0.1 to 10 ng/ml, 0.1 to 10 ng/ml, and 0.1-10 ng/ml for mice, rats, rabbits, dogs, and monkeys respectively.

Absorption

Four absorption studies are reviewed below. These studies included examination of pharmacokinetics, tissue distribution, and excretion following repeated ocular doses in rats (MRL Report No: PK010), and single ocular and intravenous doses in monkeys (MRL Report Nos.: PK014, PK015). In addition, the differential corneal penetration of preserved and preservative-free tafluprost was examined (MRL Report No.: PK013). Several other studies (MRL Report Nos.: PK008, PK009, PK011, PK012, PK016) were

not reviewed because they were considered to not add substantial information beyond the information provided in the studies that were reviewed.

Study Title: ^{3}H-AFP-168: Pharmacokinetics, tissue distribution and excretion following administration of repeated ocular doses to male and female rats ([redacted] (b) (4) Study No.: STN 030/003228; MRL Report PK010).

Methods

This study was conducted in a GLP-compliant manner, and a quality assurance statement was included in the study report. Male and female Sprague-Dawley albino rats were administered single and repeated (7, 14, or 21 days) daily topical ocular instillations of ^{3}H-AFP-168 ophthalmic solution (0.005%; 5 μl to both eyes).

Phase A: One set of animals (Group 1: 3/sex) received a single topical ocular dose of 0.005% ^{3}H-AFP-168 to both eyes. Serial blood samples (≈ 200 μl) were collected from each animal at 15 minutes, 30 minutes, 1, 2, 4, 6, 8, 12, and 24 hours for toxicokinetic analysis.

Phase B: Another set of animals (Group 2: 18/sex) received single bilateral topical ocular doses of 0.005% ^{3}H-AFP-168. Six male and female animals were exsanguinated as completely as possible and sacrificed at 30 minutes, 4 hours, and 24 hours after dosing. Terminal blood samples were obtained as well as intact eyes and tissue samples from a large panel of organs. The radioactivity associated with whole blood, hematocrit, plasma, and the various organs was determined. Also the eyes were dissected and ocular matrices including the conjunctiva, lens, aqueous humor, iris/ciliary body, vitreous humor, and choroid/retina were isolated preliminary to determination of radioactivity.

Phases C, D, and E: After 7 days (Group 3: 30/sex), 14 days (Group 4; 30/sex), or 21 days (Group 5; 54/sex) of daily bilateral topical ocular doses of 0.005% ^{3}H-AFP-168, 6 animals of each gender were exsanguinated and sacrificed at 30 minutes and 1, 2, 8 and 24 hours (Groups 3 and 4) after administration or 15 and 30 minutes, and 1, 2, 4, 6, 8, 12 and 24 hours (Group 5) after administration. Blood, ocular and systemic tissues were obtained and analyzed as for Part B.

Phase F: A final group of animals (Group 6; 4/sex) received daily bilateral topical ocular doses of 0.005% ^{3}H-AFP-168. Urine was collected from each animal during 0-6, 6-12, and 12-24 hours after the first dose, at 24 hour intervals after Doses 2 to 20 and at 0-6, 6-12, 12-24, 24-48, 24-48, 48-72, 72-76, 96-120, 120-144, and 144-168 hours after the final dose (Dose 21). In addition, feces from each rat were collected at 24-hour intervals throughout dosing and for 7 days after the final dose. At 168 hours after the final dose, rats were sacrificed and the carcass was retained for measurement of radioactivity. Cage washings were obtained and retained for radioactivity assessment on Days 7, 14, and 21 and 168 hours after the final dose.

Radioactivity in all samples was determined with liquid scintillation counting.

Results

Pharmacokinetics (Phases A, B, C, D and E)

^{3}H-AFP-168 was rapidly absorbed from the eye with T_{max} values of 15 minutes and C_{max} values of approximately 1 ng equivalents AFP-168/gram. Plasma concentrations declined thereafter in a biexponential manner until at 24 hours after dosing plasma concentrations were approximately 10% of C_{max} values. Mean plasma C_{max} concentrations were similar for males and females, but AUC_{24} values were greater in females compared to males.

Repeated dosing of ^{3}H-AFP-168 resulted in plasma concentration patterns similar to that produced with a single dose except accumulation occurred with repeated-dosing. Plasma AUC_{24} values were similar for 7, 14, and 21 days of dosing indicating near maximal accumulation was achieved after 7 days of dosing. After 7, 14, and 21 days of dosing, the AUC_{24} of plasma total radioactivity was 2.4-, 3.0-, and 3.1-fold greater respectively than after a single dose in males and 2.4-, 2.7-, and 2.8-fold higher respectively in females.

The proportion of volatile radioactivity in plasma increased rapidly with time from 11% in males and 15% in females at 30 minutes after dosing, to 82% in males and 84% in females 24 hours after dosing. The volatile radioactivity pattern indicates that oxidative transformation at the site of radiolabelling is a metabolic pathway of the ^{3}H-AFP-168 in rats.

Like the plasma T_{max}, the whole blood T_{max} was also 30 minutes after dosing (the first sampling time-point), and the decline of whole blood radioactivity followed a pattern similar to that of plasma. Whole blood concentrations were consistently lower than plasma concentrations with whole blood to plasma concentration ratios of ≈ 0.7 at 30 minutes and ≈ 0.9 at 24 hours. The mean concentrations of radioactivity in blood cells increased with time comprising 3 and 8% of the total radioactivity in males and females at 30 minutes after dosing and 30 and 33% of total radioactivity 24 hours after dosing.

Ocular Tissue Distribution (Phases B, C, D, and E)

At 30 minutes after the single ocular doses of ^{3}H-AFP-168 the ocular tissue radioactivity concentrations (ng equivalents/ml or ng equivalents/g; male and female) were highest in the cornea (1229, 1373) followed by the aqueous humor (291.1, 409.2) iris/cliary body (255.3, 397.4) sclera (51.49, 49.38) conjunctiva (29.64, 41.33) retina/choroid (25.55, 25.15) vitreous humor (18.09, 21.03) and lens (4.343, 4.110). Subsequently radioactivity steadily declined and ocular tissue concentrations were highest in the lens (1.146, 0.857), followed by the cornea (0.899, 0.866), aqueous humor (0.396, 0.249), sclera (0.127, 0.121), vitreous humor (0.114, 0.120), choroid/retina (0.104, 0.102) and conjunctiva (0.098, 0.108) with concentrations in the iris/ciliary body below the limit of detection.

Repeated dosing of ^{3}H-AFP-168 produced a similar pattern of ocular tissue distribution compared to the single ocular dose with some slight changes. Thirty minutes after dosing, after 7, 14, or 21 days of dosing, iris/ciliary body concentrations were slightly higher than the aqueous humor concentrations instead of the reverse as was the case 30 minutes after a single dose of ^{3}H-AFP-168. Generally radioactivity did not

accumulate in any of the ocular tissues with repeated dosing with the exception of the lens where concentrations increased by ≈ 50% after 21 doses.

Systemic Tissue Distribution (Phases B, C, D, and E)
In systemic tissues, the highest concentrations of ^{3}H-AFP-168 generally occurred 30 minutes after a single ^{3}H-AFP-168 dose with the highest concentrations (ng equivalents AFP-168/g tissue; mean male, female) in liver (13.43, 10.44) duodenum wall (5.554, 8.957), ileum wall (5.683, 5.128), Harderian gland (4.738, 4.872), stomach wall (3.027, 4.227) and kidney (2.061, 5.333). Much lower tissue concentrations (< 0.5 ng equivalents/g tissue) were found in the brain, spinal cord, heart, bone marrow, testis, and ovaries. This pattern of absorption suggests that AFP-168 may have drained into the oral cavity via the nasolacrimal duct with subsequent excretion via the renal and hepatobiliary routes. Also the results indicate that the AFP-168 and its metabolites did not readily cross the blood brain barrier. Following the peak values, tissue radioactivity steadily declined but the tissue to plasma concentration ratios tended to increased with time indicating that tissue levels declined slightly slower than plasma levels. The tissue to plasma concentration ratios were consistently greater than 1.0 only in the principle excretory organs, the gastrointestinal tract, liver, kidney, and urinary bladder, and in the Harderian gland. Similar systemic tissue distribution results were obtained following dosing for 7, 14, and 21 days with no substantial accumulation occurring in any particular organs.

Excretion (Phase F)
During the 21 day dosing period and for 168 hours after the last dose, mean totals approximately 87% of the total radioactive dose was recovered in urine and feces from male and females. Less than 1% of the dose remained in the animal carcasses and between 2 and 5% was recovered in cage washings. The majority of the radioactive dose was excreted in feces (58.16% in males and 43.72% in females) with 26.66% (males) and 38.21% (females) excreted in urine. The proportion of excreted radioactivity remained relatively constant in both urine and feces during the 21-day dosing period, and this pattern suggests that excretion of the total AFP-168-related material was largely complete before the administration of the next daily dose.

Study Title: Corneal penetration of tafluprost into the rabbit aqueous humor following a topical instillation of preserved and preservative-free 0.0015% tafluprost eye drops (MRL Report PK013).

Methods
A single 30 µl dose of preservative-free 0.0015% tafluprost containing either 0.05% (test product-1) or 0.075% polysorbate 80 (test product-2) was topically administered into the left corneas of male New Zealand White rabbits (4 per treatment). A reference preparation containing 0.0015% tafluprost, 0.05% polysorbate 80, and 0.01% benzalkonium chloride was administered into the right eye of each animal. Rabbits were sacrificed at 0.75, 1.5, 2, 3, 6, and 8 hours after dosing, and aqueous humor samples were obtained from both eyes of each animal. The aqueous humor samples were frozen at -80°C for later pharmacokinetic analysis and comparison.

Results
Select AFP-172 pharmacokinetic parameters following topical ocular dosing with the different test preparations and the reference preparation are summarized below in Table 14. The T_{max} for all of the preparations was 45 minutes. The AFP-172 C_{max} of test product-1 was somewhat higher than that of the reference preparation (4.48 ng/ml compared to 3.19 ng/ml at 45 minutes respectively). Mean aqueous humor concentrations were significantly higher for test-product-1 compared to the reference preparation over time. The C_{max} values for test product-2 were similar to those of test-product-1, but the C_{max} value for the reference compound in the animals receiving test product-2 was higher than the C_{max} value for the reference compound in the animals receiving test product-1 (3.99 ng/ml versus 3.19 ng/ml). Consequently the C_{max} value for test product-2 was not significantly higher than the C_{max} for its reference control. Aqueous humor exposure (AUC_{3h}) followed a similar pattern but none of the differences were significant. In all cases the aqueous humor C_{max} and AUC for both of the unpreserved preparations (test products-1 and -2) were equal to or greater than those for the preserved tafluprost preparation containing benzalkonium chloride, indicating that the removal of benzalkonium chloride did not impair corneal penetration.

Table 14: Summary of Aqueous Humor Pharmacokinetic Parameters for AFP-172 Following Topical Ocular Administration of Preservative-Free Tafluprost (Test Products-1 and -2) or Tafluprost Containing Benzalkinium Chloride (Control for Test Products-1 and -2) to Male NZW Rabbits. (Sponsor's Table)

PK parameter	Test product-1	Control for test product-1	Test product-2	Control for test product-2
C_{max} (ng/ml)	4.48	3.19	4.50	3.99
T_{max} (min)	45	45	45	45
AUC_{3h} (95% CI) (ng h/ml)	4.74 (3.55, 5.93)	4.14 (3.38, 4.90)	5.14 (3.63, 6.65)	4.54 (3.72, 5.36)

CI; confidence interval
The Cmax and AUC values were rounded to the nearest two decimal figures

Study Title: Pharmacokinetics and Tissue Distribution of ^{3}H-AFP-168 After Administration of a Single Ocular Dose of 0.005% to Male Cynomolgus Monkeys. (MRL Report PK016)

Methods
This GLP-compliant study included a quality assurance statement in the final report. A single topical ocular dose of ^{3}H-AFP-168 was administered to each eye of 30 Cynomolgus monkeys. Three monkeys per time-point were sacrificed at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours after dosing and blood and tissues were collected for pharmacokinetic and tissue distribution analysis. The tissues/organs listed below in Table 15 were collected for radioanalysis. Each eye for each monkey was enucleated and dissected into palpebra conjunctiva, bulbar conjunctiva, extraocular muscles, cornea, aqueous humor, vitreous humor, lens, iris-ciliary body, remaining eye, retina,

chroroid pigment epithelium, and sclera. Radioactivity was assessed by liquid scintillation counting.

Table 15: The Tissues and Organs Prepared for Radioanalysis. (Sponsor's Table)

Adrenal gland	Liver
Aorta	Lungs
Biliary bladder	Lymph nodes (mesenteric)
Biliary bladder contents	Muscle (thigh)
Biliary bladder wash	Medulla oblongata
Bone (femur)	Pancreas
Bone marrow (femur)	Parotid salivary gland (both)
Cecum[a]	Prostate
Cerebrum	Rectum[a]
Cerebellum	Skin (shaved, dorsal)
Colon[a]	Spinal cord
Duodenum	Spleen
Duodenum contents and wash	Stomach
Eyes (both)	Stomach contents and wash
Fat (abdominal)	Submaxillary salivary gland (both)
Heart	Testes
Hypophysis	Thymus
Ileum	Thyroid
Ileum contents and wash	Trachea
Jejunum	Urinary bladder
Jejunum contents and wash	Urinary bladder contents
Kidneys	Urinary bladder wash
Lacrimal glands (both)	

a The contents were discarded.

Aliquots of plasma were analyzed for protein binding using ultrafiltration and liquid scintillation counting.

Results

Blood and Plasma Pharmacokinetics

Select pharmacokinetic parameters for plasma radioactivity are shown below in Table 16. After ocular administration of ^{3}H-AFP-168, radioactivity was rapidly absorbed into systemic circulation with plasma and blood T_{max} values at 5 minutes post-dose and a plasma to blood ratio of about 1.5. The $t_{1/2}$ for radioactivity concentration in plasma was 9.1 hours.

Table 16: Select Pharmacokinetic Parameters in Plasma After a Single Ocular dose of ^{3}H-AFP-168 to Male Monkeys. (Sponsor's Table)

Group	Mean Actual Dose (µg/eye)	C_{max} (ng equiv/g)	t_{max} (Hour)	$t_{½}$ (Hours)	$AUC_{(0-t)}$ (ng equiv•hour/g)	$AUC_{(0-\infty)}$ (ng equiv•hour/g)
1	0.988	0.907	0.083	9.1	0.7923	0.8731

equiv Equivalents.

Distribution of Radioactivity in Ocular Tissues

Following rapid ocular absorption, high mean concentrations (ng equivalents/gram tissue) of ^{3}H-AFP-168 were observed 5 minutes after dosing in the cornea (576), bulbar conjunctiva (323), and palpebral conjunctiva (180). Lower concentrations ≤ 6 ng equivalents ^{3}H-AFP-168/gram tissue were measured in iris, sclera, choroid-pigment epithelium, and aqueous humor. The lowest levels occurred in the retina (0.185) and vitreous humor (0.166). With increasing time after dose administration, corneal and conjunctival concentrations decreased with increasing concentrations in peripheral tissues. Overall radioactive concentrations in ocular tissues decreased with time, but low concentrations were still detectable in most ocular tissues 24 hours after dosing.

Distribution of Radioactivity in Systemic Tissues

^{3}H-AFP-168 was rapidly distributed into systemic tissues following ocular administration. The highest maximal concentration occurred in the lacrimal gland 5 minutes after dosing. Peripheral tissues with high maximum concentrations (ng equivalents of ^{3}H-AFP-168/gram of tissue) were kidney (11.1) and liver (4.80) at 15 minutes after dosing.

Ex-vivo Protein Binding by Ultrafiltration

Levels of ^{3}H-AFP-168-derived radioactivity bound to plasma proteins decreased over time from 84.3% 15 minutes after dosing to 37.8% 24 hours after dosing. Conversely, unbound radioactivity increased from 15.7% at 0.25 hours to 62.2% at 24 hours post-dose. The decrease in the percentage of radioactivity bound to plasma proteins suggests ^{3}H-AFP-168 was further metabolized to more polar metabolites.

Study Title: Pharmacokinetics and Excretion of ^{3}H-AFP-168 Following Administration of a Single Ocular or Intravenous Dose to Cynomolgus Monkeys (MRL Report PK015).

Methods

This was a GLP-compliant study and a quality assurance statement was included in the study report. Male and female Cynomolgus monkeys (3/sex/group) were administered either a single topical ocular dose of 1 µg ^{3}H-AFP-168 in ophthalmic solution to each eye or a single intravenous dose of approximately 2 µg ^{3}H-AFP-168 in a saline solution containing 0.05% Tween 80. After each dose, the concentrations of total radioactivity were determined in plasma (0 to 24 hours postdose) and in urine and feces (0 to 168 hours postdose). The actual collection times for blood (plasma), urine, and feces are summarized below in Table 17. Radioactivity was measured with liquid scintillation counting.

Table 17: Study Design for Study No.: MRL PK015. (Sponsor's Table)

Group	Number of Animals Male	Female	Target Dose Level	Target Radioactive Level	Collections (Hours Postdose)
1[a]	3	3	1.0 µg/eye (0.005%)	1.8 MBq/eye	Blood: Predose, 0.083, 0.167, 0.333, 0.5, 1, 2, 4, 6, 8, 12, and 24 Urine: Predose, 0-6, 6-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 Feces: Predose, 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168
2[b]	3	3	2.0 µg/animal	3.6 MBq/animal	Same as above for blood, urine, and feces

a Dosed ocular, both eyes, 20 µL/eye.
b Dosed intravenously, bolus, at 2 mL/animal (0.0001% ^{3}H-AFP-168 solution).

Results

Select plasma pharmacokinetic parameters associated with ocular and intravenous dosing of ^{3}H-AFP-168 are summarized below in Table 18. Following ocular (total dose of ≈ 2 µg) or IV (total dose of ≈ 2 µg) administration of ^{3}H-AFP-168, the T_{max} value was 0.083 hours, and the C_{max} values were similar indicating that ^{3}H-AFP-168 was rapidly and readily absorbed following administration by the ocular route. In both genders, the mean concentration of radioactivity was higher in plasma than in blood indicating that AFP-168 and its metabolites preferentially distributed into the extracellular fraction of whole blood.

Table 18: Plasma Pharmacokinetic Parameters for Total Radioactivity Following Ocular or Intravenous Administration of 2 µg ^{3}H-AFP-168 to Cynomolgus Monkeys. (Sponsor's Table)

Group	Sex	Mean Actual Dose (µg)	C_{max} (ng equiv/g)[a]	t_{max} (hours)	$t_{1/2}$ (hours)	$AUC_{(0-t)}$ (ng equiv•hours/g)	$AUC_{(0-\infty)}$ (ng equiv•hours/g)
1	M	0.788/eye	0.870	0.083	12.1	0.621	0.694
	F	0.911/eye	1.25	0.083	7.86	0.654	0.688
2	M	2.27/animal	1.26	0.083	9.64	1.27	1.36
	F	2.23/animal	1.79	0.083	9.61	1.35	1.43

M Male.
F Female.
equiv Equivalents.
a Values are based on concentrations of nonvolatile radioactivity in plasma.

Following ocular administration of ^{3}H-AFP-168, excretion of the radioactivity occurred primarily through urine and feces with 37.9% (male) and 47.9% (female) of the administered dose recovered in urine and 35.5% (male) and 28.6% (female) recovered in feces. Most of the radioactivity in both genders was excreted within 48 hours of dosing, and the overall recovery at 168 hours postdose was approximately 87% for both genders.

Following intravenous administration of ^{3}H-AFP-168, excretion patterns and total recovery of radioactivity were similar to those seen with ocular administration. Means of 40.8% (male) and 54.6 % (female) of the administered dose was recovered in urine and 26.7% (male) and 25.5% (female) was recovered in feces. The overall recovery of radioactivity in males and females was 89.5% and 93.7% respectively 168 hours after dosing.

In both genders for both routes of administration, the mean concentration of radioactivity was higher in plasma than in blood indicating that AFP-168 and its metabolites preferentially distributed into the extracellular fraction of whole blood. Half-life measurements were similar for both routes of administration with mean values ranging from 7.86 to 12.1 hours for both genders and administration routes.

Distribution

Five distribution studies are reviewed below. Three studies examined the systemic distribution of ^{3}H-AFP-168 and its metabolites following ocular administration in rats (MRL Study Report No.: PK018) and monkeys (MRL Study Report No.: PK021) and intravenous administration in rats (MRL Study Report No.: PK020). Two other studies examined AFP-172 binding to serum albumin (MRL Study Report No.: PK017) and milk secretion and placental transfer of AFP-168 in rats (MRL Study Report No.: PK019).

Study Title: Evaluation of the binding ratios of AFP-172 to rat, rabbit, dog, and human serum albumin by ultrafiltration method (Study Report PK017)

Methods
The binding ratios of AFP-172 to rat, rabbit, dog, and human serum albumin were evaluated by an ultrafiltration method followed by LC-MS/MS detection. AFP-172 was tested at concentrations of 10, 100, and 500 ng/ml for albumin from all test species and additionally at 1 ng/ml for rabbit and human serum albumin.

Results
Only negligible amounts of AFP-172 were shown to bind to the ultrafiltration filter unit.

The binding ratios of AFP-172 to rat, rabbit, and dog serum albumin occurred in the range of 92.6 to 97% at AFP-172 concentrations of 10-500 ng/ml. The binding ratio of 500 ng/ml AFP-172 to human serum albumin was 99.2%. The binding ratios of AFP-172 to human serum albumin at AFP-172 concentrations of 1-100 ng/ml could not be obtained. This was apparently the case because at these lower concentrations, due to the high degree of protein binding, concentrations of free AFP-172 in the filtrate were below the limit of quantification for the LC-MS/MS detection method.

Study Title: ^{3}H-AFP-168: Systemic tissue distribution study in rat using quantitative whole-body autoradiography following administration of a single ocular dose (Study Report PK018).

Methods

This GLP-compliant study included a quality assurance statement in the study report. Eight male and eight female albino rats received single topical ocular doses of 0.005% ^{3}H-AFP-168 in 5 μl volumes to both eyes. Two animals of each gender were euthanized at 1, 4, 8, and 24 hours after dosing and blood samples were obtained from each rat in order to assess plasma concentrations of radioactivity. Subsequently, the tissue distribution of radioactivity was assessed in frozen animals carcasses using a quantitative whole-body autoradiography technique. Tissue radioactivity measurements were performed for adrenal glands, whole-blood in the heart, bone (surface and marrow), brain, eye, fat (abdominal and brown), Harderian gland, heart, kidney, lacrimal gland, liver, lung, muscle, ovary, pancreas, pituitary gland, salivary gland, skin, spinal cord, spleen, testes, thymus, thyroid, urinary bladder, uterus, stomach (walls and contents), small intestine (wall and contents), large intestine (wall and contents), and esophageal contents.

Results

The ratios of mean tissue to plasma radioactivity for each of the tissues examined are summarized below in Table 19 for males and Table 20 for females. The greatest concentrations of radioactivity occurred in most tissues at the first evaluation time-point, 1 hour after dosing, with the exception of the large intestine contents. At 1 hour post-dose, radioactivity concentrations were highest in the eye, followed by small intestine, esophageal contents, urinary bladder, stomach contents, liver, small intestine wall, and kidney. Low levels were also detected in large intestine contents, whole blood and lung, but radioactivity was below the limit of detection in all the other organs or tissues that were examined. After reaching peak values, tissue radioactivity levels rapidly declined and at the last measured time-point, 24 hours after dosing, low concentrations of radioactivity was only quantifiable in the large intestine contents and the eye (females only).

The pattern of tissue distribution suggests that AFP-168 is not retained in tissues for prolonged periods and that following topical ocular administration, the highest concentrations are primarily expected in the eye and the principle organs of drug absorption and excretion.

Table 19: Mean Tissue Concentrations of Radioactivity Following a Single Bilateral Ocular Administration of ^{3}H-AFP-168 to Male Rats. (Sponsor's Table)

Results are expressed as ng equivalents AFP-168/g tissue and represent the mean of two animals per time-point

Tissue/organ	Time (hours)			
	1	4	8	24
Plasma †				(b) (4)
Whole-blood				
Brain				
Spinal cord				
Eye				
Heart				
Kidney				
Urinary bladder ††				
Liver				
Lung				
Pancreas				
Spleen				
Adrenal gland				
Harderian gland				
Lacrimal gland				
Pituitary gland				
Salivary gland				
Thymus				
Thyroid				
Prostate				
Testes				
Bone surface				
Bone marrow				
Fat (abdominal)				
Fat (brown)				
Muscle				
Skin				
Stomach wall				
Stomach contents				
Small intestine wall				
Small intestine contents				
Large intestine wall				
Large intestine contents				
Oesophageal contents†††				

† Determined by liquid scintillation analysis
†† Includes measurement of urine
††† Includes measurement of oesophageal mucosa
[a] n=1; urinary bladder not sectioned for rat no. 6M
[b] n=1; urinary bladder not sectioned for rat no. 9M
ND No radioactivity detected; mean values are given as ND when the results for one or both animals were ND
Note: (i) Limit of quantification ≡ 1.382 ng equivalents AFP-168/g tissue
(ii) Values in parentheses represent concentrations in tissues which were below the limit of accurate quantification but which were visibly defined on the radioluminogram

Table 20: Mean Tissue Concentrations of Radioactivity Following a Single Bilateral Ocular Administration of ^{3}H-AFP-168 to female rats. (Sponsor's Table)

Results are expressed as ng equivalents AFP-168/g tissue and represent the mean of two animals per time-point

Tissue/organ	Time (hours)			
	1	4	8	24
Plasma †				(b) (4)
Whole-blood				
Brain				
Spinal cord				
Eye				
Heart				
Kidney				
Urinary bladder ††				
Liver				
Lung				
Pancreas				
Spleen				
Adrenal gland				
Harderian gland				
Lacrimal gland				
Pituitary gland				
Salivary gland				
Thymus				
Thyroid				
Ovaries				
Uterus				
Bone surface				
Bone marrow				
Fat (abdominal)				
Fat (brown)				
Muscle				
Skin				
Stomach wall				
Stomach contents				
Small intestine wall				
Small intestine contents				
Large intestine wall				
Large intestine contents				
Oesophageal contents†††				

† Determined by liquid scintillation analysis
†† Includes measurement of urine
††† Includes measurement of oesophageal mucosa
[a] n=1; urinary bladder not sectioned for rat no. 4F
ND No radioactivity detected; mean values are given as ND when the results for one or both animals were ND
Note: (i) Limit of quantification ≡ 1.382 ng equivalents AFP-168/g tissue
(ii) Values in parentheses represent concentrations in tissues which were below the limit of accurate quantification but which were visibly defined on the radioluminogram

Study Title: ^{3}H-AFP-168: Milk secretion and placental transfer studies in female rats following administration of a single ocular dose (MRL Report PK019).

Methods

Phase A (Milk secretion): Following pregnancy and between 10 and 13 days after parturition, 32 female CD(SD)IGS rats nursing suckling pups each received a single 5 ml ocular dose of ^{3}H-AFP-168 solution to both eyes. Milk was collected from groups of four rats at each of the following times post-administration: 30 minutes and 1, 2, 4, 8, 24, 48, and 96 hours. Pups were removed from the dams and euthanized at 3 hours before the scheduled time of milk sampling. Oxytocin was administered about 15 minutes before sampling to stimulate milk production. After collection of the milk sample, each dam was euthanized and blood samples were obtained. Radioactivity was measured in blood, plasma, and blood cell fractions.

Phases B and C (Placental transfer): Single 5 µl ocular doses of 0.005% ^{3}H-AFP-168 solution were administered to both eyes of 23 pregnant female rats on Day 12 of gestation, and to 20 other pregnant rats on Day 18 of gestation. Animals were euthanized in groups of four at 0.25, 1, 4, 12, and 24 hours after dosing. Blood samples were obtained at each time-point from each animal. In addition, in conjunction with the Day 12 dosing and sacrifices, the following tissues were removed from each dam and sampled: amniotic fluid, cerebellum, cerebrum, fetuses, heart, kidneys, liver, lung, mammary glands, ovary, placenta, and uterus. In conjunction with the Day 18 dosing and sacrifices, the following tissues were removed and sampled: amniotic fluid, cerebellum, cerebrum, heart, kidneys, liver, lungs, mammary gland, ovary, placenta, uterus, fetus, fetal blood, fetal brain, fetal carcass, fetal kidney, fetal liver, and fetal lung. The total number of fetuses per animal was recorded. For the Day 18 sacrifices, three fetuses from each dam were retained intact.

Phases D and E (Placental transfer-quantitative whole-body autoradiography): Single 5 µl ocular doses of 0.005% ^{3}H-AFP-168 were administered to both eyes of 10 pregnant female rats on Day 12 of gestation, and to 10 other pregnant female rats on Day 18 of gestation. Dams were sacrificed in pairs at 0.25, 1, 4, 12, and 24 hours after dosing, and blood samples were collected and processed to plasma. After sacrifice, the carcasses were quickly freeze-dried then analyzed for tissue-specific radioactivity concentrations using quantitative whole-body autoradiography. Since the carcass sections were freeze-dried, the expected measurements are only of non-volatile radioactivity. Radioactive concentrations were assessed in the following organs: adrenal gland, whole-blood, bone (surface and marrow), brain, eye, fat (abdominal and brown), fetuses, harderian gland, heart, kidney, lacrimal gland, liver, lung, muscle, ovary, pancreas, pituitary gland, placenta, salivary gland, skin, spinal cord, spleen, thymus, thyroid, urinary bladder, uterus, esophageal contents, and the walls and contents of the stomach, small intestine, and large intestine.

Results

Phase A: Mean maternal plasma concentrations of total radioactivity were greatest at 30 minutes after dosing with declining concentrations until a second peak at 8 hours post-

dose and still measureable concentrations at 96 hours after dosing. Whole blood concentrations followed a similar pattern. The maximum mean concentrations of total radioactivity in milk occurred two hours after dosing and concentrations declined steadily thereafter with the lowest concentrations still measureable at 96 hours after dosing. During the terminal phase, plasma, whole blood, and milk radioactivity levels declined with an estimated $t_{1/2}$ in the range of 43.4 to 45.1 hours. The mean concentrations of radioactivity in milk, plasma, and whole-blood are shown below in Table 21, and select compartmental pharmacokinetic parameters are shown below in Table 22.

Table 21: Mean Concentrations of Total Radioactivity in Milk, Plasma, and Whole-Blood following Single Ocular Administrations of 0.005% ^{3}H-AFP-168 to Both Eyes of Lactating Maternal Rats. (Sponsor's Table)

Results are expressed as ng equivalents AFP-168/g and represent the mean of four animals per sacrifice time

Time	Milk concentrations		Plasma concentrations		Whole-blood concentrations	
	Mean	SD	Mean	SD	Mean	SD
30 min	0.189	0.034	0.466	0.070	0.305	0.043
1 hr	0.221	0.052	0.179	0.014	0.127	0.011
2 hr	0.262	0.046	0.106	0.007	0.085	0.003
4 hr	0.120	0.042	0.059	0.015	0.051	0.013
8 hr	0.082	0.014	0.067	0.005	0.061	0.003
24 hr	0.031	0.007	0.035	0.009	0.031	0.008
48 hr	0.019	0.003	0.023	0.005	0.019	0.004
96 hr	0.010	0.003	0.011	0.003	0.010	0.003

SD Standard deviation

Table 22: Select Milk, Plasma, and Whole-Blood Total Radioactivity Pharmacokinetic Parameters Following a Single Ocular Administration of 0.005% ^{3}H-AFP-168 Solution to Lactating Maternal Rats. (Sponsor's Table)

Matrix	C_{max} (ng equiv/g)	T_{max} (hr)	k (hr^{-1})	$t_{½}$ (hr)	AUC_{96} (ng equiv.hr/g)
Milk	0.262	2	0.0154	45.1	3.38
Plasma	0.466	0.5	0.0160	43.4	3.17
Whole-blood	0.305	0.5	0.0154	45.1	2.68

Note: Values for k and $t_{½}$ could not be calculated in accordance with the acceptance criteria described in the Data Processing section and are included for guidance only

Phases B and C: Following the Gestation Day 12 dosing, radioactive concentrations were highest 15 minutes after dosing for most of the maternal tissues/organs analyzed

with the exception of the amniotic fluid samples and the fetuses where concentrations were maximal four hours after dosing.

Following dosing on Gestation Day 18, mean radioactive concentrations in amniotic fluid and fetal tissues/organs were both maximal 4 hours after dosing and at 4 hours after dosing, the highest mean concentrations of total radioactivity in fetal tissues were present in fetal liver, brain, and lung. For both Days 12 and 18, maternal plasma radioactivity concentrations were higher than concentrations in fetuses. Based on $AUC_{0\text{-}24hr}$, the fetus to plasma ratios were 0.62 and 0.71 on Day 12 and Day 18 respectively. The mean total radioactivity measurements for different maternal and fetal tissues is shown below in Table 23 for the Day 12 dosing and in Table 24 for the Day 18 dosing.

Table 23: Mean Total Radioactivity in Tissues Following a Single Ocular Administration of ^{3}H-AFP-168 to Pregnant Rats on Gestation Day 12. (Sponsor's Table)

Results are expressed as % dose and represent the mean of four animals per sacrifice time

Tissue/organ	Sacrifice time									
	15 minutes		1 hour		4 hours		12 hours		24 hours	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cerebellum	0.0067	0.0021	0.0043	0.0018	0.0045	0.0013	0.0033	0.0005	0.0029	0.0002
Cerebrum	0.0287	0.0104	0.0203	0.0025	0.0185	0.0064	0.0139	0.0028	0.0134	0.0021
Heart	0.1499	0.0143	0.0410	0.0064	0.0176	0.0033	0.0148	0.0018	0.0129	0.0018
Kidney	3.739	0.666	1.015	0.163	0.1124	0.0188	0.0612	0.0073	0.0397	0.0041
Liver	22.77	2.26	10.94	2.22	1.480	0.170	0.4340	0.0701	0.2389	0.0070
Lungs	0.1511	0.0350	0.0464	0.0084	0.0251	0.0060	0.0196	0.0036	0.0171	0.0041
Amniotic fluid	0.0070	0.0026	0.0099	0.0016	0.0104	0.0031	0.0083	0.0006	0.0105	0.0038
Foetuses	0.0054	0.0018	0.0067	0.0016	0.0079	0.0017	0.0082	0.0022	0.0127	0.0014
Mammary glands	0.1818	0.0362	0.0852	0.0288	0.0430	0.0087	0.0289	0.0099	0.0289	0.0053
Ovaries	0.0182	0.0026	0.0057	0.0006	0.0024	0.0005	0.0016	0.0002	0.0017	0.0001
Placentae	0.0294	0.0092	0.0186	0.0063	0.0128	0.0019	0.0093	0.0025	0.0133	0.0117
Uterus	0.1467	0.0216	0.0923	0.0139	0.0398	0.0111	0.0298	0.0113	0.0380	0.0047

SD Standard deviation

Table 24: Mean Total Radioactivity in Tissues Following a Single Ocular Administration of ^{3}H-AFP-168 to Pregnant Rats on Gestation Day 18. (Sponsor's Table)

Results are expressed as % dose and represent the mean of four animals per sacrifice time

Tissue/organ	Sacrifice time									
	15 minutes		1 hour		4 hours		12 hours		24 hours	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cerebellum	0.0067	0.0011	0.0041	0.0007	0.0037	0.0008	0.0034	0.0012	0.0038	0.0010
Cerebrum	0.0198	0.0056	0.0150	0.0049	0.0149	0.0029	0.0159	0.0057	0.0146	0.0018
Heart	0.1447	0.0399	0.0414	0.0053	0.0201	0.0032	0.0133	0.0033	0.0167	0.0021
Kidney	3.563	0.422	1.001	0.216	0.1516	0.0236	0.0638	0.0082	0.0477	0.0064
Liver	23.31	1.55	14.49	3.47	1.933	0.448	0.9007	0.2348	0.3003	0.0288
Lungs	0.1491	0.0573	0.0515	0.0060	0.0280	0.0046	0.0208	0.0065	0.0211	0.0014
Amniotic fluid	0.0279	0.0136	0.0718	0.0211	0.0938	0.0298	0.0551	0.0246	0.0486	0.0101
Foetal brain	0.0063	0.0013	0.0148	0.0007	0.0178	0.0035	0.0123	0.0027	0.0202	0.0013
Foetal carcass*	0.1010	0.0211	0.1871	0.0157	0.2482	0.0665	0.2224	0.0434	0.3201	0.0615
Foetal kidney	0.0015	0.0003	0.0022	0.0004	0.0025	0.0008	0.0025	0.0004	0.0032	0.0005
Foetal liver	0.0275	0.0091	0.0430	0.0059	0.0361	0.0113	0.0247	0.0058	0.0327	0.0029
Foetal lung	0.0072	0.0023	0.0109	0.0012	0.0132	0.0029	0.0116	0.0020	0.0177	0.0034
Foetuses (intact; n=3)	0.0517	0.0108	0.1009	0.0146	0.1062	0.0198	0.0924	0.0106	0.1483	0.0301
Mammary glands	0.2554	0.0532	0.1548	0.0315	0.0702	0.0061	0.0356	0.0111	0.0426	0.0038
Ovaries	0.0464	0.0151	0.0124	0.0038	0.0042	0.0005	0.0028	0.0003	0.0025	0.0004
Placentae	0.1529	0.0267	0.1403	0.0078	0.1196	0.0282	0.0885	0.0164	0.1037	0.0133
Uterus	0.2351	0.0480	0.1510	0.0084	0.0963	0.0167	0.0698	0.0122	0.0688	0.0047
Total % dose (all foetuses)†	0.1951	0.0335	0.3588	0.0321	0.4239	0.1038	0.3659	0.0423	0.5421	0.0951
No. of foetuses per rat	13	3	12	2	13	1	13	2	12	1
% dose per foetus**	0.0173	0.0036	0.0336	0.0049	0.0354	0.0066	0.0308	0.0035	0.0494	0.0100

SD Standard deviation
* After removal of foetal organs
† Excluding blood from dissected foetuses
** Mean % dose per intact foetus

Phases D and E: Radioactivity concentrations in fetuses were either below the limit of accurate quantification or not detected by autoradiography for both the Day 12 and Day 18 doses. Since only non-volatile radioactivity is expected to be measured with autoradiography, the results suggest that much of the radioactivity in fetuses at sacrifice was in the form of tritiated water.

Study Title: ^{3}H-AFP-168: Systemic tissue distribution study in the rat using quantitative whole-body autoradiography following administration of a single intravenous dose (MRL Report PK020).

Methods

This GLP-compliant study included a quality assurance statement in the study report. Single 0.5 μg intravenous doses of ^{3}H-AFP-168 were administered as a bolus to eight male and eight female albino rats. Animals were euthanized in groups of two per gender at 1, 4, 8, and 24 hours after administration, and radioactivity was measured in selected tissues and organs by quantitative whole-body autoradiography. Measurements were performed in the following tissues: adrenal gland, whole blood, bone (surface and marrow), brain, eye, fat (abdominal and brown), Harderian gland, heart, kidney, lacrimal gland, liver, lung, muscle, ovary, pancreas, pituitary gland, salivary gland, skin, spinal cord, spleen, testes, thymus, thyroid, urinary bladder, uterus, stomach (wall and contents) small intestine (wall and contents), large intestine (wall and contents) and

esophageal contents. Blood samples were also obtained at the same time-points, and plasma radioactivity concentrations were measured by liquid scintillation counting.

Results
The greatest concentrations of radioactivity in most tissues occurred a the first measurement point, 1 hour after administration. Exceptions to this pattern were small intestine contents (T_{max} at 4 hours in males) and large intestine contents (T_{max} values of 8 hours in males and 4 hours in females). At 1 hour after dosing, radioactivity was highest in the small intestine contents followed by liver, small intestine wall, stomach contents, and kidney. Radioactivity was not detected at 1 hour post-dose in any other organ or tissue including whole-blood. After reaching peak levels, tissue radioactivity levels declined rapidly and at 24 hours after dosing, radioactivity was only detected at low levels in the stomach contents, small intestine contents, and large intestine contents.

Study Title: Systemic tissue distribution of ^{3}H-AFP-168 by quantitative whole-body autoradiography in Cynomolgus monkeys after topical administration on the eyes (MRL Report PK021).

Methods
This study was conducted in a GLP-compliant manner but a quality assurance statement was not included with the study report. Four male and four female Cynomolgus monkeys received single bilateral administrations of 1 μg/eye of ^{3}H-AFP-168. Animals (one per gender) were euthanized at 1, 4, 8, and 24 hours after dosing, and carcasses were analyzed for tissue and organ distribution of radioactivity using whole-body autoradioagraphy. Tissues and organs analyzed for radioactivity by autoradiography included: ocular tissues (cornea, iris, palpebra, sclera), esophagus, gall bladder/bile, lacrimal apparatus, large intestine contents, liver, palate, small intestine (wall and contents), stomach (wall and contents), urinary bladder, and urine. Blood was also collected at the same post-dose timepoints as well as pre-dose, and plasma radioactivity concentrations and radioactivity associated with the blood cell fraction were assessed with liquid scintillation counting.

Results
After ocular dosing in male monkeys, radioactivity was rapidly absorbed into ocular tissues and systemic circulation. The highest concentrations of radioactivity were observed in the cornea followed by approximately ten fold lower levels in the iris, and schlera at 1 hour after dosing. Radioactivity concentrations in the eyelids 4 hours after dosing were similar to those seen in the iris and sclera at the 1 hour time-point. Maximal concentrations of radioactivity in peripheral tissues were at least 10 fold lower than in the cornea with the highest concentrations in the urinary bladder followed by the small intestine contents 1 hour after dosing and similar levels in the lacrimal apparatus 4 hours after dosing. Radioactivity concentrations in the large intestine contents peaked at 8 hours after dosing and 24 hours after dosing, the cornea and large intestinal contents were the only tissues with measureable radioactivity. Tissue distribution in females followed a similar pattern with some temporal deviations including peak concentrations

in the lacrimal apparatus 1 hour after dosing. The results were consistent with rapid uptake of ^{3}H-AFP-168 into ocular tissues accompanied by systemic absorption via the lacrimal apparatus and gastrointestinal tract.

Metabolism

Several studies examining the metabolism of AFP-168 are reviewed below. These include two studies examining the comparative *in vitro* metabolites of AFP-168 in rat, dog, monkey, and human hepatocytes (MRL Report Nos.: PK022 and PK023). Other *in vitro* studies included studies elucidating the contribution of specific esterase enzymes in rabbit corneal tissue in the metabolism of AFP-168 (MRL Report No.: PK024), the contribution of specific CYP-450 isozymes in AFP-172 metabolism (MRL Report No.: PK032), and the ability of AFP-168 to induce phase I and phase II hepatic enzymes (MRL Report No.: PK025). *In vivo* studies included examination of AFP-168 metabolism following intravenous administration in rats (MRL Report No.: PK027) and topical ocular administration in monkeys (MRL Report No.: PK029). Other studies (MRL Report Nos.: PK026, PK028, PK030) were not reviewed because they were considered to not add substantially new information.

Study Title: AFP-168: Comparative *in vitro* metabolism studies with rat, dog, Cynomolgus monkey and human hepatocytes. (MRL Report PK022).

Methods
This study was conducted in a GLP-compliant manner and a quality assurance statement was included in the study report. Cryopreserved hepatocytes from rat, Cynomolgus monkey, and human were cultured and incubated at a cell density of 1 x 10^{6} cells/ml with [^{3}H]AFP-168 concentrations of 2.5 and 25 μM for 4 hours. The number of viable dog hepatocytes obtained after thawing was lower than for the other species and the cell density of incubated dog hepatocytes was lower. After incubation, the hepatocyte cultures were homogenized and AFP-168 metabolites were assessed using a gradient elution radiochemical HPLC method. Aliquots of each sample were subject to enzymatic deconjugation in order to assess the degree and scope of conjugated metabolites.

Results
[^{3}H]AFP-168 was rapidly converted to its active metabolite [^{3}H]AFP-172 when incubated with hepatocytes from all species particularly at the lower [^{3}H]AFP-168 concentrations. The results from the deconjugation treatment indicate that in all species a small percentage of the total [^{3}H]AFP-168 metabolites were conjugated. Due to endogenous esterase activity present in the enzyme used for deconjugation, the deconjugation treatment also resulted in complete conversion of [^{3}H]AFP-168 to [^{3}H]AFP-172. Further metabolism of [^{3}H]AFP-172 was rapid and extensive (> 50%) in hepatocytes from all species except dog where the low number of viable cells per incubation may have affected the outcome. The metabolic profiles for each species indicate that [^{3}H]AFP-172 was further metabolized to 7, 3, 12, and 7 other unidentified metabolite fractions in a time-dependent manner in rat, dog, monkey, and human respectively (Table 25). Two

human metabolites (HMF-2 and HMF-5) were not formed in any of the other experimental species. However, these metabolites comprised a low percentage (< 5%) of the total metabolites (Table 26). The major metabolites were formed in each species and corresponded to the human metabolites HMF-4, HMF-7 (AFP-172) and HMF-8 (AFP-168).

Table 25: Human Metabolites and the Corresponding Animal Equivalents. (Sponsor's Table)

Untreated samples

Human	Rat	Dog	Cynomolgus monkey
-	RMF-1	-	MMF-1
-	-	-	MMF-2
-	-	-	MMF-3
HMF-1	RMF-2	-	MMF-4
-	RMF-3	-	MMF-5
HMF-2	-	-	-
-	-	-	MMF-6
HMF-3	RMF-4	-	MMF-7
-	-	-	MMF-8
-	-	-	MMF-9
HMF-4	RMF-5	DMF-1	MMF-10
HMF-5	-	-	-
HMF-6	RMF-6	-	MMF-11
HMF-7‡	RMF-7‡	DMF-2‡	MMF-12‡

- not formed
‡ co-chromatographed with AFP-172

Table 26: Metabolism of ^{3}H-AFP-168 by Cyopreserved Pooled Human Hepatocytes after 4 hours Incubation. (Sponsor's Table)

			% of injected radioactivity attributed to							
AFP-168 Conc (μM)	Treatment	Rep	HMF-1	HMF-2	HMF-3	HMF-4	HMF-5	HMF-6	HMF-7‡	HMF-8†
2.5	Untreated	A	4.6 (1)	3.0 (0)	5.3 (1)	25.3 (3)	3.2 (0)	0.7 (0)	49.3 (7)	0.1 (0)
		B	3.6 (0)	2.5 (0)	4.9 (1)	26.5 (4)	3.3 (0)	1.0 (0)	50.0 (7)	0.0 (0)
	Control		0.0	0.0	0.0	0.0	0.0	0.0	5.6	94.2
	Deconjugated	A	4.3 (1)	2.6 (0)	5.5 (1)	27.7 (4)	3.6 (0)	1.0 (0)	45.5 (6)	0.8 (0)
		B	3.3 (0)	1.9 (0)	5.2 (1)	28.2 (4)	3.3 (0)	1.1 (0)	49.2 (7)	1.0 (0)
	Control		0.0	0.0	0.0	0.1	0.1	0.1	98.5	0.9
25	Untreated	A	1.2 (2)	1.2 (2)	2.6 (3)	18.9 (25)	3.2 (4)	0.8 (1)	66.5 (89)	0.0 (0)
		B	1.1 (1)	1.4 (2)	2.3 (3)	19.4 (26)	3.1 (4)	1.4 (2)	66.4 (88)	0.0 (0)
	Control		0.0	0.0	0.0	0.0	0.1	0.1	4.0	94.3
	Deconjugated	A	1.1 (1)	1.3 (2)	1.8 (2)	21.2 (28)	2.5 (3)	1.0 (1)	64.7 (86)	1.3 (2)
		B	1.1 (1)	1.2 (2)	2.3 (3)	19.6 (26)	2.7 (4)	1.6 (2)	65.6 (87)	1.0 (1)
	Control		0.1	0.1	0.0	0.1	0.1	0.2	95.9	1.4

( ) denotes rate of metabolism/formation (nmoles/hr/mg protein)
‡ co-chromatographed with AFP-172
† co-chromatographed with AFP-168

Study Title: *In vitro* metabolism of [^{3}H]-AFP-168 and AFP-172 by rat, monkey, and human hepatocytes (MRL Report PK023).

Methods
This study was conducted in a GLP-compliant manner and a quality assurance statement was included in the study report. Cryopreserved hepatocytes from Sprague-Dawley rats, Cynomolgus monkeys, and humans were thawed, assessed for viability and standard metabolic activity and incubated with 100 μM concentrations of [^{3}H]-AFP-168 or AFP-172 for 0, 120, and 240 minutes. Upon termination, AFP-168, AFP-172 and metabolites were analyzed using a LC/MS method.

Results
In hepatocytes from all species, [^{3}H]-AFP-168 was completely metabolized to various metabolites after 240 minutes of incubation. In monkey and human hepatocytes, eleven metabolites were detected and 7 metabolites were detected in rat hepatocyte incubations. Following incubation with AFP-172, AFP-172 and the same 10 metabolites were detected in monkey and human hepatocytes, and AFP-172 and 10 metabolites were detected in rat hepatocytes. The metabolites and for each species and the putative metabolite identities are summarized in Table 27. Five metabolites (1,2-dinor-AFP-172, 1,2,3,4-tetranor-AFP-172, hydroxy-1,2-dinor-AFP-172, hydroxy-1,2,3,4-tetranor-AFP-172 and AFP-172) were detected in hepatocytes from all three species

incubated with either [^{3}H]-AFP-168 or AFP-172. Also, a total of seven potential glucuronide products were detected with one (No. 7) apparently occurring only in rat hepatocytes.

Table 27: Metabolites Identified in Rat, Monkey, and Human Hepatocytes After Incubation with Either ^{3}H-AFP-168 or AFP-172. (Sponsor's Table)

	Incubation with [^{3}H]-AFP-168			Incubation with AFP-172		
Metabolite	Rat	Monkey	Human	Rat	Monkey	Human
AFP-172	x	x	x	x	x	x
1,2-dinor-AFP-172	x	x	x	x	x	x
1,2,3,4-tetranor-AFP-172	x	x	x	x	x	x
Hydroxy-1,2-dinor-AFP-172	x	x	x	x	x	x
Hydroxy-1,2,3,4-tetranor-AFP-172	x	x	x	x	x	x
AFP-172 glucuronide No. 1	x	x	x	x	x	x
AFP-172 glucuronide No. 2		x	x	x	x	x
AFP-172 glucuronide No. 3		x	x	x	x	x
AFP-172 glucuronide No. 4		x	x		x	x
AFP-172 glucuronide No. 5		x	x	x	x	x
AFP-172 glucuronide No. 6		x	x	x	x	x
AFP-172 glucuronide No. 7	x			x		

x Metabolite detected.

Study Title: Determination of esterase enzyme related to AFP-168 metabolism in corneal tissue of rabbits (MRL Report PK024).

Methods

AFP-168 (0.001%) was incubated with rabbit eye S9 metabolizing enzymes or purified preparations of individual esterases (acetylcholinesterase, butyrylcholinesterase, or carboxyesterase) in the absence or presence of specific esterase inhibitors. The specific esterase inhibitors included: BW284c51, an acetylcholinesterase inhibitor, iso-OMPA, a butyrylcholinesterase inhibitor, p-NPA, a carboxylesterase inhibitor, and α-NA, a carboxyesterase inhibitor. After 30 minutes incubation, the reaction products were separated by HPLC.

Results

AFP-168 was hydrolyzed to AFP-172 after incubation with rabbit corneal S9. The carboxyesterase inhibitors, p-NPA, and α-NA inhibited the AFP-168 hydrolysis at concentrations of 1×10^{-3} mol/L. Iso-OMPA, a butyrylcholinesterase inhibitor, also inhibited AFP-168 hydrolysis at a concentration of 1×10^{-4} mol/L. The acetylcholinesterase inhibitor, BW284c51 did not inhibit AFP-168 hydrolysis at a concentration of 1×10^{-5} mol/L.

In the experiments with purified esterases, AFP-168 was completely hydrolyzed with 0.1U of carboxyesterase and partially hydrolyzed with a larger activity of butyrylcholinesterase (10U). High 10U activities of acetylcholinesterase failed to

hydrolyze AFP-168 to any extent. The results from the purified enzyme and enzyme inhibitor experiments suggest AFP-168 is primarily metabolized by carboxyesterases.

Study Title: Metabolism of ^{3}H-AFP-168 in rats following intravenous administration (MRL Report PK027).

Methods

This study was conducted in a GLP-compliant manner and a quality assurance statement was included in the study report. Three bile duct cannulated male rats were administered two consecutive daily intravenous doses of [^{3}H]-AFP-168 (100 μg/kg/day). Urine, feces and bile were collected at specified intervals following the first daily dose. Urine was collected at 0-4, 4-8, and 8-24 hours after the first dose administration. Feces was collected for 24 hours after the first dose administration. Bile was collected at 0-2, 2-4, 4-6, 6-8 and 8-24 hours after the first dose administration. At 0.25 hours after the second daily dose, the rats were sacrificed and blood (plasma), liver, kidneys, and lungs were collected. Plasma, urine, feces and bile were analyzed for radioactivity by liquid scintillation counting. The amount of tritiated water in plasma and urine samples was determined. Plasma, urine, and bile samples were analyzed for the AFP-168 metabolic profile using an HPLC/MS method.

Results

The Day 1 and Day 2 predose and postdose radiopurity values from HPLC analysis of the [^{3}H]-AFP-168 ranged from 99.6 to 99.7%.

The average concentrations of radioactivity in the plasma of the three experimental rats are shown below in Table 28. Blood was obtained from one rat at 0.10 hours after dosing and at 0.25 hours after dosing for the other two rats. The much lower plasma concentrations of radioactivity, and much greater percent tritiated water in plasma for the two animals sampled at the later timepoint suggests rapid metabolism and plasma clearance of [^{3}H]-AFP-168 after dosing.

Table 28: Concentrations of Radioactivity and the Amount of Tritiated Water in Plasma at Sacrifice After the Day 2 Intravenous Administration of 100 μg/kg/day ^{3}H-AFP-168 to Male Rats Dosed Once Daily for Two Days. (Sponsor's Table)

Matrix	Collection Time Point (hours)	ng Equivalents [^{3}H]-AFP-168/g				
		Animal Number				
		C24686[a]	C24687	C24688	Mean[b]	SD
Wet[c] Plasma	0.25	321[a]	76.6	59.0	67.8	NA
Dry[d] Plasma	0.25	316[a]	70.6	53.5	62.1	NA
		Percent Tritiated Water in Plasma				
Plasma	0.25	1.56[a]	7.83	9.32	8.58	NA

NA Not applicable.
SD Standard deviation.
Note: Percent tritiated water is calculated by taking wet minus dry, divided by wet, times 100.
a Animal was sacrificed at 0.10 hours after the Day 2 dose. Values for plasma concentration values and % tritiated water are excluded from the mean.
b Average of 2 values.
c Samples were analyzed by direct LSC.
d Samples were allowed to dry and were then reconstituted before counting by LSC.

The amounts of radioactive dose in urine, feces, bile, cage wash, cage wipe, bile cannula, and jacket rinse are shown in Table 29 below. Radioactivity was rapidly excreted with bile as the primary route of elimination. A mean of 49.9% of the administered dose was excreted in bile in the first 24 hours with most of the bile excretion (41.1%) occurring in the first two hours after dosing. The secondary route of excretion was urine accounting for 25.3% of the administered dose 24 hours after dosing, and excretion in feces was a minor route of elimination (1.31% of the dose in 24 hours).

Table 29: Percent of Radioactive Dose in Urine, Feces, Bile, Cage Wash, Cage Wipe, Bile Cannula, and Jacket Rinse, at Specified Intervals After a Single Intravenous Administration of ^{3}H-AFP-168 to Male Rats Dosed Once Daily for Two Days. (Sponsor's Table)

Collection Interval (Hours)	Percent of Radioactive Dose				
	Animal Number				
	C24686	C24687	C24688	Mean	SD
			Urine		
0-4	BLQ	13.1	26.6	13.2	13.3
4-8	23.7	6.79	BLQ	10.2	12.2
8-24	2.22	1.41	2.22	1.95	0.47
Subtotal	25.9	21.3	28.8	25.3	3.8
			Feces		
0-24	1.32	1.21	1.39	1.31	0.09
			Bile		
0-2	40.1	44.2	39.1	41.1	2.7
2-4	6.92	7.23	7.32	7.16	0.21
4-6	0.82	0.73	0.78	0.78	0.05
6-8	0.24	0.18	0.19	0.20	0.03
8-24	0.79	0.52	0.60	0.64	0.14
Subtotal	48.9	52.9	48.0	49.9	2.6
			Cage Wash, Cage Wipe, Bile Cannula, and Jacket Rinse		
24[a]	0.04	0.36	0.03	0.14	0.19
24[b]	0.14	0.10	0.11	0.12	0.02
24[c]	0.05	0.03	0.05	0.04	0.01
24[d]	BLQ	BLQ	0.00	0.00	0.00
Total	76.3	75.9	78.4	76.9	1.3

BLQ Below the limit of quantitation.
SD Standard deviation.
a Cage wash.
b Cage wipe.
c Bile cannula.
d Jacket rinse.

The proposed identities of the AFP-168 metabolites and the matrices where they occurred (plasma, urine, and bile) are summarized below in Table 30. [^{3}H]-AFP-168 was not found in plasma urine or bile, and was rapidly de-esterified to [^{3}H]-AFP-172. Subsequently, [^{3}H]-AFP-172 was rapidly oxidized and metabolized by β-oxidation, hydroxylation, and conjugation with glucuronic acid or sulfate.

Four metabolites were found in plasma. The first predominant metabolite was AFP-172 (M22) which was superceded as the major metabolite by 1,2,3,4-tetranor-AFP-172 (M15) at 15 minutes after dosing. The two other metabolites in plasma were 1,2,3,4-tetranor-AFP-172-glucuronide (M10) and hydroxyl-1,2,3,4-teranor-AFP-172-sulfate (M20B).

In urine, seven metabolites were found including four low incidence (≈ 1 to 4% of sample radioactivity), unidentified metabolites. The three major metabolites were 1,2,3,4-tetranor-AFP-172 (M15; 29.2 to 41.5% of sample radioactivity), hydroxyl-1,2,3,4-tetranor-AFP (M9, 19.5 to 23.0% of sample radioactivity), and hydroxyl-1,2,3,4-teranor-AFP-172-sulfate (M20B; 29.7 to 38.5% of sample radioactivity). Two additional metabolites, hydroxyl-1,2-dinor-AFP-172 (M11) and 1,2-dinor AFP-172 (M20A) were observed at radioactivity levels too low to quantify.

Eight metabolites were found in bile. The three major metabolites were: 1,2,3,4-tetranor-AFP-172 (M15; 10.2 to 17.0% of sample radioactivity), hydroxyl-1,2,3,4-tetranor-AFP-172 sulfate (M20B; 29.5 to 34.9%), and 1,2,3,4-tetranor-AFP-172 glucuronide (M10; 38.6 to 55.3%). Two other unidentified metabolites were found at low levels (≤ 4.02% of the sample radioactivity) and only in one animal. Three additional metabolites, hydroxyl-1,2-dinor-AFP-172 (M11), AFP-172 glucuronide (M17B), and 1,2-dinor-AFP-172 (M20A) were observed at radioactive levels too low to be quantified.

Table 30: Metabolites found in Plasma, Urine, and Bile in Male Rats Dosed Once Daily for Two Days with Intravenous 100 µg/kg/day ^{3}H-AFP-168. (Sponsor's Table)

Peak	Retention Time Minutes	Proposed Identification	Metabolite found in: Plasma	Urine	Bile
M1	3.90	Unknown		x	
M2	4.60-4.70	Unknown		x	
M9	14.60-14.70	Hydroxy-1,2,3,4-tetranor-AFP-172		**x**	
M10	17.40-17.60	1,2,3,4-tetranor-AFP-172 glucuronide	x		**x**
M11[a]	17.30[b]	Hydroxy-1,2-dinor-AFP-172		ms	ms
M13	21.70	Unknown			x
M15	23.30-23.60	1,2,3,4-tetranor-AFP-172	x	**x**	**x**
M17B[a]	24.00[b]	AFP-172 glucuronide			ms
M20A[a]	25.90[b]	1,2-dinor-AFP-172		ms	ms
M20B	27.90-30.70	Hydroxy-1,2,3,4-tetranor-AFP-172 sulfate	x	**x**	**x**
M22	32.20-32.40	AFP-172	x		
M23	34.40	Unknown		x	
M24	46.30-47.00	Unknown		x	x

ms Indicates the metabolite was present in the matrix, but was only detectable by LC/MS.

x Indicates the metabolite was present in the matrix as analyzed by HPLC. Bold (**x**) designates a major metabolite.

a Metabolite observed by LC/MS; levels of radioactivity were too low for quantitation.
b Retention time from LC/MS analysis.

Study Title: Metabolism of [^{3}H]-AFP-168 in Cynomolgus Monkeys After Topical Administration on the Eyes (MRL Report PK029).

Methods

This study was conducted in a GLP-compliant manner and a quality assurance statement was included in the study report. The metabolism of [^{3}H]-AFP-168 was determined in monkeys following a single topical ocular administration to each eye. Four monkeys of each gender per Phase of the Study were dosed with 1 μg/eye of [^{3}H]-AFP-168 (Phase 1) and with 10 μg/eye for Phases 2 and 3 (Table 31). Plasma, urine, and feces were collected for metabolic profiling in Phases 1 and 2. Plasma samples were collected at 0.083, 0.167, 0.333, 0.5, 1, 2, 4 and 6 hours after dosing. Urine was collected at 0-6, 6-12, 12-24, 24-48, and 48-72 hours after dosing. Feces were collected 0-24, 24-48, and 48-72 hours after dosing. In Phase 3, one monkey/sex was sacrificed at 0.25, 1, 2, and 8 hours after dosing for metabolic profiling of [^{3}H]-AFP-168 in ocular tissues (cornea, aqueous humor iris and ciliary body) as well as in the kidneys, liver, and lungs. Samples were analyzed with liquid scintillation counting, and radio-HPLC and LC/MS/MS methods.

Table 31: Study Design for MRL Study PK029. (Sponsor's Table)

Phase	Number of Animals Male	Number of Animals Female	Dose Level[a] (μg/eye)	Dose Concentration (μg/mL)	Radioactive Dose Level (MBq/eye)	Sample Collections (Hours Postdose)
1	4	4	1.0	50	1.6	Blood, Urine, Feces[b]
2	4	4	10.0	500	10	Blood, Urine, Feces[b]
3	4	4	10.0	500	10	Tissues[c]

a Ocular dose, both eyes (20 μL/eye).

b Blood (approximately 2 mL) was collected predose, and at 0.083, 0.167, 0.333, 0.5, 1, 2, 4, and 6 hours postdose. Urine was collected predose, and at 0-6, 6-12, 12-24, 24-48, and 48-72 hours postdose. Feces were collected predose, and at 0-24, 24-48, and 48-72 hours postdose.

c Blood (approximately 10 mL) was collected predose and at the time of sacrifice; one animal per sex per time point was sacrificed at 0.25, 1, 2, and 8 hours postdose. At sacrifice, kidney, liver, lung, and each eye were excised.

Results

Radiopurity was determined to be 98.5% to 99.7%, and actual [^{3}H]-AFP-168 concentrations were 91.8%, 98.8%, and 99.4% of the nominal concentrations for Phases 1, 2, and 3 respectively.

The mean C_{max} values in blood and plasma increased in an approximately dose-dependent manner between the 1 μg/kg and 10 μg/kg doses administered in Phase 1 and Phase 2 of this study respectively. T_{max} values were at the first measured time-point as is expected after intravenous administration. Males consistently demonstrated slightly higher blood and plasma concentration levels compared to females. Also the concentration of radioactivity was higher in plasma compared to blood.

After ocular administration of 1 μg/kg ^{3}H-AFP-168 in Phase 1 of the study, the greatest proportion of the total radioactivity were recovered in urine and feces (Table 32). The total radioactivity recovered in urine and feces was approximately 26% and 21% respectively through 72 hours after dosing. The overall recovery of radioactivity through 72 hours was approximately 73%. A very similar pattern of excretion was evident following topical ocular administration of 10 μg/kg ^{3}H-AFP-168 in Phase 2 of the study.

Table 32: Mean Percent Radioactive dose in Urine, Feces, Cage Rinse, Cage Wash, and Cage Wipe at Specificed Intervals After Topical Ocular Administration of ^{3}H-AFP-168 to Male and Female Cynomolgus monkeys. (Sponsor's Table)

Collection Interval (Hours)	Percent of Radioactive Dose			
	Male		Female	
	Mean	SD	Mean	SD
		Urine		
0-6	9.07	3.50	6.91	7.03
6-12	3.59	2.97	3.29	2.06
12-24	8.10	2.05	6.69	6.30
24-48	4.62	1.50	5.87	1.84
48-72	1.92	0.27	2.53	1.30
Subtotal	27.3	5.1	25.3	8.5
		Feces		
0-24	10.5	5.9	7.46	4.92
24-48	8.40	2.37	6.93	5.99
48-72	3.68	2.31	5.64	4.11
Subtotal	22.5	7.8	20.0	11.5
		Cage Rinse		
0-24	13.6	2.8	13.5	2.9
24-48	3.61	1.72	4.27	2.37
Subtotal	17.2	3.4	17.8	4.8
		Cage Wash and Cage Wipe		
72[a]	2.52	1.48	1.97	0.58
72[b]	3.59	0.51	6.80	3.14
Total	73.1	7.9	71.9	6.5

SD Standard deviation.
a Cage wash.
b Cage wipe.

In Phase 3 of the study, distribution of [^{3}H]-AFP-168 to specific ocular and systemic tissues was measured. The radioactivity concentrations and the percent of radioactivity in each measured tissue are shown below in Table 33 and Table 34 respectively. Relatively high tissue concentrations of radioactivity occurred in ocular tissues. The highest concentrations on a ng equivalents ^{3}H-AFP-168 per gram basis were in the cornea (3350 in males and 5950 in females), followed by kidneys (185 in males and 138 in females), iris (170 in males and 122 in females) liver (55.3 in males and 69.4 in females) and the aqueous humor (30.1 in males and 42.4 in females). Eight hours after administration the highest radioactivity concentrations were in ocular tissues suggesting slower elimination from the eye compared to systemic tissues. The highest tissue and blood concentrations were measured 15 minutes after dosing. The greatest percentage of radioactivity distributed to the liver (15.1% in males and 18.6% in females), followed by kidney (9.64% in males and 5.96% in females), then cornea (1.80% in males and 2.87% in females).

Table 33: Concentrations of Radioactivity in Blood, Plasma, and Specific Ocular and Systemic Tissues at Specified Times After Topical Ocular Administration of 10 µg/eye of ^{3}H-AFP-168. (Sponsor's Table)

	ng equivalents ^{3}H-AFP-168/g							
	0.25 Hours		1 Hour		2 Hours		8 Hours	
	I08203	I08208	I08204	I08209	I08205	I08211	I08207	I08212
Matrix	Male	Female	Male	Female	Male	Female	Male	Female
Aqueous Humor	30.1	42.4	150	189	220	208	36.0	30.6
Blood	5.38	6.03	0.842	1.01	0.550	0.265	0.176	0.203
Ciliary Body	14.2	21.3	30.7	31.6	40.6	38.7	15.2	16.0
Cornea	3350	5950	3840	3720	2310	2780	386	363
Iris	170	122	254	281	363	428	139	114
Kidneys	185	138	18.3	25.4	12.2	8.93	2.47	2.56
Liver	55.3	69.4	16.2	18.6	9.85	6.94	3.74	3.33
Lungs	3.72	4.23	0.960	0.937	0.981	0.446	0.182	0.191
Plasma	7.60	8.50	1.13	1.47	0.770	0.343	0.207	0.248

Table 34: Percent of Radioactive Dose Recovered in Blood, Plasma, and Specific Ocular and Systemic Tissues at Specified Times After Topical Ocular Administration of 10 μg/eye of ^{3}H-AFP-168. (Sponsor's Table)

	Percent of Radioactive Dose							
	0.25 Hours		1 Hour		2 Hours		8 Hours	
	I08203	I08208	I08204	I08209	I08205	I08211	I08207	I08212
Matrix	Male	Female	Male	Female	Male	Female	Male	Female
Aqueous Humor	0.05	0.06	0.24	0.31	0.31	0.28	0.05	0.05
Ciliary Body	0.02	0.02	0.03	0.04	0.03	0.03	0.02	0.02
Cornea	1.80	2.87	1.61	2.51	1.31	1.62	0.25	0.25
Iris	0.03	0.02	0.04	0.04	0.06	0.05	0.02	0.02
Kidneys	9.64	5.96	0.88	1.45	0.51	0.48	0.11	0.13
Liver	15.1	18.6	3.88	4.49	1.95	1.96	0.82	0.84
Lungs	0.22	0.21	0.05	0.06	0.04	0.03	0.01	0.01
Total	26.9	27.7	6.73	8.90	4.21	4.45	1.28	1.32

The metabolic profile in plasma in males and females was similar. Eight metabolites were detected in plasma and seven (Table 35) were tentatively identified.

Table 35: AFP-168 Metabolites Following Topical Ocular Administration in Monkeys

Metabolite	Tentative identification
AFP-172	Tafluprost acid
M10	1,2,3,4-tetranor-AFP-172-glucuronide
M14	1,2,3,4-tetranor-AFP-172-glucuronide-lactone form
M16	1,2-dinor-AFP-172-glucuronide
M18	1,2,3,4-tetranor-AFP-172 (acid form)
M21	1,2-dinor-AFP-172
M22	1,2,3,4-tetranor-AFP-172 (δ-lactone form)

Most of the plasma radioactivity was associated with three metabolites (AFP-172, M18, M21). AFP-172 was the major circulating metabolite in plasma up to 2 hours after dosing. At 15 minutes after dosing, AFP-172 comprised approximately 88% of the sample radioactivity for both males and females. M18 contributed 11.7 % of the total radioactivity in males and 23.3% in females 0.5 hours after dosing. The third major metabolite, M21 contributed 10.5 and 12.2% of the total plasma radioactivity at 15 minutes after dosing in males and females respectively. M10, M14, and M16 were minor metabolites each contributing less than 10% of the total radioactivity in any sample.

The metabolic profiles in ocular tissues samples from males and females were similar. Five metabolites were detected in aqueous humor, ciliary body, cornea, and iris in addition to parent drug, and three metabolites were tentatively identified. No unchanged AFP-168 was detected in the aqueous humor, and most of the radioactivity in all of the ocular tissues was associated with two metabolites, AFP-172 and M21. At T_{max} (1-2

hours), AFP-172 and M21 composed 72.6% and 23.8% of the sample radioactivity aqueous humor in males with similar proportions in females. A minor metabolite, M18 was also detected in aqueous humor and two unidentified metabolites, M1 and M13 were detected at low levels (< 1.5%) of the sample radioactivity.

In the ciliary body, the maximum concentrations of the AFP-172 and M 21 were 45.8 and 47.7% or the sample radioactivity respectively with similar values for females. A minor metabolite, M18 was also identified.

In cornea, AFP-172 was the major metabolite, comprising 93.4% and 81.7% of the sample radioactivity at maximal concentrations at 15 minutes and 1 hour after dosing respectively. Minor metabolites M21 and M18 were also identified in the cornea.

AFP-172 and M 21 were also the major metabolites in iris from males and females. Low levels of AFP-168 and at later time points low concentrations of M18 and M13 were also detected in the iris.

The metabolic profiles in systemic tissues from males and females were similar. Up to 17 metabolites were detected in kidney, liver, and lungs in addition to parent drug, but only three major metabolites, M21, 1,2,3,4-tetranor-AFP-172 (acid and lactone forms), and 1,2,3,4-tetranor-AFP-172-glucuronide (acid and lactone forms) were tentatively identified. In the kidney, at 15 minutes after dosing, the C_{max} values of M21, 1,2,3,4-tetranor-AFP-172 (acid and lactone forms), and 1,2,3,4-tetranor-AFP-172-glucuronide (acid and lactone forms), corresponded to 34.1, 30.4, and 14.3% of the sample radioactivity in males and similar values in females. Lower concentrations of AFP-172 and several unidentified metabolites were also detected in kidney.

Most of the radioactivity in liver was associated with two major metabolites, M21 and 1,2,3,4-tetranor-AFP-172-glucuronide (acid and lactone forms) accounting for 28.7 and 14.1% of the sample radioactivity at 15 minutes post-dose in males and similar percentages in females. The minor metabolites were AFP-172, and 1,2,3,4-tetranor-AFP-172 (lactone form), and unidentified metabolites.

Most of the radioactivity in lungs was associated with three major metabolites, AFP-172, M 21, and 1,2,3,4-tetranor–AFP-172 (acid form). Maximum concentrations of these respective metabolites corresponded to 57.3, 20.5, and 11.5% of the sample radioactivity at 15 minutes post-dose in males and similar percentages in females. In addition, M16 and M10 were detected in aqueous lung fractions 2 hours after dosing, and other minor metabolites included unidentified metabolites.

Urine and Feces: The metabolite profiles for both urine and feces were similar for both genders and are summarized in Table 36 below. In urine, twenty metabolites were detected of which five were tentatively identified. During 72 hours of urine collection, 25.3 and 28.6% of the administered dose was excreted via urine in males and females. The major metabolites were 1,2,3,4-tetranor-AFP-172 (acid and lactone forms) and M21. Each of these metabolites accounted for approximately 6 to 8% of the dose in

males and females. Two other metabolites, 1,2,3,4-tetranor-AFP-172-glucuronide (acid and lactone forms) and M16 were also identified in urine and respectively accounted for 5.78 and 2.39% in males and similar percentages in females. All remaining metabolites were not identified and accounted for less than 1% of the administered dose.

The amount of radioactivity excreted in feces over a 72 hour period following dose administration accounted for 22.3 and 19.5% of the administered dose in males and females respectively. Nine metabolites were detected in feces from males and females of which three metabolites were tentatively identified. M21 was the major metabolite accounting for 13.4 and 9.82% of the dose in males and females respectively. Other metabolites occurring at lower incidence in feces included 1,2,3,4-tetranor-AFP-172 (acid and lactone forms) and M16. Other unidentified metabolites accounted for less than 3.5% of the administered dose.

Table 36: Metabolite profile for Urine and Feces Following Topical Ocular Dosing of ^{3}H-AFP-168 in Monkeys (Sponsor's Table)

	Percent of Dose					
	Urine (0-72 hours)		Feces (0-72 hours)		Total	
Identified Metabolite	Male	Female	Male	Female	Male	Female
1,2-dinor-AFP-172 (M 21)	7.33	8.05	13.4	9.82	20.7	17.9
1,2-dinor-AFP-172-glucuronide (M 16)	2.39	2.39	0.67	0.73	3.06	3.12
1,2,3,4-tetranor-AFP-172 (acid form, M 18)	5.17	6.47	2.75	2.30	7.92	8.77
1,2,3,4-tetranor-AFP-172 (lactone form, M 22)	0.87	0.60	0.10	0.20	0.97	0.80
1,2,3,4-tetranor-AFP-172-glucuronide (acid form, M 10)	2.94	3.98	ND	ND	2.94	3.98
1,2,3,4-tetranor-AFP-172-glucuronide (lactone form, M 14))	2.84	2.92	ND	ND	2.84	2.92

ND Not detected.

Study Title: Cytochrome P450 in vitro reaction phenotyping study of AFP-172 with human recombinant cytochrome P450s (MRL Report PK032).

Methods

In vitro cytochrome P450 (CYP-450) reaction phenotyping of AFP-172 was conducted using 12 human recombinant CYP-450 isozymes (1A2, 2A6, 2B6, 2C9*1, 2C9*2, 2C19, 2D6*1, 2E1, 3A4, 4A11, 4F2, 4F3B). Eighty μmol/L of AFP-172 was incubated with each isozyme for 4 and 6 hours. After the reactions were stopped, the concentrations of AFP-172 and its major metabolites (tetranor AFP-172 and dinor AFP-172) in each reaction mixture were determined using HPLC-MS/MS.

Results

AFP-172 was not significantly metabolized in any of the CYP-450 isozyme reactions. After incubation in each reaction mixture, AFP-172 concentrations were measured as 84.8% to 103.3% of the starting concentration and metabolic conversion was not observed.

Study Title: AFP-168: Effect of levels of hepatic cytochrome P450 and Phase II drug metabolizing enzymes in male and female rats after intravenous administration for 26 weeks (MRL Report PK025).

Methods

This study was conducted in a GLP-compliant manner and a quality assurance statement was included in the study report. Frozen livers were obtained from a separate toxicology study (Study Report No.: TT #01-5526) in which groups of male and female Crl:WI(Glx/BRL/Han)BR rats were intravenously dosed with AFP-168 at dose levels of 0 (control), 10, 30, and 100 μg/kg/day for 26 weeks. Microsomal and cytosolic subcellular fractions of the liver samples from the control (8 male and 8 female) and high dose (6 male and 8 female) animals were prepared. The liver fractions were assayed for the following parameters: micosomal and cytosolic protein concentrations, cytochrome P450 concentration, the activities of 7-ethoxyresorufin O-deethylase, various testosterone metabolizing enzymes and lauric acid 11-(ω-1) and 12 (ω) hydrolases. The phase II marker activities of p-nitrophenol UDP-glucuronosyltransferase and glutathione S-transferase, were also measured.

Results

As shown in Table 37, treatment with intravenous 100 μg/kg/day AFP-168 for 26 weeks in rats did not significantly alter most of the measured liver microsomal or cytosolic parameters. The only change was a marginal although significant decrease in cytosolic protein concentration (mg/g liver) for high-dose females, but not males, relative to control animals. A few phase I enzyme activities were below the limit of quantification. However, none of the measurable phase I or phase II marker enzyme activities were significantly altered. Overall AFP-168 appeared to have very little effect on phase I and phase II marker enzyme activities.

Table 37: Mean Values for Liver Microsomal and Cytosolic Parameter Measurements for the AFP-168 High-Dose Group Expressed as a Percentage of the Corresponding Vehicle Control Group Mean (Sponsor's Table).

Parameter	AFP-168 (100 μg/kg/day)	
	Male	Female
Microsomal protein - (mg/g liver)	98%	97%
Cytochrome P450 - (nmoles/mg protein) - (nmoles/g liver)	101% 99%	107% 103%
7-Ethoxyresorufin O-deethylase - (nmoles/min/mg protein) - (nmoles/min/g liver)	98% 96%	112% 110%
Testosterone 2α- (+ 2β-)hydroxylases[1] - (nmoles/min/mg protein) - (nmoles/min/g liver)	108% 105%	a
Testosterone 6β-hydroxylase - (nmoles/min/mg protein) - (nmoles/min/g liver)	90% 89%	a
Testosterone 7α-hydroxylase - (nmoles/min/mg protein) - (nmoles/min/g liver)	a	106% 103%
Testosterone 16α-hydroxylase - (nmoles/min/mg protein) - (nmoles/min/g liver)	101% 99%	a
Testosterone 17β-dehydrogenase - (nmoles/min/mg protein) - (nmoles/min/g liver)	98% 97%	a
Lauric acid 11-hydroxylase - (nmoles/min/mg protein) - (nmoles/min/g liver)	90% 89%	104% 101%
Lauric acid 12-hydroxylase - (nmoles/min/mg protein) - (nmoles/min/g liver)	96% 95%	103% 100%
p-Nitrophenol UDP-glucuonosyltransferase - (nmoles/min/mg protein) - (nmoles/min/g liver)	101% 98%	104% 102%
Cytosolic protein - (mg/g liver)	98%	95%*
Glutathione S-transferase - (μmoles/min/mg protein) - (μmoles/min/g liver)	103% 102%	102% 98%

[1] Predominantly 2α-, where quantifiable

[a] Mean activity below limit of quantification: *i.e.* <0.35 nmoles/min/mg protein (males) or <0.175 nmoles/min/mg protein (females). Statistical analysis not performed

* $p<0.05$ for changes measured following administration of AFP-168, when compared with the appropriate control

Excretion

In addition to the reviewed absorption studies that also examined excretion, the biliary excretion, enteroheptatic circulation and metabolism of ^{3}H-AFP-168 was examined in rats (MRL Report No.: PK031), and this study is reviewed below.

Study Title: ^{3}H-AFP-168: Biliary excretion, enterohepatic circulation, and metabolism in the rat following a single ocular administration (MRL Report PK031).

Methods

This study was performed in a GLP-compliant manner and contained a quality assurance statement in the final study report. A single bilateral dose of 0.005% ^{3}H-AFP-168 was administered to both eyes of male and female bile-cannulated Sprague-Dawley rats. The rat were euthanized 48 hours after administration and concentrations of radioactivity were measured in bile, plasma, tissues, and excreta to obtain information on the absorption, metabolism, and excretion of total drug-related material.

Phase A: Sprague-Dawley rats were anesthetized and the common bile duct of each animal was cannulated to allow complete collection of bile. A second cannula was inserted into the stomach of each rat and a crude solution of sodium taurocholate was infused into the stomach to serve as a replacement for lost bile salts. Once the animals had recovered from surgery, and an adequate bile flow had been established, single bilateral ocular doses of 0.005% ^{3}H-AFP-168 were administered to four male and four female rats. Bile was collected separately for each animal during the 0-1, 1-2, 2-4, 4-6, 6-8, 8-24, and 24-48 hour periods after dosing. Urine was collected during the 0-6, 6-12, 12-24, and 24-48 hour periods after dosing and feces were collected during the 0-24 and 24-48 hour periods after dosing. At 48 hours after dosing, rats were euthanized, exsanguinated, and the gastrointestinal tract and liver were removed from each carcass. Each terminal blood sample was processed to plasma and the gastrointestinal tracts were dissected to separate walls and contents.

Phase B: Using the same surgical procedure as in Phase A, a flexible cannula was inserted into the bile duct of “donor” rats then a second cannula was inserted into the stomach of each rat. The unattached end of the biliary cannula from each “donor” rat was then surgically implanted into the duodenum of a second “recipient” rat of the same gender to allow transfer of bile. A second cannula was then established in the common bile duct of each “recipient rat to allow complete collection of bile from each recipient rat. Once the animals had recovered from surgery, and an adequate bile flow had been established, single bilateral ocular doses of 0.005% ^{3}H-AFP-168 were administered to four male and four female “donor” rats. Bile was collected separately from each “recipient” rat during the 0-1, 1-2, 2-4, 4-6, 6-8, 8-24, and 24-48 hour periods after dosing. Urine was collected during the 0-6, 6-12, 12-24, and 24-48 hour periods after dosing and feces were collected during the 0-24 and 24-48 hour periods after dosing from all “donor” and “recipient” rats. At 48 hours after dosing, rats were euthanized and exsanguinated and the gastrointestinal tract and liver was removed from each carcass.

Each terminal blood sample was processed to plasma and the gastrointestinal tracts were dissected to separate walls and contents. Radioactivity was measured with liquid scintillation counting. In addition, radioactive components in urine, bile, and feces were separated by HPLC and quantification of the separated metabolites was performed by fraction collection followed by liquid scintillation counting.

Results

Phase A: A total of approximately 80% of the radioactive dose was excreted during the 48 hour sample collection period and a total of approximately 5-7% remained in the carcasses. An additional approximately 10% of the total radioactivity was measured in association with food residues, plastic gloves, and cage washings. Of the excreted radioactivity, approximately 50% (male) and 33% (female) was excreted in bile, 25% (male) and 41% (female) was excreted in urine, and 3% (male) and 5% (female) was excreted in feces. Approximately 75% of the excretion in bile occurred rapidly in the first four hours after dosing.

Phase B: A total of approximately 89% (male) or 83% (female) of the radioactive dose was recovered at the end of the 48-hour collection period. Mean totals of approximately 28% (male) and 34% (female) and 53% (male) and 38% (female) of the dose were excreted by the donor and recipient rats respectively. An additional approximately 10% of the total radioactivity was measured in association with food residues, plastic gloves, and cage washings. The male donor rats excreted mean totals of approximately 25% and 2.8% in urine and feces respectively. The female donor rats excreted mean totals of approximately 32% and 1.4% in urine and feces respectively. The male recipient rats excreted approximately 14%, 6% and 33% in bile, urine and feces respectively, and the female recipient rats excreted approximately 13%, 5.7% and 19% in bile, urine and feces respectively. The mean extent of reabsorption via enterohepatic recirculation of total drug-related material from bile therefore amounted to approximately 20% of the total dose of ^{3}H-AFP-168 administered to donor rats for both genders.

Metabolic Profile: Overall the metabolite profiles were qualitatively similar in male and female rats, but quantitative differences were found between the genders in the amounts of the various metabolites appearing in bile, urine and feces. The total amounts of AFP-168 metabolites in bile urine and feces are shown below in Table 38.

Table 38: Total Amounts of the Principal AFP-168 Metabolites Excreted in Bile and Urine 24 Hours After Dosing and in Feces 48 hours After Dosing in Bile Cannulated Male and Female Rats. (Sponsor's Table)

Results are expressed as % dose

Radioactive component†	Male				Females			
	Bile	Urine	Faeces	Total	Bile	Urine	Faeces	Total
M1	18.20	4.49	0.16	22.85	4.44	4.63	0.21	9.28
M2	5.18	6.16	0.22	11.56	4.59	7.05	0.29	11.93
M3	2.61	1.30	0.03	3.94	0.84	1.84	0.00	2.65
M3a	1.17	0.22	0.04	1.43	0.64	0.71	0.00	1.35
M3b	0.70	0.24	0.00	0.94	0.65	0.34	0.00	0.99
M4	1.80	7.89	0.29	9.98	3.23	15.46	0.42	19.11
M5	6.17	1.68	0.12	7.97	4.37	4.10	0.00	8.47
M6	3.73	0.38	0.28	4.39	2.52	4.83	0.82	8.17
M7	4.48	0.87	0.84	6.19	4.58	8.53	0.98	14.09
M8	1.42	0.12	0.26	1.80	1.95	2.49	0.56	5.00
M9	1.22	0.09	0.19	1.50	1.27	0.58	0.43	2.28
M10	1.06	0.14	0.28	1.48	0.93	1.16	0.39	2.48
M11[1]	0.35	0.00	0.13	0.48	0.38	0.15	0.35	0.88
M12[2]	0.09	0.65	0.07	0.81	0.50	0.15	0.15	0.80
Total	48.18	24.23	2.91	75.32	30.89	51.99	4.60	87.48
% dose	49.06	24.51	2.97	76.53	31.03	50.69	4.70	86.42

[1] Corresponds chromatographically to AFP-172

[2] Corresponds chromatographically to AFP-168

† The radioactive components detected in this study have been assigned identities (*ie* M1-M12) that are consistent with those used in [redacted] (b) (4) confidential report STN 035/014195 (Midgley *et al*, 2002). This is for comparative purposes only; it should not be inferred that the radiolabelled components are necessarily the same in both studies

6 General Toxicology

6.1 Single-Dose Toxicity

Single-dose toxicology studies in rats were performed with oral (Study Report No.: TT #99-5543) and intravenous (Study Report No.: TT #99-5544) doses of AFP-168, and a single-IV-dose toxicology study was performed in dogs (Study Report No.: TT#99-5546). Toxicology studies employing multiple topical ocular doses of AFP-168 administered in one day were performed in rabbits (Study Report No.: TT#99-5545) and monkeys (Study Report No.: TT #99-5547). The oral and intravenous administration studies are summarized in Table 39, and the ocular dosing studies are summarized in Table 40.

Table 39: Single-Dose Studies with Oral or Intravenous AFP-168.

Study Report No./ Species/ number per group	Route/Dose	Results
TT#99-5543/ Sprague-Dawley Rat/ Oral/ 5/sex/group	Oral doses of 0, 10, 30, and 100 mg/kg AFP-168; animals were observed for 14 days after dosing.	1. No deaths occurred in any treatment group. 2. Clinical signs included: hunched posture and wasted appearance beginning one day after dosing in one 10 mg/kg and one 100 mg/kg animal. 3. One 10 mg/kg animal and one 100 mg/kg animal exhibited chest sores or loss of chest fur, at 7 days after dosing that resolved by Day 14. 4. No noteworthy findings in body weight, food intake, water intake or gross pathology
TT#99-5544/ Sprague-Dawley Rat/ 5/sex/group	IV doses of 0, 1, and 3 mg/kg AFP-168; animals were observed for 14 days after dosing.	1. No deaths occurred in any treatment group. 2. No noteworthy findings occurred for clinical signs, body weight, food intake, water intake, and gross pathology.
TT#99-5546/ Beagle Dogs/ 2 males/group	IV doses of 0.3, 3, and 30 μg/kg AFP-168; animals were observed for 14 days after administration.	1. No deaths occurred at any AFP-168 dose. 2. At doses ≥ 3 μg/kg, animals experienced signs of nausea, vomiting, moderate to severe miosis, irregular respiration and increased respiratory rate, increased heart rate, transient prolongation of QTc, and transient increased in serum ALT (GPT) activity. 3. There were no noteworthy findings for body weight, food intake, hematology parameters, urinalysis, body temperature, or ophthalmologic findings.

Table 40: Studies Employing Multiple Topical Ocular Doses of AFP-168 in a Single Day.

Study Report No./ Species/ number per group	Route/Dose	Results
TT#99-5545/ Japanese White Rabbits/ 6 males/ group	Topical ocular doses of 0, 0.005%, 0.05%, or 0.5% AFP-168 instilled into the left eye 10 times in one day	1. No clinical signs or body weight changes were attributed to AFP-168 treatment. 2. The high-dose (0.5%) of AFP-168 produced an increase in blinking frequency. 3. AFP-168 produced very slight to moderate conjunctival redness at the two highest doses, but since the vehicles also produced these effects, the results were hard to interpret. 4. All of the eye redness resolved within 24 hours. 5. No treatment-related changes were observed with slit-lamp examination and fluorescein staining of the cornea.
TT#99-5547/ Cynomolgus monkey/ 2 females/ group	Topical ocular doses of 0, 0.0005%, 0.005%, and 0.05% AFP-168 to the left eye 10 times in one day.	1. No clinical signs or body weight changes were attributed to AFP-168 treatment. 2. Conjunctival redness and fluorescein staining of the cornea occurred more frequently at the 0.005% AFP-168 compared to vehicle. 3. In addition to conjunctival redness, administration of the 0.05% AFP-168 dose resulted in slight cornea opacity, more frequent fluorescein staining of the cornea, transient anisocoria and sporadic conjunctival chemosis.

6.2 Repeat-Dose Toxicity

Non-pivotal repeated-intravenous-dose toxicology studies of 28-days duration were performed in rats (MRL TT #99-5548) and dogs (Study Report No.: TT #99-5550). In addition, non-pivotal, repeated-ocular dose studies of 28-days (Study Report No.: TT #99-5553) and 13 weeks (Study Report No.: TT #00-5537) duration were performed in monkeys. The non-pivotal intravenous and ocular studies are summarized below in Table 41 and Table 42 respectively. Additional intravenous–dose studies that were considered pivotal included a 26-week study in rats (Study Report No.: TT #01-5526) and a 39-week study in dogs (Study Report No.: TT #01-5530). Pivotal ocular-dose studies included a 13-week monkey study with tafluprost and/or timolol dosing (Study Report No.: TT #11-7800) and a 52-week ocular-dose study in monkeys with tafluprost dosing (Study Report No.: TT # 01-5531). The pivotal intravenous and ocular-dose toxicology studies are reviewed below.

Table 41: Non-Pivotal Repeated-Intravenous-Dose Toxicology Studies

Study Report No./ GLP Compliance	Species/ Number per Group/ Duration/ Route and Dose	Results
TT #99-5548/ GLP-compliant	Rat/ 12/sex/group/ 28 days of dosing followed by a 14-day recovery period/ intravenous doses of 10, 30, or 100 μg/kg AFP-168	1. No AFP-168-related mortality, clinical signs, gross pathology or histopathology or changes in body weight, water consumption, clinical chemistry, urinalysis parameters, ophthalmic parameters, electrocardiograph traces, and organ weights were noted. 2. Slightly low hemoglobin concentration, erythrocyte numbers, and packed cell volume occurred in high-dose males and erythrocyte numbers were marginally reduced in low and intermediate dose males. Also platelet numbers were marginally low in all AFP-168 treated females and intermediate and high-dose males. Significant differences did not occur for these parameters, and all of these parameters returned to normal levels during the recovery period. 3. The NOAEL was considered to be 100 μg/kg/day
TT #99-5550/ GLP-compliant	Dog/ 4/sex/group/ 28 days of dosing followed by a 14-day recovery period/ intravenous doses of 0, 0.1, 1, or 10 μg/kg/day AFP-168	1. All animals survived throughout the dosing and recovery periods. Also, no AFP-related changes in body weight, food consumption, hematology, body temperature, ophthalmology, gross pathology, organ weights or histopathology were noted at any dose. 2. At 1 μg/kg AFP-168, animals experienced slight miosis, and sporadic salivation and vomiting in animals of both genders. 3. At the high dose of 10 μg/kg, animals of both genders demonstrated salivation, vomiting, miosis, increased respiratory rate, increased heart rate, and prolonged QTc interval. Increased GPT(ALT) activity, increased urine volume, and decreased urinary potassium concentration were found in both sexes during the dosing period, but these effects disappeared after the recovery period. Increased diastolic pressure was found only in males, and reduced urinary chloride concentration occurred only in females.

Table 42: Non-Pivotal Repeated-Ocular-Dose Toxicology Studies

Study Report No./ GLP Compliance	Species/ Number per Group/ Duration / Route and Dose	Results
TT #99-5553/ GLP-compliant	Cynomolgus monkey/ 3/sex/group/ 28-days/ topical ocular BID doses of 0, 0.15, 1.5, and 15 µg/left eye/time AFP-168	1. AFP-168-related changes included changes in left eye iris color in two animals receiving 1.5 µg/left eye/time. 2. In addition, transient corneal precipitates, anterior chamber cells, superficial corneal opacity and erosion, positive epithelial fluorescein staining and conjunctival redness were occasionally observed after administration of the two highest doses of AFP-168. The latter three effects were also observed in vehicle control animals and untreated eyes and may have been related to corneal surface drying due to the anesthesia. 3. No AFP-168-related macroscopic ocular findings, lens or vitreous body changes, or changes in intraocular pressure, electroretinogram parameters, or eye lash color were observed. 4. All animals survived the treatment period, and there were no AFP-168-related gross pathology or histopathology findings or changes in body weight gain, food intake, macroscopic ocular findings, intraocular pressure, ECG parameters, blood pressure, hematology parameters, clinical chemistry, or organ weight changes.
TT #00-5537/ GLP-compliant	Cynomolgus monkey/ 4/sex/group/ 13-weeks with 4-week recovery/ topical ocular BID doses of 0, 0.15, 1.5, and 15 µg/left eye/time AFP-168	1. AFP-168-related changes included a reversible finding of sunken eyelids in the treated eye, slight punctuate fluorescein staining of the cornea, a tendency to deceased intraocular pressure, and irreversible iris color darkening in the left treated eye. 2. No AFP-168-related findings were observed during the fundus and slit lamp examinations and no changes in electroretinogram parameters, or eye lash color were observed. No AFP-168-related ocular histopathology was observed. 3. No AFP-168-related mortality, clinical signs, gross pathology, or histopathology, or changes in weight gain, food intake, ECG parameters, blood pressure, hematology parameters, clinical chemistry parameters, urinalysis parameters, or organ weights were observed.

Study title: AFP-168: 26 week intravenous administration toxicity study in the rat [redacted]. Study No.: 1241/031; MRL TT #01-5526).

Study no.:	[redacted] Study No.: 1241/031; MRL TT #01-5526
Study report location:	Electronic transmission
Conducting laboratory and location:	[redacted]
Date of study initiation:	February 27, 2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AFP-168, Lot No.: FP-0002, purity of

98.6%

Key Study Findings

Fifteen animals (eleven males and four females) in the Main Study died or were killed during the treatment period. The apparent cause of death for one low dose and two intermediate dose males was glomerulonephropathy, but the cause of death for the rest of the fifteen animals (all intermediate and high dose) was not apparent. Some slight hematological effects including decreased hemoglobin concentration, erythrocyte numbers and packed cell volume were evident in males and females. Analysis of bone marrow smears revealed reduced numbers of early erythropoietic and myelopoietic cell types and increased late erythropoietic and myelopoietic cell types in high-dose animals. Slightly high spleen weights were observed in high-dose males and females and in intermediate-dose females. Histopathology included: dose-related hyperostosis and myelofibrosis in femoral and sternum bone marrow at all treated doses. Also hematopoesis was increased in spleen, liver, and male femoral bone marrow of AFP-168 treated animals and increased corticomedullary mineralization in treated females. The histopathology was consistent with known pharmacological activity of proastaglandin F_{2a} analogues. A NOAEL value was not established.

Methods

Doses:	0, 10, 30, and 100 μg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Intravenous
Dose volume:	10 ml/kg
Formulation/Vehicle:	The vehicle was sterile saline (0.9% NaCl)
Species/Strain:	Crl:CD®(SD)IGSBR rats
Number/Sex/Group:	15/sex/group
Age:	Approximately 5.5 weeks old at the start of dosing.
Weight:	Males: 133.2 to 183.5 grams; Females: 109.0 to 150.4 grams respectively for the Main Study
Satellite groups:	8/sex/group for toxicokinetic analysis
Unique study design:	See below
Deviation from study protocol:	The study report noted minor deviations from the study protocol. However these deviations were not considered to have altered the outcome or integrity of the study.

Table 43: Study Design for Study No.: MRL TT #01-5526. (Sponsor's Table)

Group Number	Description	Dose level (µg/kg/day)	Dose concentration (%)	Animals/group			
				Main study		Satellite study#	
				Male	Female	Male	Female
1	Control (vehicle)	0	0	15	15	8	8
2	Low	10	0.0001	15	15	8	8
3	Intermediate	30	0.0003	15	15	8	8
4	High	100	0.001	15	15	8	8

for toxicokinetic investigations and limited necropsy procedures only; clinical signs, body weight and food consumption data were obtained but not reported

Observations and Results

Mortality

Animals were examined for morbidity and mortality at the beginning and the end of the working day throughout the experiment.

Eleven males and four females in the Main Study, and three male toxicokinetic animals, all from AFP-168 treatment groups, were sacrificed due to morbidity or died during the treatment period. Some of the animals were sacrificed due to impaired mobility. All of the animals died or were sacrificed between Weeks 6 and 26 of the experiment. The incidence of mortality was AFP-168-dose dependent with intermediate and high-dose male animals predominantly affected (Table 44). For three of the male animals, the cause of death was determined to be kidney glomerulonephropathy; however, no specific cause of death was apparent for the other animals.

Table 44: Mortality Results in the 26-Week Intravenous-Dose Rat Toxicology Study. (Sponsor's Table)

Main study	Group and sex							
	1M	2M	3M	4M	1F	2F	3F	4F
Group size:	15	15	15	15	15	15	15	15
Decedents:	0	1	5	5	0	0	2	2

Satellites	Group and sex					
	2M	3M	4M	2F	3F	4F
Group size:	8	8	8	8	8	8
Decedents:	0	1	2	0	0	0

Clinical Signs

All animals were examined daily for signs of ill health or overt toxicity. In addition, each animal was given a detailed physical examination at weekly intervals.

No clinical signs in surviving animals were attributed to AFP-168 treatment.

Body Weights

Individual body weights were recorded before treatment, on the first day of dosing, at weekly intervals during the first 14 weeks, at 4-weekly intervals thereafter, and before necropsy.

No effects on body weight or body weight gain were attributed to treatment with AFP-168.

Feed Consumption

The amount of food consumed by each cage of animals was determined during the final week of the acclimation period, weekly during the first 14 weeks, and at 4-weekly intervals thereafter. Consumption was calculated as g/animal/week.

Food consumption was similar for all groups

Water Consumption

The amount of water consumed by each cage of animals was determined during the final week of the acclimation period, weekly during the first 14 weeks and at 4-weekly intervals thereafter. Consumption was calculated as g/animal/week.

Water consumption was similar for all groups.

Ophthalmoscopy

All animals received ophthalmoscopy examinations pretreatment and the six lowest numbered animals/group/sex in Week 26. The animal eyes were dilated and the fundus was examined with an indirect ophthalmoscope.

No AFP-168-related ophthalmoscopy effects were observed.

ECG

Electrocardiogram measurements were performed on the 6 lowest numbered animals/group/sex pre-treatment and in Week 26 before dosing. Recordings were taken using the fixed limb leads I, II, and III and the augmented leads aVR, aVL, and aVF. Heart rate was derived form lead II.

The few ECG findings were of uncertain relationship to AFP-168 administration. Heart rates for the high-dose males were approximately 12% lower than for controls in Week 26 and this difference was significant, but high-dose males also had a non-significant lower mean heart rate of approximately 10% compared to controls before the start of treatment. Intermediate-dose females also had a significant, approximately 9% lower mean heart rate compared to controls in Week 26, but a similar effect was not observed in high-dose females.

Hematology

Blood samples were collected in Week 26 from all animals for hematology and coagulation parameter analysis. The parameters listed in Table 100 were examined.

Several hematology parameters changed in a dose-dependent manner. In Week 26, hemoglobin concentration and erythrocyte numbers were slightly decreased in females in all AFP-168 dose groups. Reticulocyte numbers, mean cell volume, mean cell hemoglobin, and red cell distribution widths were slightly higher in females in the 30 and 100 μg/kg/day dose groups. Males in the high-dose group demonstrated similar patterns but generally to a lesser extent for most parameters. Platelet numbers and platelet crit were slightly low at all dose levels in males, and the mean platelet volume and platelet distribution width were slightly high in males in the 30 and 100 μg/kg/day groups. For these parameters, females in the high-dose group demonstrated similar patterns but generally to a lesser extent for most parameters. Neutrophils were also slightly elevated in high-dose males, but not females. Activated partial thromboplastin time was decreased only in the low-dose AFP-168 group and no dose-dependent effect was apparent.

Table 45: Significant Changes in Group Mean Hematology Parameters

Hematology Parameter	Group 1		Group 2		Group 3		Group 4	
	Male	Female	Male	Female	Male	Female	Male	Female
Hemoglobin (g/L)	15.4	14.9	15.4	14.4[b]	15.1[c]	14.0	14.9[a]	13.7[c]
Red Blood Cell (10^6/ml)	8.67	7.83	8.58	7.44[b]	8.36	7.16[c]	8.18[b]	6.82[c]
Packed Cell Volume (%)	46.6	44.2	46.7	43.2	45.6	42.2[b]	45.5	41.3[c]
Reticulocytes (10^6/ml)	3.0	2.3	3.1	2.9	3.2	3.3[b]	4.0[b]	3.7[c]
Absolute Reticulocytes (10^6/ml)	0.26	0.18	0.26	0.21	0.27	0.23[b]	0.32[b]	0.25[c]
Mean Cell Volume (flow cytometry)	53.8	56.5	54.4	58.1[a]	54.6	58.9[c]	55.7[b]	60.7[c]
Mean Cell Hemoglobin (pg)	17.8	19.0	17.9	19.3	18.1	19.6[a]	18.2	20.2[c]
Hemoglobin Distribution Width (g/L)	2.61	2.04	2.46	2.06	2.45	2.12	2.53	2.15[a]
Red Cell Distribution Width (%)	13.3	10.7	13.0	11.1	13.1	11.6[c]	13.8	11.8[c]
Platelets (%)	1313	1032	1088[c]	937	1010[c]	936	1005[c]	844[b]
Plateletcrit (%)	0.76	0.63	0.64[b]	0.59	0.62[c]	0.59	0.61[c]	0.54[a]
Mean Platelet Volume (flow cytometry)	5.8	6.1	5.9	6.3	6.1[b]	6.3	6.1[b]	6.4
Platelet Distribution Width (%)	55.7	54.7	58.6	56.8	63.0[c]	58.1	62.1[b]	58.0
Activated Partial Thromboplastin Time (s)	19.4	16.5	17.8[a]	14.7[b]	18.7	15.4	18.0	15.9
Total Neutophils (10^3/ml)	1.6	1.0	1.9	1.1	1.9	1.1	2.7[c]	1.2
Neutrophils (%)	18	17	21	17	21	17	27[c]	20
Lymphocytes (%)	76	76	72	74	73	76	67[c]	73
[a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$								

Clinical Chemistry

Blood was collected in Week 26 and processed to plasma for clinical chemistry analysis. The parameters listed in Table 101 were examined.

No AFP-168-related changes in any of the hematology parameters were noted.

Urinalysis

Urine samples were collected overnight from all animals (fasted) in Week 25. The following parameters were determined: microscopy of sediment, color, specific gravity, protein, ketones, blood, reducing substances, potassium, chloride, turbidity, volume, pH, glucose, bilirubin, urobilinogen, sodium, and calcium.

Males receiving AFP-168 produced slightly smaller volumes of urine with slightly greater specific gravity than controls. The concentration of urine sodium, potassium, and calcium was consequently slightly higher than for the controls, but the total output of each electrolyte was similar to controls. Females did not demonstrate a similar pattern, and the changes in males were not thought to have toxicological significance.

Gross Pathology

Main Study animals were sacrificed after fasting overnight and euthanized animals received a full gross pathology examination.

Females in the AFP-168 treatment groups demonstrated gross pathology changes in the bone marrow firmness and thickness which correlated with histopathology findings.

Organ Weights

Animals were weighed before necropsy, and the organs listed in Table 102 were weighed. Paired organs were weighed together.

Mean Spleen weights were slightly but significantly higher in males receiving 100 μg/kg/day (0.898 g) and in females receiving 30 (0.612 g) and 100 (0.635 g) μg/kg/day AFP-168 compared to controls (0.766 g for males and 0.535 g for females).

Histopathology

Adequate Battery
Yes. The organs and tissues listed in Table 102 were examined for histopathology.
Peer Review
No
Histological Findings
In animals in the AFP-168 treatment groups histopathology findings were noted in bone marrow in femoral and sternum bone, spleen, liver, and kidney.

In bone marrow in femoral and sternum bone, there was hyperostosis in animals in all AFP-168 groups. In the most severe cases, bone marrow cavities were almost completely filled with bony trabeculae. The effect was dose-dependent and females were more affected than males. Also, myelofibrosis was seen in femoral and sternum

bone marrow. The myelofibrosis was characterized by lightly eosinophilic fibrous tissue in the marrow cavity, particularly around small blood vessels and the bony trabeculae. This lesion was more prominent and absent in sternum bone marrow in low-dose females. Dose-related severity was evident in femur, but less apparent in the sternum. The incidence and severity of bone marrow histopathology is summarized below in Table 46.

Table 46: Histopathology in Femoral and Sternum Bone Marrow. (Sponsor's Table)

Group incidence of selected microscopic findings – terminal kill									
		Males				Females			
		1M	2M	3M	4M	1F	2F	3F	4F
Tissue and finding	Level (µg/kg/day)	0	10	30	100	0	10	30	100
Femur + marrow	No. examined:	15	14	10	10	15	15	13	13
hyperostosis	Grade -	15	7	0	0	15	1	0	0
	1	0	5	1	1	0	11	0	0
	2	0	1	5	3	0	2	3	0
	3	0	1	4	5	0	1	9	6
	4	0	0	0	1	0	0	1	7
myelofibrosis	Grade -	15	3	0	0	15	14	2	0
	1	0	11	4	2	0	1	11	8
	2	0	0	6	7	0	0	0	5
	3	0	0	0	1	0	0	0	0
Sternum + marrow	No. examined:	15	14	10	10	15	15	13	13
hyperostosis	Grade -	15	4	0	0	15	6	0	0
	1	0	9	4	3	0	7	0	0
	2	0	1	6	3	0	2	6	1
	3	0	0	0	4	0	0	5	8
	4	0	0	0	0	0	0	2	4
myelofibrosis	Grade -	15	9	4	5	15	15	6	3
	1	0	5	4	5	0	0	7	10
	2	0	0	2	0	0	0	0	0

Key: "-" = finding not present, 1 = minimal, 2 = slight, 3 = moderate, 4 = moderately severe

Increased hematopoiesis was observed in spleen, liver and male femoral bone marrow. In the spleen, a degree of background hematopoiesis was apparent in vehicle control animals. AFP-168 treatment produced a dose-dependent increase in severity and females were more affected than males. Control animals did not demonstrate liver or femoral bone marrow hematopoiesis. Generally minor liver hematopoiesis occurred as foci in liver parenchyma in all AFP-168 treatment groups without a clear dose-dependent trend for severity. Increased femoral bone marrow hematopoiesis was only apparent in males and was dose-dependent for severity. The incidence and severity of the spleen, liver, and femoral bone marrow hematopoiesis is summarized below in Table 47.

Table 47: The Incidence and Severity of Spleen, Liver, and Femoral Bone Marrow Hematopoiesis. (Sponsor's Table)

Group incidence of selected microscopic findings - terminal kill									
		Males				Females			
		1M	2M	3M	4M	1F	2F	3F	4F
Tissue and finding	Level (µg/kg/day)	0	10	30	100	0	10	30	100
Spleen	No. examined:	15	14	10	10	15	15	13	13
haemopoiesis	Grade 1	10	3	1	0	8	1	0	0
	2	5	10	7	4	7	7	3	0
	3	0	1	2	6	0	7	10	10
	4	0	0	0	0	0	0	0	3
Liver	No. examined:	15	14	10	10	15	15	13	13
haemopoiesis	Grade -	15	7	3	0	15	1	0	0
	1	0	7	7	10	0	14	12	12
	2	0	0	0	0	0	0	1	1
Femur + marrow	No. examined:	15	14	10	10	15	15	13	13
increased haemopoiesis	Grade -	15	8	2	0	15	15	13	13
	1	0	4	3	1	0	0	0	0
	2	0	2	5	9	0	0	0	0

Key: "-" = finding not present, 1 = minimal, 2 = slight, 3 = moderate, 4 = moderately severe

An AFP-168 dose-dependent increase in the incidence and severity of corticomedullary mineralization was observed only in females. The incidence and severity of kidney corticomedullary mineralization is shown below in Table 48.

Table 48: The Incidence and Severity of Kidney Corticomedullary Mineralization (Sponsor's Table).

Group incidence of selected microscopic findings - terminal kill									
		Males				Females			
		1M	2M	3M	4M	1F	2F	3F	4F
Tissue and finding	Level (µg/kg/day)	0	10	30	100	0	10	30	100
Kidney	No. examined:	15	14	10	10	15	15	13	13
corticomedullary mineralisation	Grade -	15	14	10	10	9	5	2	4
	1	0	0	0	0	4	3	4	2
	2	0	0	0	0	2	5	6	3
	3	0	0	0	0	0	2	1	2
	4	0	0	0	0	0	0	0	2

Key: "-" = finding not present, 1 = minimal, 2 = slight, 3 = moderate, 4 = moderately severe

Toxicokinetics

Blood samples for toxicokinetics were obtained from all toxicokinetic animals on Day 1 and during Weeks 13 and 26 predose and 1, 5, 10, 30, and 60 minutes after dosing.

Samples were processed to plasma, and plasma AFP-168 and AFP-172 concentrations were determined by a validated LC/MS/MS method.

Plasma concentrations of AFP-168 were below the limit of quantification for all of the AFP-168 dose groups with the exception of one predose sample from the 30 μg/kg/day dose group in Week 13. Plasma AFP-172 was detected at all dose levels. Also two vehicle control samples tested positive for low levels of AFP-172, but these results were thought to be false positives due to contamination. In the AFP-168-treatment groups, C_{max} and AUC_{0-1h} values increased in an approximately dose-proportional manner with the exception noted below. Male values were similar for all three AFP-168 doses at all three sampling time points, but the female C_{max} values for the high-dose group were approximately 80% lower for the week 13 and 26 samples compared to the Day 1 samples. AUC_{0-1h} values were similar for male and females at all time-points. T_{max} was consistently 1 hour (the first sample timepoint) except for the Day 26 high dose female samples where T_{max} was 5 hours (Table 49).

Table 49: Toxicokinetic Parameters Associated with Intravenous Administration of AFP-168 to Rats for 26 Weeks. (Sponsor's Table).

Group	Dose (μg/kg/day)	Sex	C_{max} (ng/mL)			T_{max} (min)			$AUC_{(0-1h)}$ (ng.h/mL)		
			Day 1	Week 13	Week 26	Day 1	Week 13	Week 26	Day 1	Week 13	Week 26
2	10	Male	36.24	23.36	122.02	1	1	1	-	2.78	8.70
		Female	16.94	27.76	184.66	1	1	1	-	3.18	9.48
3	30	Male	116.96	120.40	219.62	1	1	1	7.25	13.99	13.82
		Female	87.94	43.86	219.02	1	1	1	6.56	5.76	15.71
4	100	Male	632.29	704.74	556.89	1	1	1	30.32	52.18	54.17
		Female	788.29	125.02	192.96	1	1	5	31.11	25.18	48.20

Dosing Solution Analysis

The Sponsor performed stability and homogeneity analysis. All stock formulations were analyzed for concentration before preparation of the dosing solutions. Concentration analysis of the dosing solutions was performed on formulations prepared for use on Day 1, and in Weeks 13 and 26. The dosing solutions were also examined for osmotic pressure and pH on Day 1 and in Weeks 13 and 26. The target range was 90-110% of the nominal concentration for the stock solutions and 100 to 130% of the nominal concentration for the dosing solutions. The target range for osmotic pressure relative to physiological saline was 0.9 to 1.1 fold.

The 0.0015% AFP-168 solution was stable for 6-weeks at room temperature.
The 0.00001% and 0.0015% solutions were stable for 3 weeks at 5°C and 30°C. Almost all of the stock solution concentrations fell within the target range and those that were outside the target range were not used. All of the dosing solution concentrations fell within the target range. The range of pH values for the dosing solutions was 5.04 to

6.78, and osmotic pressures ranged from 97 to 103% of the osmotic pressure of physiological saline.

Study title: AFP-168: 39-week intravenous administration toxicity study in the dog.

Study no.:	(b) (4) Study No.: 1241/030; MRL TT #01-5530.
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 17, 2000
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AFP-168, batch # FP-0002, purity of 98.6%.

Key Study Findings

Intravenous dosing of AFP-168 for 39 weeks to dogs resulted in specific toxicity primarily in high-dose (10 µg/kg/day) animals. Findings included: transient clinical signs of nausea, transient miosis, transient slight elevations in heart rate, blood pressure, and respiratory rate, enlarged salivary glands, and adrenal and salivary gland histopathology. In addition one high-dose male was killed in extremis in the 19th week of the experiment, and this animal displayed multiple indications of hepatic failure.

Methods

Doses:	0, 0.1, 1, and 10 µg/kg/day
Frequency of dosing:	Once daily for 39 weeks
Route of administration:	intravenous
Dose volume:	1 ml/kg administered over a period of approximately two minutes.
Formulation/Vehicle:	The vehicle was 0.9% sodium chloride
Species/Strain:	Beagle dogs
Number/Sex/Group:	4/sex/group
Age:	At the start of dosing, males were 9 to 10 months old, and females were 9 to 11 months old.
Weight:	At the start of dosing, males weighed 11.58 to 14.94 kg and females weighed 8.81 to 13.78 kg.
Satellite groups:	None
Unique study design:	See below
Deviation from study protocol:	Multiple deviations from the study protocol were noted; however, none of the deviations were considered to have changed the results or altered the integrity of the study.

Table 50: Study Design for Study No.: MRL TT #01-5530. (Sponsor's Table)

Group number	Description	Dose level (μg/kg/day)	Animals/group Male	Female
1	Control	0	4	4
2	Low	0.1	4	4
3	Intermediate	1	4	4
4	High	10	4	4

Observations and Results

Mortality

All animals were observed for morbidity and mortality at the beginning and the end of the working day.

One male in the high dose group was killed in extremis during Week 19 of treatment. A number of clinical signs, gross pathology, coagulation parameter, clinical chemistry, and histopathology findings were observed for this animal as noted below in appropriate sections.

Clinical Signs

Each animal was given a detailed physical examination once daily and observed for signs of ill health or overt toxicity daily. In addition, post-dosing observations were usually performed daily before dosing, immediately after dosing, and at approximately 0.5 to 4 hours after dosing.

Several clinical signs were observed in high-dose animals on an almost daily basis including salivation, emesis/retching, and pacing. Emesis/retching and loose feces were less frequently observed in dogs receiving 1 μg/kg/day AFP-168. Other clinical signs that were observed less frequently in high-dose animals included panting, subdued mood, vocalization, aggression, loose feces, poor mobility, and vasodilation. Clinical signs were occasionally observed during the dosing procedure, but generally occurred between 5 and 30 minutes after dosing.

For the single high-dose male killed in extremis, clinical signs included: inappetence, progressive body weight loss, thin appearance, sluggishness, and yellow coloration of the whole body.

Body Weights

Individual body weights were recorded pre-treatment, before treatment on the first day of dosing, at weekly intervals, and before necropsy.

Body weights were not significantly different between groups.

Feed Consumption

The amount of food consumed by each animal was determined during the pre-treatment period and weekly throughout the treatment period. Consumption was calculated as g/animal/week.

Food consumption was similar for the AFP-168 treatment groups compared to controls.

Ophthalmoscopy

Opthalmoscopy examinations were performed on all animals pre-treatment and in Weeks 13, 26, and 39. An indirect binocular ophthalmoscope was used to examine the eyelids, optic disc, tapedal and non-tapedal fundus, and retinal blood vessels. In addition the pupillary light response of all animals was examined 5, 10, and 120 minutes after dosing in Week 14, and 10 and 120 minutes after dosing in Weeks 27, 38 (females) and 39 (males).

No ocular changes were considered related to AFP-168 administration other than dose-dependent changes in pupillary light response. High-dose animals demonstrated marked miosis shortly after dosing during Weeks 14, 27, and 39. Miosis was slight to moderate two hours after dosing in Weeks 27 and 39 for most high-dose animals. Slight to moderate miosis occurred in most intermediate-dose (1 μg/kg/day) animals after dosing in Weeks 14, 27, and 39. Low dose (0.1 μg/kg/day) and control animals exhibited a similar range of pupillary responses including normal pupil size, slight miosis, or moderate mydriasis shortly after dosing and for up to two hours after dosing.

ECG

Electrocardiograms were performed on all animals using fixed limb leads I, II, and III and the augmented leads aVR, aVL, and aVF. Electrocardiogram measurements were obtained pre-treatment, and before dosing and 5, 30 and 120 minutes after dosing in Weeks 4, 8, 13, 26, and 39. Heart rate, and the P, R, and T-wave amplitudes as well at PR, RR, and QT-intervals and QTc (Bazett's formula) were measured or derived.

Heart rates increased in an AFP-168 dose-dependent manner 5 and/or 30 minutes after dosing as shown in Table 51 below.

Table 51: Mean Heart Rates. (Sponsor's Table)

	Selected Group mean heart rates (beats/min) attaining statistical significance						
Group / sex	Week 8 5 min AD	Week 13 5 min AD	Week 13 30 min AD	Week 26 5 min AD	Week 26 30 min AD	Week 39 5 min AD	Week 39 30 min AD
1M	-	-	117	-	-	-	98
2M	-	-	134	-	-	-	145**
3M	-	-	136	-	-	-	142*
4M	-	-	162**	-	-	-	171***
1F	115	120	-	98	118	116	-
2F	160*	163	-	140	126	175*	-
3F	152	179**	-	173**	144	166*	-
4F	164*	181**	-	182***	154*	174*	-

* $p<0.05$; ** $p<0.01$

As a consequence of increased heart rate, RR-intervals were slightly shortened. The QT values corrected for heart rate (QTc) were normal.

Blood Pressure

In addition, blood pressure recordings were made on all animals pre-treatment, before dosing and 5, 30 and 120 minutes after dosing in Weeks 4, 8, 13, 26, and 39.

The mean arterial blood pressure (MAP) of high-dose females was slightly but significantly elevated compared to vehicle control animals 30 minutes after dosing during Weeks 4, 26, and 39. Also MAP was significantly elevated in intermediate- and high-dose males at a single time-point, 5 minutes after dosing in Week 26.

Body Temperature

Rectal temperatures for all animals were obtained pre-treatment and before dosing and 5, 30 and 120 minutes after dosing in Weeks 4, 8, 13, 26, and 39.

No AFP-168-related changes in body temperature were observed.

Respiratory Rate

The respiratory rates for all animals were assessed by observation over a period of 30 seconds for each reading. Assessments were performed on all animals pre-treatment and before dosing, and 5, 30, and 120 minutes after dosing in Weeks 4, 8, 13, 26 and 39.

Respiratory rates were slightly but significantly elevated in high-dose males 5 and 30 minutes after dosing during Weeks 8, 13, 26, and 39. In high-dose females, respiratory rates were significantly increased only in Weeks 26 and 39, 30 minutes after dosing. Respiratory rates were generally less elevated or similar to control values two hours after dosing indicating a transient effect.

Hematology

Blood samples for hematology, coagulation, and clinical chemistry parameter evaluation were collected from all animals before treatment, and in Weeks 13, 26, and 39. The hematology and coagulation parameters that were assessed are shown in Table 100.

No AFP-168-related changes in hematology or coagulation parameters were observed in any of the study animals except the high-dose male killed in extremis. For this animal, prothrombin and activated partial thromboplastin times were prolonged.

Clinical Chemistry

Blood samples were collected and processed to serum according the schedule shown above for hematology sample collection. The panel of plasma chemistry parameters assessed in this study is listed in Table 101.

No consistent or AFP-168-dose-dependent changes in any of the plasma chemistry parameters were observed except in the high-dose male killed in extremis. For this animal, aspartate aminotransferase, alanine aminotransferase, gamma

glutamyltransferase and alkaline phosphatase activities and total bilirubin concentration were elevated.

Urinalysis

Overnight urine samples were collected from all animals pre-treatment and in Weeks 12, 26, and 38. The following urinalysis parameters were assessed: specific gravity, protein, ketones, reducing substances, blood, chloride, calcium, urobilinogen, volume, pH, glucose, bilirubin, colour, potassium, sodium, and turbidity.

No AFP-168- related changes in any of the urinalysis parameters were observed.

Gross Pathology

All animals including early decedents and those euthanized at the terminal sacrifice were examined for gross pathology.

The primary gross pathology finding was injection site reddening but not in a dose-dependent manner for incidence. No other AFP-168 related gross pathology findings were observed. In addition, in the high-dose male killed in extremis, a small, firm, mottled liver, a small thymus, and a slightly enlarged kidney were observed. The intestinal tract was also discolored in this animal.

Organ Weights

Following the terminal sacrifice, organ weights were obtained for the panel of organs shown in Table 102. Left and right organs were weighed together. Both absolute organ weights and organ weights relative to body weight at termination were determined.

The relative salivary gland weights of high-dose males and females were increased 28 and 37% respectively compared to controls. None of the other measured organ weights were changed by treatment with AFP-168.

Histopathology

Adequate Battery
Yes. The large panel of organs and tissues shown in Table 102 were examined for all study animals.
Peer Review
No
Histological Findings
As summarized below in Table 52, for all of the animals except the high-dose male killed in extremis, minor adrenal cortical eosinophilia and acinar cell hypertrophy in salivary glands was observed primarily in high-dose males and females. For the affected animals, the salivary gland acini tended to be larger due to an increased amount of normal appearing cytoplasm. No other AFP-168-related histopathology findings were noted.

Table 52: Group Incidence of AFP-168-Related Histopathology Findings.
(Sponsor's Table)

Group incidence of selected microscopic findings – terminal kill									
		Males				Females			
		1M	2M	3M	4M	1F	2F	3F	4F
Tissue and finding	Level (µg/kg/day)	0	0.1	1	10	0	0.1	1	10
Adrenal	No. examined:	4	4	4	3	4	4	4	4
Cortical eosinophilia	Incidence	0	0	0	2	0	0	0	3
Salivary gland	No. examined:	4	4	4	3	4	4	4	4
acinar cell hypertrophy	Incidence:	0	0	1	3	0	0	1	4

For the male killed in extremis, extensive histopathology occurred in the liver. The centrilobular cords were atrophic and consisted mainly of collapsed sinusoids with pigmented cells and few normal hepatocytes. The periportal region consisted of proliferating bile ducts variably surrounded by basophilic hypertrophic hepatocytes interspersed with necrotic cells and mitosis.

Toxicokinetics

Blood samples were obtained and processed to plasma before dosing, on Day 1, and in Weeks 4, 13, 26, and 39. On each day, samples were collected within one minute after dosing, and at approximately 5, 10, 20, 30, 60, and 90 minutes after dosing. A LC/MS/MS method was used to measure plasma AFP-168 and AFP-172.

AFP-168 was measureable only at very low levels in a few samples, but this may have been due to sample storage beyond the established limits for AFP-168 in dog plasma (42 days at -70°C). AFP-168 was not detected in blood samples obtained from vehicle control animals.

APF-172 was detected in plasma at all AFP-168 dose levels on each sampling occasion with the exception of Group 2 (0.1 µg/kg/day) in Week 4 where no plasma AFP-172 was detected. AFP-172 was not detected in blood samples obtained from vehicle control animals. C_{max} (Table 53), AUC (Table 54), and T_{max} (Table 55) values for AFP-172 are shown below. Substantial gender differences were not apparent for C_{max} and AUC values, and both of these parameters increased in an approximately dose-proportional manner. Values were similar on all the sampling occasions indicating an absence of AFP-172 accumulation in plasma. T_{max} values ranged from 1 to 5 minutes.

Table 53: C_{max} Values for Plasma AFP-172 Following 39 Weeks of AFP-168 Intravenous Dosing in Male and Female Dogs. (Sponsor's Table)

Group	Dose (µg/kg/day)	Sex	C_{max} (ng/mL)				
			Day 1	Week 4	Week 13	Week 26	Week 39
2	0.1	Male	0.29	0.23	0.11	0.14	0.17
		Female	0.27	-	0.16	0.17	0.16
3	1	Male	2.26	1.45	1.33	1.74	1.39
		Female	2.05	1.67	1.27	5.71	1.48
4	10	Male	14.71	11.06	12.70	15.80	14.24
		Female	22.63	13.14	11.94	14.85	10.86

Table 54: AUC Plasma Exposure Values for AFP-172 Following 39 Weeks of AFP-168 Intravenous Dosing in Male and Female Dogs. (Sponsor's Table)

Group	Dose (µg/kg/day)	Sex	$AUC_{(0\text{-}90min)}$ (ng.min/mL) *				
			Day 1	Week 4	Week 13	Week 26	Week 39
2	0.1	Male	NC	NC	NC	NC	NC
		Female	NC	NC	NC	NC	NC
3	1	Male	25.97	19.21	14.55	21.16	18.02
		Female	27.00	23.00	13.74	38.19	20.29
4	10	Male	267.53	167.18	175.04	221.06	188.01
		Female	274.25	181.82	155.97	219.73	184.17

* AUC values were calculated using zero where concentrations were less than the LLOQ
NC = Not calculated due to insufficient data

Table 55: T_{max} Values for Plasma AFP-172 Following 39 Weeks of AFP-168 Intravenous Dosing in Male and Female Dogs. (Sponsor's Table)

Group	Dose (µg/kg/day)	Sex	T_{max} (min)				
			Day 1	Week 4	Week 13	Week 26	Week 39
2	0.1	Male	2.00	5.00	2.33	5.00	4.00
		Female	4.00	-	1.00	3.00	5.00
3	1	Male	2.00	4.00	1.00	3.00	4.00
		Female	1.00	5.00	3.00	5.25	5.00
4	10	Male	3.00	2.00	2.00	2.33	1.00
		Female	1.00	3.25	2.00	4.00	5.00

Dosing Solution Analysis

Stability and homogeneity analysis was performed by the Sponsor. Concentration analysis was performed on formulations prepared for use on Day 1 and in Weeks 4, 13,

26, and 39. The osmotic pressure and pH of the dosing solutions were measured in Weeks 13, 26, and 39. The target range for percent nominal concentration was 100 to 130%, and the target range for osmotic pressure ratio to physiological saline was 0.9 to 1.1.

The 0.0015% solution was shown to be stable for 6 weeks at room temperature. The 0.00001% and 0.0015% solutions were shown to be stable for 3 weeks at 5°C and 30°C. All of the solutions were used within one to two weeks after preparation to minimize the risk of bacterial contamination.

All of the actual formulation solution concentrations were within the target range except those denoted with an asterisk in the table below.

Table 56: Actual Formulation Concentrations. (Sponsor's Table)

		Results as % nominal		
Week	Sex	0.001%	0.0001%	0.00001%
4	Female	(b) (4)		
26	Male			
26	Female			
39	Female			

Study Title: DE-111 Ophthalmic Solution: 13-Week Ocular Toxicity Study in the Cynomolgus Monkey

Study no.:	MRL TT #11-7800
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 17, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Test Item #1: DE-111 ophthalmic solution (0.0015% tafluprost and 0.5% timolol), Lot # 100002-02, purities of 102.2% and 100.4% for tafluprost and timolol respectively. . Test Item #2: 0.0045%/1.5% DE-111 ophthalmic solution, Lot # 100002-03, purities of 103.9% and 99.5% for tafluprost and timolol respectively. . Test Item #3: 0.0045%/0% DE-111 ophthalmic solution, Lot # 100002-04, purity of 102.4% for tafluprost. . Test Item #4: 0% / 1.5% DE-111 ophthalmic solution, Lot # 100002-05,

purity of 99.5% for timolol.

Key Study Findings

Tafluprost and timolol in two different concentrations each were administered in combination BID by the topical ocular route for 13 weeks. Generally all of the dosing solutions were well tolerated. The only test item-related finding was iridial color darkening in five animals receiving tafluprost in combination with timolol or tafluprost alone. Assuming the iridial color change is not considered toxic, the NOAEL dose was the highest combination dose of 0.0045% tafluprost/1.5% timolol.

Methods

Doses:	See Table 57 below
Frequency of dosing:	Twice daily (BID)
Route of administration:	Topical ocular to the left eye
Dose volume:	30 µl/eye/dose
Formulation/Vehicle:	The vehicle was identified only as "vehicle for DE-111 ophthalmic solution." Its specification data indicated that it contained benzalkonium chloride, had a pH of approximately 7.0, and an osmolar ratio of approximately 1.
Species/Strain:	Cynomolgus monkeys (Macaca fascicularis)
Number/Sex/Group:	3/sex/group
Age:	4 to 5 years old
Weight:	Predose body weights were 3.7 to 5.7 kg for males and 2.5 to 3.6 kg for females.
Satellite groups:	None
Unique study design:	See Table 57 below. Animals were dosed BID with left eye topical ocular administrations for 13 weeks. The right eyes were untreated. Tafluprost was administered in combination with timolol (DE-111 ophthalmic solution) to animals in Groups 2 and 3. In Group 2, animals received 0.0015% tafluprost and 0.5% timolol. In Group 3, animals received 0.0045% tafluprost and 1.5% timolol. In addition, animals in Group 1 received vehicle, and animals received 0.0045% tafluprost or 1.5% timolol in Groups 4 and 5 respectively. .
Deviation from study protocol:	Multiple protocol deviations occurred including single instances of misdosing, minor deviations in the standard operating procedures associated with ophthalmic examinations, and single minor deviations in the environmental humidity and temperature. However, the study deviations were not considered to have altered the results or the validity and integrity of the study.

Table 57: Study Design for Study No.: MRL TT #11-7800. (Sponsor's Table)

Group number	Group description	Treatment schedule		Dose level (µg/eye/time)		Animals/group	
		Right eye	Left eye	Right eye	Left eye Tafluprost/Timolol	Male	Female
1	Control [a)]	Non treated	Vehicle	0	0	3	3
2	DE-111 [b)]	Non treated	DE-111	0	0.45/150	3	3
3	0.0045%/1.5% DE-111 [c)]	Non treated	0.0045%/1.5% DE-111	0	1.35/450	3	3
4	0.0045%/0% DE-111 [d)]	Non treated	0.0045%/0% DE-111	0	1.35	3	3
5	0%/1.5% DE-111 [e)]	Non treated	0%/1.5% DE-111	0	450	3	3

a) Vehicle for DE-111 ophthalmic solution

b) DE-111 ophthalmic solution (Test item formulation 1): solution containing 0.0015% Tafluprost and 0.5% Timolol

c) 0.0045%/1.5% DE-111 ophthalmic solution (Test item formulation 2): solution containing 0.0045% Tafluprost and 1.5% Timolol

d) 0.0045%/0% DE-111 ophthalmic solution (Test item formulation 3): solution containing 0.0045% Tafluprost

e) 0%/1.5% DE-111 ophthalmic solution (Test item formulation 4): solution containing 1.5% Timolol

Observations and Results

Mortality

Animals were visually inspected for morbidity and mortality twice daily.

No unscheduled deaths occurred during the study period.

Clinical Signs

Animals were assessed for clinical signs (behavior, appearance, eye irritation) twice daily.

Except for eye color, no AFP-168- or timolol-related changes in appearance, behavior, feces, or the appearance of the fur occurred during the study period. The left eye receiving dosing was darker in one animal in Group 2, one animal in Group 3 and three animals in Group 4 compared to the untreated right eye. This change was attributed to tafluprost administration.

Body Weights

Body weights were measured twice predose, once during dosing, and the day before necropsy.

No AFP-168- or timolol-related changes in body weight were noted. Female animals in Groups 3 and 4 were significantly higher in body weight compared to controls, but these animals were also higher in body weight for the predose measurement.

Feed Consumption: Not performed.

Ophthalmoscopy

Macroscopic ocular examination as well as fundus examination, slit lamp examination and fluorescein staining of the cornea, and intraocular pressure (IOP) measurements were performed predose, and 2 to 6 hours after dosing in Weeks 4, 8, and 13. Iridial color and eyelash color examinations were performed predose, and 2 hours 26 minutes to 5 hours 13 minutes after dosing in Weeks 4, 8, and 13.

No findings related to tafluprost or timolol administration were noted for the macroscopic fundus, or slit lamp examinations. Also no significant differences were noted for IOP measurements.

There was a slight tendency for decreased IOP in the left eyes of males in Groups 2 to 4 and in the left eyes of females in Groups 2 to 5 during the study compared to untreated eyes. The reduced IOP in the treated eyes fell within the normal variation but may have been due to the pharmacological actions of the test items.

The iridial color appeared to be darkened relative to predose colors for one male animal in Group 2, one male in Group 3 and two males in Group 4. No changes in eyelash color were attributed to treatment with the test items.

ECG

Electroretinography (ERG) was performed in all animals once predose, and once during Week 13. Scotopic ERG, oscillatory potentials, 30 Hz flicker, and photopic ERG were measured.

No changes in ERG were considered related to treatment with the test items.

Hematology, Clinical Chemistry, and Urinalysis: not performed

Gross Pathology

Animals were assessed for gross pathology immediately after termination on Day 92.

There were no unusual or test item-related gross pathology findings.

Organ Weights

The organs listed in Table 102 were assessed for organ weights. Paired organs were weighed separately and in combination.

One male in Group 5 demonstrated increased spleen weight. However, this finding and other minor changes in organ weights were not considered related to treatment with the test items.

Histopathology

Adequate Battery
Yes. The tissues listed in Table 102 were assessed for histopathology.
Peer Review
Internal peer review
Histological Findings
There were no test-item related histopathology findings.

Special Evaluation

None

Toxicokinetics

Blood samples were collected and processed to plasma on Day 1 and in Week 13 at 5, 15, 30, 60, 240, and 480 minutes after dosing from all the DE-111 treatment groups. Negative control blood samples were only obtained 5 minutes after dosing and Week 13 samples were also obtained predose. Tafluprost and timolol plasma concentrations were determined using LC-MS/MS methods of analysis.

Plasma concentrations of AFP-172 were quantifiable in samples following administration of DE-111 ophthalmic solution confirming systemic exposure. In Groups 1 and 5, where tafluprost was not part of the dosing solution, AFP-172 was not quantifiable in plasma. In the groups receiving tafluprost either alone or in combination, plasma concentrations of AFP-172 increased rapidly with a T_{max} of 0.083 hours. Due to the rapid elimination of AFP-172 from plasma, AUC could not be calculated for most animals, however C_{max} levels were consistently measurable. The C_{max} and T_{max} values for AFP-172 in males and females on Day 1 and in Week 13 are summarized below in Table 58. AFP-172 C_{max} values increased in a generally dose-proportional manner for both Day 1 and Week 13 measurements. There was a trend toward minimal plasma accumulation of AFP-172 in Week 13 compared to Day 1 in females but not in males. Plasma C_{max} values were similar for males and females on Day 1 but higher for females in Week 13 compared to Day 1. However, the Week 13 female values were not significantly higher than the Week 1 values and at least in some instances, the higher mean resulted primarily from a higher value for a single female.

Table 58: Plasma Toxicokinetic Parameters for AFP-172 in Monkeys Following Topical Administration of DE-111 Ophthalmic Solution.

Dose Group	Dose (μg/eye)	Gender	C_{max} (ng/ml)	T_{max} (h)
Day 1				
Group 2	0.45	**Male**	0.168*	0.083
		Female	0.145 ± 0.00608	0.083
Group 3	1.35	**Male**	0.455 ± 0.0918	0.083
		Female	0.316 ± 0.218	0.083
Group 4	1.35	**Male**	0.345 ± 0.137	0.083
		Female	0.553 ± 0.0747	0.083
Week 13				
Group 2	0.45	**Male**	0.139 ± 0.0323	0.083
		Female	0.297 ± 0.0935	0.083
Group 3	1.35	**Male**	0.311 ± 0.0287	0.083
		Female	0.840 +0.345	0.083
Group 4	1.35	**Male**	0.395 ± 0.183	0.083
		Female	0.809 ± 0.507	0.083

C_{max} values are expressed as mean ± standard deviation.

* Mean data obtained from n = 1.

Plasma concentrations of timolol were also quantifiable following administration of DE-111 ophthalmic solution. Timolol was not quantifiable in Group 1 receiving vehicle or in Group 4 receiving tafluprost alone except in one Group 4 male at one timepoint suggesting crosscontamination for the single sample. Plasma timolol C_{max} and AUC values were measurable and t_{max} values ranged from 0.083 to 0.50 hours (Table 59). Both AUC and C_{max} values increased in an approximately dose-proportional manner. Combination dosing of tafluprost and timolol did not result in plasma accumulation based on both AUC and C_{max} measurements. There was evidence of decreased systemic exposure with timolol administered alone in Group 5 with both AUC and C_{max} values decreasing in males and females after 13 weeks of dosing compared to Day 1. Male and female values were similar for each measurement timepoint.

Table 59: Plasma Toxicokinetic Parameters for Timolol in Monkeys Following Topical Administration of DE-111 Ophthalmic Solution.

Dose Group	Dose (µg/eye)	Gender	AUC_{0-t} (ng x h/ml)	C_{max} (ng/ml)	T_{max} (h)
Day 1					
Group 2	0.45	**Male**	14.8 ± 3.48	6.87 ± 2.19	0.25 – 0.50
		Female	24.4 ± 5.23	12.4 ± 3.58	0.25
Group 3	1.35	**Male**	51.2 ± 9.36	26.7 ± 3.20	0.50
		Female	63.8 ± 51.3	28.4 ± 21.4	0.083 – 0.25
Group 5	1.35	**Male**	45.0 ± 0.808	30.2 ± 0.458	0.25 – 0.50
		Female	49.6 ± 12.5	31.1 ± 10.5	0.083 – 0.25
Week 13					
Group 2	0.45	**Male**	17.3 ± 6.37	6.36 ± 2.25	0.25 – 0.50
		Female	25.8 ± 11.2	10.3 ± 4.96	0.25
Group 3	1.35	**Male**	46.2 ± 10.9	18.6 ± 4.05	0.50
		Female	62.6 ± 52.4	37.9 ± 37.7	0.083 – 0.25
Group 5	1.35	**Male**	28.7 ± 8.06	15.2 ± 3.69	0.25 – 0.50
		Female	25.0 ± 3.64	13.6 ± 0.917	0.083 – 0.25
AUC and C_{max} values are expressed as mean ± standard deviation. T_{max} is expressed as a range.					

Dosing Solution Analysis

The dosing solutions were assessed for osmolar ratio, pH, and purity upon initial dosing, and at 8 weeks, 13 weeks, and 18 weeks with storage at room temperature in between measurements.

For all dosing solutions, the osmolar ratio (approximately 1.0), pH (approximately 7.0), and purity or tafluprost and/or timolol remained almost unchanged at each later measurement (8, 13, and 18 weeks) compared to the initial measurement.

Study title: AFP-168: 52-week ocular toxicity study in the Cynomolgus monkey.

Study no.:	MRL TT # 01-5531
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	6/19/2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AFP-168, Lot # D01114, purity of 98% Lot # D01115, purity of 100%; Lot # D01116, purity of 102%.

Key Study Findings

AFP-168 (0.0005%, 0.005%, 0.05%) or vehicle was administered BID topically into the left eye of Cynomolgus monkeys for 52 weeks. No systemic toxicity was observed. All AFP-168-related toxicity was associated with the left treatment eyes. Sunken left eyes occurred in the intermediate- and high-dose groups. Iris color darkening was observed in all AFP-168 treatment groups. Blue gray discoloration of the lower eyelid occurred in the intermediate and high-dose groups. In addition two males and two females in the high-dose group displayed minimal focal inflammation in the epithelium of the left eyelid.

Methods

Doses:	0, 0.15, 1.5, 15 µg/left eye/administration
Frequency of dosing:	Twice daily (approximately 12 hours apart).
Route of administration:	Topical ocular
Dose volume:	30 µl/dose
Formulation/Vehicle:	The vehicle for the 0.005% and 0.0005% AFP-168 ophthalmic solutions contained 0.05% Tween 80, (b) (4) $NaH_2PO_4 \times H_2O$, (b) (4) EDTA x 2Na, (b) (4) glycerin, 0.01% benzalkonium chloride, and NaCl. The vehicle for the 0.05% AFP-168 ophthalmic solution contained exactly the same excipients except the Tween 80 concentration was (b) (4) instead of (b) (4).
Species/Strain:	Cynomolgus monkeys (macaca fascicularis)
Number/Sex/Group:	4/sex/group
Age:	Approximately 3 to 4 years old.
Weight:	2.8 to 4.6 kg for male and 2.8 to 3.5 kg for female animals.
Satellite groups:	None
Unique study design:	See Table 60 below. AFP-168 was administered topically into the cornea of the left eye of the Cynomolgus monkeys at concentrations of 0.0005%, 0.005%, and 0.05%. The right eye of vehicle control animals received the vehicle of 0.05% AFP-168, the left eye of vehicle control animals received the vehicle for 0.005% and 0.0005% AFP-168, and the right eye of animals from Groups 2 to 4 received sterile saline. Animals were dosed twice daily for 52 weeks.
Deviation from study protocol:	Multiple protocol deviations were noted; however, none was considered to have altered the results of the study or compromised the study's integrity.

Table 60: Study Design for Study No.: MRL TT #01-5531

Group No.	Dose (μg/left eye/ administration)	Number of Animals		Left Eye Treatment	Dose Volume (μl per Administration)
		Males	Females		
1	0	4	4	Vehicle	30 μl
2	0.15	4	4	0.0005% AFP-168	
3	1.5	4	4	0.005% AFP-168	
4	15	4	4	0.05% AFP-168	

Observations and Results

Mortality

All animals were observed for morbidity and mortality at the beginning and the end of the work day.

All animals survived the treatment period.

Clinical Signs

All animals were observed four times daily for appearance and behavior (before and after each dosing) and twice daily for feces. Macroscopic examination of the eye was performed before the first dosing and after the second dosing each day.

Only eye-related clinical signs were considered related to treatment with AFP-168. However, in the highest two AFP-168-treatment groups, Groups 3 and 4, at least 3 of 4 animals of each gender demonstrated a sunken left treatment eye beginning as early as Day 23, and this change was permanent throughout the experiment. Four animals (three males and one female) demonstrated sunken eyes on single occasions. Also, at least 3/4 animals of each gender in all of the AFP-168-treatment groups demonstrated treatment-related darker iris color in the left eye beginning as early as Day 23. The iris color change, once it occurred, was permanent throughout the experiment.

Body Weights

Individual body weights were recorded once pre-dose and once weekly during the study and before necropsy.

AFP-168 at any of the administered doses did not appear to alter body weights compared to the control animals in Group 1.

Feed Consumption

Individual food intake was determined daily from the amount of food left throughout the study period and calculated as g/animal/week.

AFP-168-related changes in food consumption were not observed.

Ophthalmoscopy

Macroscopic ocular examinations of the eyes were performed by an ophthalmologist blinded to the study design.

Macroscopic Examinations: Macroscopic examinations were performed on unanesthetized animals once pre-dose and 2 to 6 hours after the first dosing during Weeks 13, 26, 39 and 52. In order to evaluate pupillary size, photographs of the conscious monkey faces were taken once pre-dose and monthly during the study period (Weeks 4, 8, 13, 17, 21, 26, 30, 34, 39, 43, 47, and 52).

As noted above, AFP-168-related sunken eyes and darkened iris color were observed. Also many of the same animals demonstrated blue-gray discoloration of the lower eyelid. Treatment with AFP-168 did not alter pupil size during the treatment period.

Slit Lamp Examinations: Zeiss SL 120 slit lamp examinations of the cornea, conjunctiva, anterior chamber, iris, lens, and vitreous body were performed on sedated monkeys once pre-dose and once during Weeks, 13, 26, 39, and 52 (2 to 6 hours after the first dosing). The different ocular structures were scored for irritation.

During Week 26, two animals of each gender in Group 4 demonstrated pigment around the lower nasal limbus. However, this change was not evident in Weeks 39 and 52, and did not correlate with histopathology. No AFP-168 related changes in the irritation score for any of the ocular structures occurred during the treatment period.

Corneal Examination with Fluorescein Staining: Fluorescein staining of the cornea was performed on sedated animals once pre-dose, and once in Weeks 13, 26, 39, and 52 (2 to 6 hours after dosing). The cornea was scored for irritation.

In single animals from both control and AFP-168 treatment animals, punctuate corneal keratopathy was observed during Weeks 13, 26, and 52 with decreased incidence and severity in the latter examinations.

Ophthalmoscopy: Fundus examination was performed on all animals (sedated with eye dilation) once pre-dose and once during Weeks 13, 26, 39, and 52 (2 to 6 hours after the first dosing). Eyes were examined with an indirect ophthalmoscope, and the ocular fundus with macula lutea, papilla, ocular vessels, and retina were examined.

None of the findings in the fundus examinations were considered related to AFP-168 administration.

Intraocular Pressure (IOP): IOP was determined in all animals (sedated) once pre-dose and once in Weeks 13, 26, 39, and 52 (2 to 6 hours after the first dosing).

A statistically significant decrease in ocular pressure occurred in Group 4 males and females during Week 26. Left eye intraocular pressure remained lower compared to the right eye for the rest of the study, but not to a significant degree. Slight decreases in IOP also occurred in Group 2 and 3 animals during Week 26.

Iridial Color Observations: sedated animals were assessed for iridial color once pre-dose and once in Weeks 13, 26, 39, and 52.

As noted above as a clinical sign, clear darkening of the left eye iris color was noted in most animals in all of the AFP-168 treatment groups from Week 13 onward. The degree of darkening was not dose-dependent, however, and no correlating histopathology was noted.

Eyelash Observations: Slightly sedated animals were photographed with a 5-fold magnification once pre-dose and once in Weeks 13, 26, 39, and 52 in order to examine each animal's eyelashes.

Many animals in Groups 3 and 4 demonstrated darkening of the lower eyelid which appeared to be related to AFP-168 administration.

Electroretinography (ERG): ERG measurements were determined in sedated animals once pre-dose, one during Weeks 25 and 26, once during Weeks 29 or 30, and once during Weeks 51 or 52. Measurements included scotopic ERG, oscillatory potentials, 30 Hz flicker, and photopic ERG.

No changes in ERG were considered related to treatment with AFP-168.

ECG

Electrocardiograms (EKG) were performed on all animals (unanesthetized, temporarily restrained) once pre-dose and in Weeks 13, 26 and 52 (prior to and 15 minutes after the first dosing). Heart rates (beats/minute), ECG intervals, RR, P, PR, QRS, and QT intervals (QTc and Qtdispersion), as well as voltage measurement of P, R, S, and T were measured.

No AFP-168-related changes in ECG intervals were noted in the experiment. Isolated cases of tachycardia or bradycardia and QTC lengthening were observed in single animals across all groups including controls.

Blood pressure (BP) was recorded in non-anesthetized animals once pre-dose and twice in Weeks 13, 26 and 52 (prior to and 15 minutes after dosing). Measurements included systolic pressure (SP), diastolic pressure (DP) and mean arterial pressure (MAP).

No AFP-168 related changes in blood pressure were observed. All animals demonstrated blood pressure changes similar to pre-dose determinations and within the normal range.

Hematology

Blood samples intended for hematology and clinical chemistry analysis were collected from all animals once pre-dose and once in Weeks 13, 26, and 52 of the study. The panel of hematology parameters shown in Table 100 was examined.

None of the changes in any of the hematology parameters were attributed to treatment with AFP-168.

Clinical Chemistry

Blood collected according the schedule noted above for hematology collection was processed to serum and analyzed for the clinical parameters listed in Table 101.

No AFP-168-related changes in any of the serum chemistry parameters were observed.

Urinalysis

Urine samples were collected overnight from all animals once pre-dose and once in Weeks 13, 26, and 52. During the collection period, animals were deprived of water. Urine samples were examined for specific gravity, protein, ketones, blood, nitrite content, volume, pH, glucose, bilirubin, urobilinogen and sediment (via microscopy). Also quantitative analysis of potassium, sodium, chloride, and calcium in urine was performed.

No AFP-168 related changes in any of the urine parameters were noted.

Gross Pathology

Animals were sacrificed after fasting overnight and a full gross pathology examination was performed.

Other than the AFP-168-related eye changes already noted (left eye iris darkening, sunken left eye), no AFP-168-related gross pathology changes were noted.

Organ Weights

Following necropsy the organs listed in Table 102 were collected and weighed. Absolute weights were measured and organ weight to body weight and to brain weight ratios were calculated. Paired organs were weighed separately.

None of the changes in organ weights were ascribed to AFP-168 administration. Very high values in two males in Groups 3 and 4 caused significant increases in the group mean thymus/brain weight ratios for male animals in these groups relative to control animals. However, all other animals in these groups demonstrated similar thymus/brain weight ratios and absolute thymus and thymus weight/body weight ratios were not statistically increased.

Histopathology

Adequate Battery
Yes. The extensive battery of tissues listed in Table 102 were examined for histopathology.
Peer Review
No

Histological Findings
No systemic organ histopathology considered related to AFP-168 administration was noted. Consistent with the observed iris darkening, most of the animals in the three AFP-168-treatment groups demonstrated increased melanocyte pigment in the iris stroma of the left eye. In addition increased pigment around the hair follicles of the left eye and minimal focal inflammation in the epithelium of the left eyelid were noted predominantly in high-dose males and females.

Toxicokinetics

Blood (≈ 2.2 mls) was collected from all animals from all groups 5 and 60 minutes after the first dose on Day 1, during Weeks 13 and 39, and before the first administration and 5, 15, 30, 60, and 120 minutes after dosing during Week 25 or 26, during Week 28 and during Weeks 51 or 52. Immediately after collection, blood was processed to plasma and stored at -80°C until analysis. AFP-168 and AFP-172 (active metabolite of AFP-168) concentrations in plasma were determined using an LC/MS/MS method.

Plasma concentrations of AFP-168 were below the level of detection at all time-points. Male and female animals demonstrated systemic exposure to AFP-172 only in the 0.005% and 0.05% dose groups and toxicokinetic parameters were only calculable for the high-dose group (Table 61). No accumulation of AFP-172 was observed after repeated administration. C_{max} and $AUC_{(0-2h)}$ tended to be slightly higher in males compared to females, but values were similar for both genders.

Table 61: Select Toxicokinetic Parameters Following Topical Ocular Administration of High-Dose AFP-168 to Monkeys for 52 Weeks (Sponsor's Table).

Sex (n=4)	Animal Number	C_{max} (ng/mL)		$AUC_{(0-2h)}$ (ng.min/mL)		T_{max} (min)	
		Week 28	Week 52	Week 28	Week 52	Week 28	Week 52
M	20453	3.92	3.96	45.4	45.8	5	5
M	20466	3.41	2.44	73.2	74.0	5	15
M	20467	7.82	5.09	119.8	122.4	5	5
M	20573	5.97	2.83	89.0	57.1	5	5
Mean		5.28	3.58	81.9	74.8	-	-
SD (n-1)		2.02	1.20	31.1	33.8	-	-
F	20591	4.05	4.47	75.6	64.6	5	5
F	20595	6.67	4.80	125.2	78.8	5	5
F	20597	10.84	5.28	147.6	64.1	5	5
F	20600	10.11	6.12	137.3	83.2	5	5
Mean		7.92	5.17	121.4	72.7	-	-
SD (n-1)		3.15	0.72	31.9	9.8	-	-

Dosing Solution Analysis

The study report indicates that the test article was analyzed by the supplier [redacted] before arrival at the testing facility ([redacted]). 8 Page(s) of Draft L b li

No stability information was provided in the study report concerning any of the AFP-168 dosing solutions.

7 Genetic Toxicology

Tafluprost was tested for genetic toxicity in three GLP-compliant assays. These included an *in vitro* bacterial reverse mutation assay (Ames assay; Study Report No.: MRL TT #99-5551), an *in vitro* chromosome aberration assay in cultured Chinese hamster lung cells (Study Report No.: MRL TT #99-5552), and an *in vivo* mouse bone marrow micronucleus assay (Study Report No.: MRL TT #99-5538).

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: AFP-168: Reverse Mutation in four histidine-requiring strains of *Salmonella typhimurium* and one tryptophan-requiring strain of *Escherichia coli*.

Study no.:	MRL TT#99-5551
Study report location:	Electronic transmission
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	May 13, 1999
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AFP-168, batch number FP-9901, purity of 99%.

Key Study Findings

AFP-168 at concentrations as high as 5000 µg/plate did not induce mutation in the *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, or the *Escherichia coli* strain WP2 uvrA in an Ames test in the absence and presence of S9 activation.

Methods

Strains:	*Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537; and Ecoli strain WP2 uvrA.
Concentrations in definitive study:	Experiment #1: 0, 312.5, 625, 1250, 2500, and 5000 µg/plate with and without S9 activation. Experiment #1 Repeat Treatments: 0, 78.125, 156.25, 312.5, 625, 1250, 2500 with and without S9 activation. Experiment #2: 0, 78.125, 156.25, 312.5, 625, 1250, 2500, 5000 µg/plate with and without S9 activation.

	Experiment #2 Repeat Treatments: 0, 19.532, 39.063, 78.125, 156.25, 312.5, 625, and 1250 μg/plate with and without S9 activation.
Basis of concentration selection:	The concentrations for the definitive experiments (Experiment #1 and Experiment #2) were based on the results of a range-finding assay using AFP-168 concentrations of 0. 1.311, 3.277, 8.192, 20.48, 51.2, 128, 320, 800, 2000, and 5000 μg/plate with and without S9 activation.
Negative control:	DMSO (vehicle)
Positive control:	2-nitrofluorene (2NF) for TA98, sodium azide (NaN_3) for TA100 and TA1535, 9-aminoacridine (AAC) for TA1537, 4-nitroquinoline 1-oxide (NQO) for WP2 uvrA, and 2-aminoanthracene (AAN) for at least one *Salmonella* strain and for WP2 uvrA in the presence of S9.
Formulation/Vehicle:	AFP-168 was dissolved in DMSO
Incubation & sampling time:	All plate incubations were for 3 days.

Study Validity

The following acceptance criteria were met and the assay was considered valid.

1. The mean negative control counts fell within the normal historical control ranges for each bacterial strain.
2. The positive control chemicals induced clear increases in revertant numbers.
3. No more than 5% of the plates were lost to contamination or some other unforeseen event.

Results

In all of the definitive experiments, bacterial strains were treated with AFP-168 and vehicle and positive control articles in triplicate. AFP-168 was considered to be mutagenic if it produced a significant increase in revertants relative to the negative control plates and if the data indicated a significant dose correlation.

The range finder assay was performed in strain TA100 only, and evidence of toxicity in the form of a reduction in revertant numbers below the level of the solvent control was observed only for the high dose, 5000 μg/plate in the absence of S9 activation.

In Experiment 1, cytotoxicity was apparent for strain TA100 following treatment with AFP-168 concentrations of 5000 μg/plate. Also a slight thinning of the background bacterial lawn was apparent for a majority of AFP-168 concentrations with and without S9 activation. Repeat testing with TA1537 was conducted for Experiment #1 to obtain more data in the absence of cytotoxicity. While cytotoxicity again occurred for the higher

AFP-168 concentrations, several lower concentrations did not cause cytotoxicity thus allowing assessment of mutagenicity in the absence of cytotoxicity. Also AFP-168 precipitated at the 2500 and 5000 µg/plate concentrations in Experiments 1 and 2.

In Experiment 2, the mutagenic ability of AFP-168 in each strain was further assessed with a high concentration of 5000 µg/plate for all strains other than TA1537 and a high concentration of 1250 µg/plate for TA1537. In addition, all treatments were modified by a pre-incubation step. Evidence of cytotoxicity was observed in strains TA100 and TA1537 with and without S9 activation, and in strain TA98 in the absence of S9 only.

For both Experiment 1 and Experiment 2 and the repeat assays in these experiments, none of the AFP-168 treatments of any of the tester strains with and without S9 activation induced a significant increase in revertant numbers thus indicating negative results for mutagenicity. In contrast, all of the positive control treatments induced significant increases in revertant numbers for all strains under all experimental conditions.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: AFP-168: Induction of chromosome aberrations in cultured Chinese hamster lung (CHL) cells.

Study no.:	MRL TT#99-5552
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 12, 1999
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AFP-168, Lot # FP-9901, purity of 99.0%

Key Study Findings

CHL cells treated with AFP-168 in the absence and presence of S-9 under all treatment conditions exhibited frequencies of cells with structural aberrations which were similar to those seen in concurrent negative control cultures which in all but one case fell within historical ranges for negative controls. Cultures treated with AFP-168 for 3 hours in the absence of S-9 resulted in cultures with increased frequencies of cells with polyploidy, but for unclear reasons, the concurrent untreated control culture also demonstrated polyploidy.

Methods

Cell line:	Chinese Hamster Lung (CHL) cells
Concentrations in definitive study:	At least 10 concentrations of AFP-168 within the range of 1.186 to 4245 µg/ml were tested for all of the experimental conditions in both Experiments 1 and 2. The concentrations shown in Table 62 for each

	experimental condition were selected for cytotoxicity based on the criteria shown below.
Basis of concentration selection:	The concentrations used for cytogenetic analysis were determined based on the following criteria: 1. The top dose should be the highest dose tested or one that produces at least 50% reduction in cell number. 2. The top dose should also provide an adequate number of metaphases for scoring. 3. In addition to the top dose, two lower doses are selected such that a range of toxicity from maximal to little or none is covered.
Negative control:	DMSO (vehicle)
Positive control:	Without S9 activation: Methanesulphonate (MMS). With S9 activation: cyclophosphamide (CPA).
Formulation/Vehicle:	AFP-168 was dissolve in DMSO
Incubation & sampling time:	See Table 62 below. In the presence of S9 activation, cells were incubated with different concentrations of AFP-168 for 3 hours with 17 and 41 hours of recovery before sampling. In the absence of S9, cells and AFP-168 were incubated for 3 hours with 17 hours of recovery and 44 and 20 hours with no recovery before sampling.

Evaluation criteria:

1. The proportion of cells with structural aberrations at one or more concentrations exceeds the normal range in both replicate cultures.
2. A statistically significant increase in the proportion of cells with structural aberrations (excluding gaps) occurs at such doses.
3. Confirmation in an independent experiment.

Table 62: Concentrations Selected for Cytogenicity Analysis for Experiments 1 and 2 (Sponsor's Table).

S-9	Treatment + recovery (hours)	Vehicle Control	Concentration AFP-168 (µg/mL)	Positive Control
Experiment 1				
-	20+0	0[a]	2.966, 7.414, 18.53	35 µg/mL MMS
+	3+17	0[a]	7.414, 46.34, 115.8	12.5 µg/mL CPA
Experiment 2				
-	20+0	0[a]	1.879, 3.341, 14.08	35 µg/mL MMS
+	3+17	0[a]	79.10, 105.5, 140.6	12.5 µg/mL CPA
-	3+17	0[a]	10.56, 18.77, 33.37	-
+	3+41	0[a]	105.5	-
-	44+0	0[a]	14.08	-

[a] Vehicle control was DMSO only

Study Validity

The following criteria were met and the assays were considered valid.

1. The binomial dispersion test demonstrated acceptable heterogeneity between replicate cultures.
2. The proportion of cells with structural aberrations (excluding gaps) in negative control cultures fell within the normal range.
3. At least 160 cells out of an intended 200 were analyzable at each concentration level.
4. The positive control articles induced significant increases in the number of cells with structural aberrations.

Results

AFP-168 completely dissolved in DMSO, but precipitated in a concentration-dependent manner. AFP-168 formed a precipitate in the range of 115.8 to 187.5 µg/ml under all experimental conditions.

The aberrant cells in each culture were categorized as cells with structural aberrations including gaps, cells with structural aberrations excluding gaps, and polyploidy, endoreduplicated or hyperdiploid cells.

In Experiment 1, approximately 100% cytotoxicity occurred at a concentration of ≥ 46.34 µg/ml in the 20 hour treatment without S9 and 55% cytotoxicity occurred at the next highest concentration of 18.53 µg/ml. Similarly 100% cytotoxicity occurred at an AFP-168 concentration of ≥ 289.6 µg/ml in the 3 hour treatment with S9, and 20% cytotoxicity

occurred at the next highest concentration of 115.8 μg/ml. Based on these results, AFP-168 concentrations of 2.966, 7.414, and 18.53 μg/ml were analyzed for the 20 hour treatment without S9, and concentrations of 7.414, 18.53, and 115.8 μg/ml were analyzed for the 3 hour treatment with S9 activation.

In Experiment 2, the same treatment conditions as for Experiment 1 were repeated with the addition of a delayed sampling time treatment, and a pulse treatment in the absence of S9. The highest concentrations chosen for analysis for the 20 hour treatment in the absence of S9 and the 3 hour treatment in the presence of S9 were 14.08 and 140.6 μg/ml respectively which induced approximately 50% and 80% cytotoxicity. The effects of single concentrations of 14.08 and 105.5 mg/ml with and without S9 were investigated for the delayed (44+0, 3+41 in hours) sampling time conditions. These concentrations induced approximately 55% and 0% cytotoxicity. For the pulse treatment in the absence of S9 (3+17 hours) three concentrations (10.56, 18.77 and 33.37 μg/ml) were chosen and the highest of the high concentration of 33.37 μg/ml induced approximately 57% cytotoxicity.

In both experiments under all conditions, cultures treated with AFP-168 in the absence and presence of S9 exhibited frequencies of cells with structural aberrations which were similar to those seen in the concurrent solvent control cultures. Also the solvent control cultures displayed frequencies which fell within historical negative control ranges. One culture condition, the treatment of AFP-168 for 3 hours in the absence of S9 resulted in cultures with moderately increased frequencies of cells with polyploidy relative to the historical control range of 0 to 3 per 100 cells (Table 63). However, an increased frequency of cells with polyploidy was also seen in one negative control. These results suggest that a factor other than AFP-168 may have induced the observed polyploidy.

Table 63: Frequency of Cells With Polyploidy in the 3+17 hour Incubation Without S9. (Sponsor's Table)

3+17 hours, -S-9, Experiment 2

Treatment (μg/mL)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Solvent	A	100	0	0	0	0	0
	B	103	0	0	3	3	2.9
	Total	203	0	0	3	3	1.5
Untreated	A	107	1	0	6	7	6.5
	B	101	0	0	1	1	1.0
	Total	208	1	0	7	8	3.8
10.56	A	104	0	0	4	4	3.8
	B	100	0	0	0	0	0
	Total	204	0	0	4	4	2.0
18.77	A	105	0	0	5	5	4.8
	B	106	2	0	4	6	5.7
	Total	211	2	0	9	11	5.2
33.37	A	102	0	0	2	2	2.0
	B	104	0	0	4	4	3.8
	Total	206	0	0	6	6	2.9

** = Total cells examined for numerical aberrations

See Appendix 2 for abbreviations and classification

Numbers highlighted exceed the historical negative control range (Appendix 5)

Numerical aberrations (num abs)

E	=	endoreduplicated
H	=	hyperdiploid (28-37 chromosomes)
P	=	polyploid (greater than 37 chromosomes)

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: AFP-168: Induction of micronuclei in the bone marrow of treated mice.

Study no:	MRL TT#99-5538
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 17, 2000
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AFP-168, lot No.: FP-9901, purity of 99.0%

Key Study Findings

AFP-168 at doses of 0, 175, 350, and 700 mg/kg did not induce micronucleated polychromatic erthrocytes indicative of clastogenesis in the bone marrow of mice 24 and 48 hours after a single intraperitoneal dosing.

Methods

Doses in definitive study:	0, 175, 350, and 700 mg/kg
Frequency of dosing:	Single-dose
Route of administration:	Intraperitoneal
Dose volume:	20 ml/kg
Formulation/Vehicle:	AFP-168 was dissolved in corn oil.
Species/Strain:	Outbred CD-1 mice
Number/Sex/Group:	6 males per group at the 24 hour (all groups) and 48 hour (all groups except the positive control) sampling times.
Satellite groups:	None
Basis of dose selection:	The Main Study doses were based on the results of a range-finding assay with doses of 700, 1000, 1400, and 2000 mg/kg. Males and females were used in the range-finding assay, but because no significant gender differences were observed, only males were used in the Main Study
Negative control:	Corn oil (the vehicle)
Positive control:	Cyclophosphamide (40 mg/kg in saline).

Study Validity

The following criteria were met and the assay was considered valid.

1. The incidence of micronucleated polychromatic erythrocytes (PCE) in the vehicle control group fell within or close to the historical vehicle control range.
2. At least five animals in each group were available for analysis.

3. The positive control chemical, cyclophosphamide, induced a significant increase in the frequency of micronucleated PCE.

Results

In the range finding assay, males and female mice administered 700 mg/kg AFP-168 experienced lethargy and eye closure, but no mortality. In contrast, mice administered ≥ 1000 mg/kg experienced a number of clinical signs including abnormal breathing, abnormal gait, lethargy, tremors, piloerection and 17-67% mortality. Because it was the highest non-lethal dose, 700 mg/kg was chosen as the high dose for the Main Study. In the Main Study, one male mouse administered 700 mg/kg was found dead.

The effects of AFP-168 were considered positive if a statistically significant increase in the frequency of micronucleated PCE occurred for at least one dose and the frequency of micronucleated PCE for the positive dose exceeded the historical vehicle control range. The groups of mice receiving AFP-168 exhibited polychromatic erythrocyte (PCE) to nonchromatic erythrocyte (NCE) ratios that were similar to or lower than values for the vehicle control group. Bone marrow from animals receiving AFP-168 at 350 mg/kg (24 hour sample time) and 700 mg/kg (24 and 48 hour sample times) exhibited reduced PCE/NCE ratios indicative of bone marrow cytotoxicity. Group mean frequencies of micronucleated PCE for all the AFP-168 treatment groups were similar to those of the vehicle control group (0.58 ± 0.08, mean ± SEM) at both sampling times. No significant differences were apparent. In contrast treatment with the positive control article, 40 mg/kg cyclophosphamide for 24 hours produced a group mean frequency of micronucleated PCE of 6.67 ± 2.96 (mean ± SEM). The group mean frequencies of micronucleated PCE for the 24 and 48 hour sampling timepoints are shown below in Table 64 and Table 65 respectively.

Table 64: Summary of the Group Mean Frequencies of Micronucleated PCE for the 24 Hour Sample Timepoint. (Sponsor's Table)

Treatment group (mg/kg)	Mean ratio PCE/NCE	Group mean frequency of micronucleated PCE ± SD (per 1000 cells)
Vehicle	0.80	0.58 ± 0.80
175	0.75	0.25 ± 0.42
350	0.38	0.20 ± 0.45
700	0.38	0.33 ± 0.26
CPA, 40	1.08	6.67 ± 2.96

SD standard deviation

Table 65: Summary of the Group Mean Frequencies of Micronucleated PCE for the 48 Hour Sample Timepoint. (Sponsor's Table)

Treatment group (mg/kg)	Mean ratio PCE/NCE	Group mean frequency of micronucleated PCE ± SD (per 1000 cells)
Vehicle	1.00	0.25 ± 0.27
175	0.67	0.33 ± 0.41
350	0.72	0.33 ± 0.52
700	0.53	0.40 ± 0.55

SD standard deviation

7.4 Other Genetic Toxicity Studies

None

8 Carcinogenicity

Subcutaneous dose, 13-week, range-finding studies rats and mice were conducted in order to determine a rationale for dose selection in the long-term carcinogenicity studies in the same species. Maximum tolerated doses were not determined for either species in the range-finding studies, but doses associated with plasma exposure more than 25 fold greater than that expected in humans following topical ocular dosing were determined. The range-finding studies (Study Report Nos.: TT #03-5574 and TT #03-5576), and the 2-year and 78-week studies in rats (Study Report No.: TT #03-5575) and mice (Study Report No.: TT #04-5572) respectively are reviewed below.

Study title: AFP-168: 13 week subcutaneous administration range-finding study in the rat.

Study no.:	MRL TT #03-5574
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 10, 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AFP-168, Lot # F01X010, purity of 99.5%

Key Study Findings

Daily subcutaneous administration of AFP-172 to rats for 13 weeks resulted in very little toxicity even at the high dose of 30 µg/kg/day. A maximum tolerated dose was not determined. Only minor histopathology (spleen hematopoiesis and kidney corticomedullary mineralization) was considered related to high-dose AFP-168 administration. Plasma AFP-168 was not detectable at any dose, but AFP-172, the major active metabolite of AFP-168 was measureable in plasma for the 10 and 30 µg/kg/day dose groups. AFP did not accumulate in plasma and C_{max} and AUC values

were similar for both genders. The high-dose AUC value was approximately 6 ng x h/ml).

Study Objective

The objective of this study was to determine the toxicity of AFP-168 following subcutaneous administration for 13 weeks in support of dose selection for a subsequent 2-year carcinogenicity study in rats.

Methods

Crl:CD®(SD)IGSBR rats received daily subcutaneous administrations of 0, 3, 10, and 30 μg/kg/day for 13 weeks. Ten males and females were included in each group in the Main Study and an additional 4 animals/sex in the vehicle control group and 8 animals/sex/group in the AFP-168-treatment groups were used for toxicokinetic analysis. Mortality, clinical signs, body weights, food consumption, hematology (extensive panel), coagulation parameters (prothrombin time and activated partial thromboplastin time), clinical chemistry (alkaline phosphatase, calcium, and inorganic phosphorus), gross pathology, organ weights (extensive panel), and histopathology (vehicle and high-dose groups; extensive panel) were assessed. Also blood samples were collected for toxicokinetic analysis on Day 1, Week 4, and Week 13 at predose, and 5, 15, 30, 60, 90, and 120 minutes after dosing.

Results

One animal died immediately after dosing with 3 μg/kg AFP-168 on Day 42 from hemorrhage in the thoracic cavity. However, this death was thought to have resulted from a faulty dose procedure. No AFP-168-related clinical signs, gross pathology, or changes in body weight, food consumption, hematology, clotting parameters, clinical chemistry, or organ weights were observed.

As shown in Table 66, histopathology in the spleen and kidney was considered related to AFP-168 administration. In the spleen, a minor increase in the incidence and severity of hematopoiesis in high-dose males and females compared to vehicle control animals was noted. In the kidney, a minor increase in the incidence and severity of corticomedullary mineralization in high-dose females was noted.

Table 66: Spleen and Kidney Histopathology Associated with Daily Subcutaneous Administration of AFP-168 for 13 Weeks to Rats. (Sponsor's Table)

Group incidence of selected microscopic findings									
		Males				Females			
		1M	2M	3M	4M	1F	2F	3F	4F
Tissue and finding	Level (µg/kg/day)	0	3	10	30	0	3	10	30
Spleen	No. examined:	10	0	0	10	10	0	0	10
haemopoiesis	Grade -	1	0	0	0	4	0	0	2
	1	8	0	0	6	4	0	0	5
	2	1	0	0	4	2	0	0	3
Kidney	No. examined:	10	2	1	10	10	1	0	10
corticomedullary mineralisation	Grade -	10	2	1	10	5	0	0	3
	1	0	0	0	0	5	1	0	4
	2	0	0	0	0	0	0	0	3

Key: "-" = finding not present, 1 = minimal, 2 = slight

Toxicokinetic analysis revealed that AFP-168 was generally not detected in plasma at any sampling time. Its major active metabolite, AFP-172, was detected in all of the plasma samples from the 10 and 30 µg/kg/day dose Groups. AFP-172 was not detected in plasma from the 3 µg/kg/day group except at very low levels and neither AFP-168 nor AFP-172 was detected in plasma from the vehicle control group. Plasma C_{max} values increased in a roughly dose-proportional manner, and $AUC_{(0\text{-}2h)}$ values increased in a slightly greater than dose-proportional manner (Table 67). Plasma $AUC_{(0\text{-}2h)}$ values were similar in Weeks 4 and 13 indicating an absence of plasma accumulation. Plasma T_{max} occurred at 15 minutes after dosing for all samples where it was measureable indicating rapid absorption.

Table 67: Select Toxicokinetic Parameters for AFP-172 Following Subcutaneous Dosing to Rats for 13 Weeks.

Dose (µg/kg/day)	Gender	C_{max} (ng/ml)			T_{max} (min)			$AUC_{(0\text{-}2h)}$ (ng x h/ml)		
		Day 1	Week 4	Week 13	Day 1	Week 4	Week 13	Day 1	Week 4	Week 13
10	Male	----	2.10	2.37	-----	15	15	----	1.19	1.36
	Female	1.55	2.51	2.13	15	15	15	1.04	2.19	1.15
30	Male	2.84	7.30	7.57	5	15	15	1.71	7.07	6.43
	Female	6.87	6.59	6.59	15	15	15	4.76	7.54	5.51

Study title: AFP-168: 13 week subcutaneous administration range-finding study in the mouse

Study no.:	MRL TT #03-5576
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 2, 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AFP-168, Lot # F01X010, purity of 99.5%.

Key Study Findings

Daily subcutaneous administration of AFP-168 to mice for 13 weeks resulted in no toxicity even at the high dose of 100 µg/kg/day. A maximum tolerated dose was not determined. Plasma AFP-168 was generally not detectable at any dose, but AFP-172, the major active metabolite of AFP-168, was measureable in plasma for the 30 and 100 µg/kg/day dose groups. Plasma AFP-172 did not accumulate in plasma and C_{max} and AUC values were similar for both genders. The high-dose AUC value was approximately 8 ng x h/ml).

Study Objective

The objective of this study was to determine the toxicity of AFP-168 following subcutaneous administration for 13 weeks in support of dose selection for a subsequent 78 week carcinogenicity study in mice.

Methods

Crl:CD-1(ICR)BR mice received daily subcutaneous administrations of 0, 3, 10, 30, and 100 µg/kg/day for 13 weeks. Twelve males and females were included in each group in the Main Study and an additional 4 animals/sex in the vehicle control group, 16 animals/sex/group for the 3 and 10 µg/kg/day groups and 24 animals/sex/group for the 30 and 100 µg/kg/day groups were used for toxicokinetic analysis. Mortality, clinical signs, body weight, food consumption, hematology (extensive panel), coagulation parameters (prothrombin time and activated partial thromboplastin time), clinical chemistry (extensive panel), gross pathology, organ weights (extensive panel), and histopathology (extensive panel) were assessed. Also, blood samples were collected for toxicokinetic analysis on Day 1, Week 4, and Week 13 at predose, and 5, 15, 30, 60, 90, and 120 minutes after dosing.

Results

No AFP-168-related clinical signs, gross pathology, histopathology, or changes in body weight, food consumption, hematology, clotting parameters, clinical chemistry, or organ weights were observed.

Neither AFP-168 nor AFP-172 was detected in plasma samples collected from vehicle control groups. In the AFP-168 dose groups, AFP-168 was not detected in plasma

samples except low levels in two samples collected 5 and 30 minutes after dosing. AFP-172 was detected in all of the AFP-168 dose groups, but plasma levels were below the lower limit of quantification in the 3 µg/kg/day group, and only quantifiable in the 10 µg/kg/day group up to 15 minutes after dosing. As shown in Table 68, in the 30 and 100 µg/kg/day groups, plasma C_{max} and AUC values were similar for both genders and increased in a roughly dose-proportional manner. AUC values were similar for the Day 1, Day 28, and Week 13 sample dates indicating an absence of plasma accumulation. Plasma T_{max} values ranged from 5 to 15 minutes after dosing.

Table 68: Select Plasma Toxicokinetic Parameters Associated with Daily Subcutaneous Dosing with AFP-168 for 13 Weeks in Mice. (Sponsor's Table)

Group	Dose (µg/kg/day)	Sex	C_{max} (ng/mL)			T_{max} (min)			$AUC_{(0\text{-}2hrs)}$ (ng.hr/mL)		
			Day 1	Day 28	Week 13	Day 1	Day 28	Week 13	Day 1	Day 28	Week 13
4	30	Male	6.58	6.08	7.08	15	5	15	2.1581	2.3169	2.2265
		Female	7.16	4.03	5.57	5	15	15	2.9545	2.1109	2.1087
5	100	Male	21.06	16.41	13.23	5	15	15	10.5067	10.7874	7.2378
		Female	19.39	12.89	14.13	5	15	5	7.1631	8.4296	5.6342

Study title: A carcinogenicity study of AFP-168 in rats via subcutaneous administration

Study no.:	MRL TT #03-5575
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 10, 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AFP-168, Lot # 037010, purity of 101.5% (HPLC)
CAC concurrence:	No

Key Study Findings

Only minor and/or dose-independent changes were noted for mortality, clinical signs, and food consumption for the AFP-168 treatment groups compared to the vehicle control groups. Body weights were significantly decreased in high-dose (30 µg/kg/day) AFP-168 male and female rats. Select AFP-168-related changes in hematology (decreased red blood cells and increased white blood cells), and organ weights (increased spleen and adrenal weights) were observed. Non-neoplastic histopathology included: AFP-168-related hyperostosis of the sternum and femur in some animals and increased incidence of extramedullary hematopoiesis in the spleen in males in all AFP-168-treated groups. In this study, AFP-168 did not produce biologically significant changes in the incidence of neoplastic lesions.

Adequacy of Carcinogenicity Study

This two-year rat study was adequately conducted in terms of duration, dosing schedule, route of administration, and dose selection. The high dose of AFP-168 in this study provided systemic exposure for the active metabolite, AFP-172, which was > 500 fold greater than the human plasma AFP-172 exposure associated with clinical topical-ocular administration of AFP-168 at the dose intended for marketing. Appropriate observations and assessments were conducted allowing evaluation of general toxicity and AFP-168-related carcinogenicity.

Appropriateness of Test Models

The rat strain used for this study is commonly used for carcinogenicity studies, and two-year rodent models are considered appropriate for carcinogenicity testing.

Evaluation of Tumor Findings

Some significant differences were noted between male animals in one or both of the vehicle control groups and one or more of the AFP-168 treatment groups for the incidence of adenocarcinoma and adenoma in the pars distalis of the pituitary. Also specific mammary gland and uterine tumors in female rats were significantly increased in one or more AFP-168 treatment groups compared to one of the vehicle control groups. In general, the tumor incidence changes did not follow a dose-related pattern, and in some instances the AFP-168 treatment groups exhibited lower tumor incidences compared to the controls. Female mammary adenomas associated with a subcutaneous mass were significantly increased in the AFP-168 high-dose group but only with reference to one of the vehicle-control groups. Also female mammary gland adenomas as a whole were not increased in the AFP-168 high-dose group, and the total of female mammary gland tumors (adenomcarcinoma, adenoma, and fibroadenoma) associated with a subcutaneous mass were not increased for any of the AFP-168 treatment groups compared to either vehicle control group. These comparisons suggest that none of the pituitary gland, mammary gland, or uterine tumor incidence changes were toxicologically significant.

Methods

Doses:	0, 3, 9, and 30 μg/kg for 24 months
Frequency of dosing:	Once per day for 24 months
Dose volume:	3 ml/kg
Route of administration:	subcutaneous
Formulation/Vehicle:	The AFP-168 stock solution was 0.0015% AFP-168 dissolved in the vehicle (isotonic sodium chloride solution).
Basis of dose selection:	The doses were chosen based on a previous 13-week range-finding study. No mortality and only limited toxicity resulted from AFP-168 subcutaneous administration in this study, and a maximum tolerated dose was not determined. However, the plasma exposure for AFP-172 (the major active metabolite of AFP-

	168) associated with the AFP-168 high dose (30 μg/kg/day) in the range-finding study was determined to be much more than 25 fold greater than the human plasma exposure following daily topical ocular dosing with the tafluprost (AFP-168) dose intended for marketing. Based on this data, an AFP-168 high dose of 30 μg/kg/day was chosen for the 24 month carcinogenicity study.
Species/Strain:	Crj:CD(SD)IGS rats
Number/Sex/Group:	Main Study: 60 animals/sex/group
Age:	5 weeks for both genders at initiation of dosing. Main Study males and females weighed 143-199 g and 114-165 g respectively at the initiation of dosing.
Animal housing:	Animals were housed individually in stainless steel wire mesh cages (29W x 22D x 21H cm) on wood chips with a 12 hour/12 hour light/dark cycle.
Paradigm for dietary restriction:	None; animals were allowed free access to autoclaved feed.
Dual control employed:	Yes; two saline groups
Interim sacrifice:	No
Satellite groups:	Toxicokinetic analysis: 12 animals/sex/group
Deviation from study protocol:	Multiple deviations from the protocol occurred in this study, however, none were considered to have affected the evaluation of the study results or the study integrity.

Observations and Results

Mortality

Animals were assessed for morbidity and mortality in conjunction with clinical sign assessments. Survival was calculated as a weekly rate and as the mean survival time. Animals that died during the study were necropsied and examined for gross pathology as soon as possible. Blood samples were obtained when possible from moribund animals, before the animals were euthanized. For both the animals that died and the moribund animals, following necropsy the organs were weighed and histopathology sections were prepared.

Deaths occurred during the dosing period as shown below in Table 69. The number of dead animals and mean survival times for the AFP-168 treatment groups did not differ significantly from the same values for the vehicle control groups (Groups 1 and 2). The Group 4 (9 μg/kg/day; mid-dose) male cumulative survival rate of 25% was significantly lower than the same measurement in the vehicle control group. The difference was not dose-related however, suggesting it was not related to AFP-168 administration.

Table 69: Rat Mortality Parameters for the Two year Rat Cancer Study

Group	AFP-168 Dose (μg/kg/day)	Number of Dead Animals		Cumulative Survival Rate (%)		Mean Survival Time (weeks)	
		Male (n = 60)	Female (n = 60)	Male	Female	Male	Female
Group 1	0 (saline)	34	37	43.3	38.3	93.9	91.2
Group 2	0 (saline)	36	36	40.0	40.0	91.9	92.2
Group 3	3	37	32	38.3	46.7	92.3	91.8
Group 4	9	45	39	25.0*	35.0	85.2	92.7
Group 5	30	38	45	36.7	25.0	86.9	90.0
*Significantly different from the vehicle control Groups 1 and 2							

Clinical Signs

All animals were assessed for clinical signs beginning on Day -3 until the terminal necropsy date. Animals were assessed twice daily (before and after the daily dose) during the dosing period and once daily before initiation of dosing.

A number of clinical signs were observed in study animals; however, no clear relationship with the time of onset, the incidence, or the severity of the clinical signs and AFP-168 administration was evident. Clinical signs included body masses detectable by palpation in males and females in all the study groups including the vehicle control groups. Masses were evident at sites including the face, lower jaw, ear, tail and the cervical, thoracic, abdominal, axillary, inquinal, and perianal/perigenital regions. Primary clinical signs observed in animals that died during the experiment and or moribund animals included: emaciation, prone position, lateral position, decreased movement, irregular respiration, and wryneck. Other clinical signs included decubitus ulcer, loss of fur, rough fur, oozing mass surface, lacrimation and discoloration of the eyes.

Body Weights

All Main Study animals were evaluated for body weight once on Day -3, once a week during the period from Day 1 to Week 13, and once a month during the period from Week 14 until the termination of dosing.

Significantly decreased body weight occurred in the high-dose (30 μg/kg/day) group in males on Day 708 and in females during Days 484-652 compared to vehicle control 1 and in females during Days 484-708 compared to vehicle control 2. At the end of the experiment, mean body weights were decreased approximately 10% for high-dose males compared to vehicle control 1, and approximately 5% for high-dose females compared to vehicle control group 2. These changes were considered to be related to AFP-168 administration. Other changes were not, including significant increases in body weight in males in Group 3 (3 μg/kg/day) on individual days relative to one or the other vehicle control groups. Male and female body weights are shown below in Figure 1 and Figure 2 respectively.

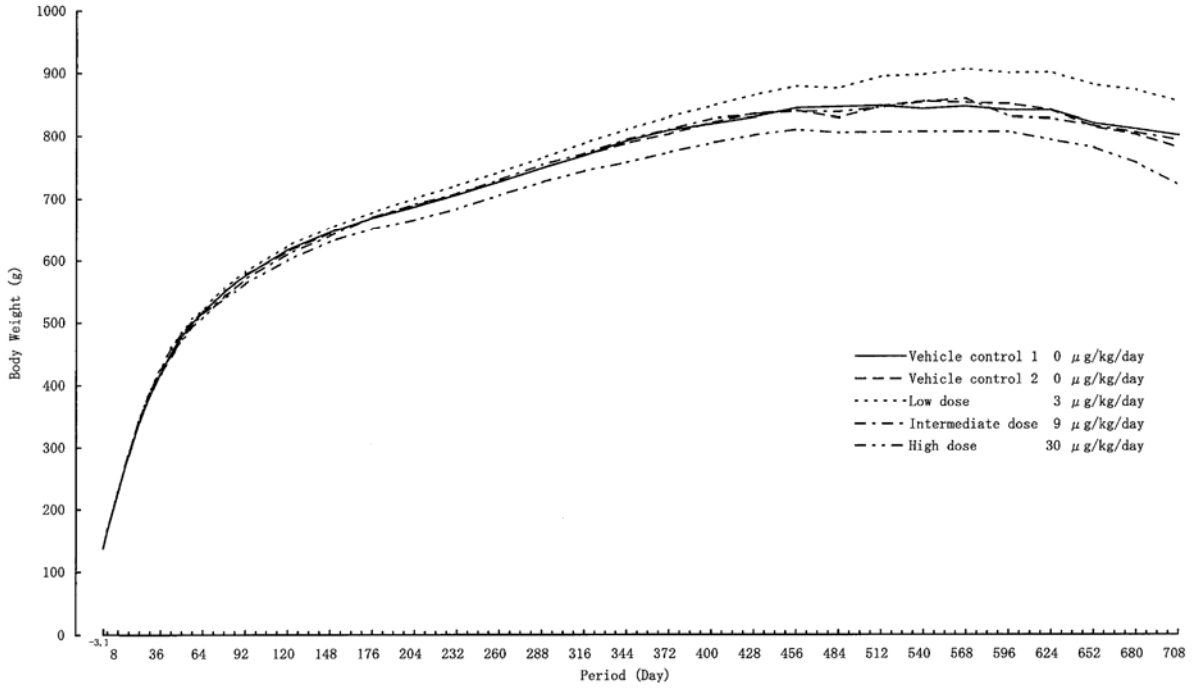


Figure 1: Group Mean Body Weights for Male Rats. (Sponsor's Figure)

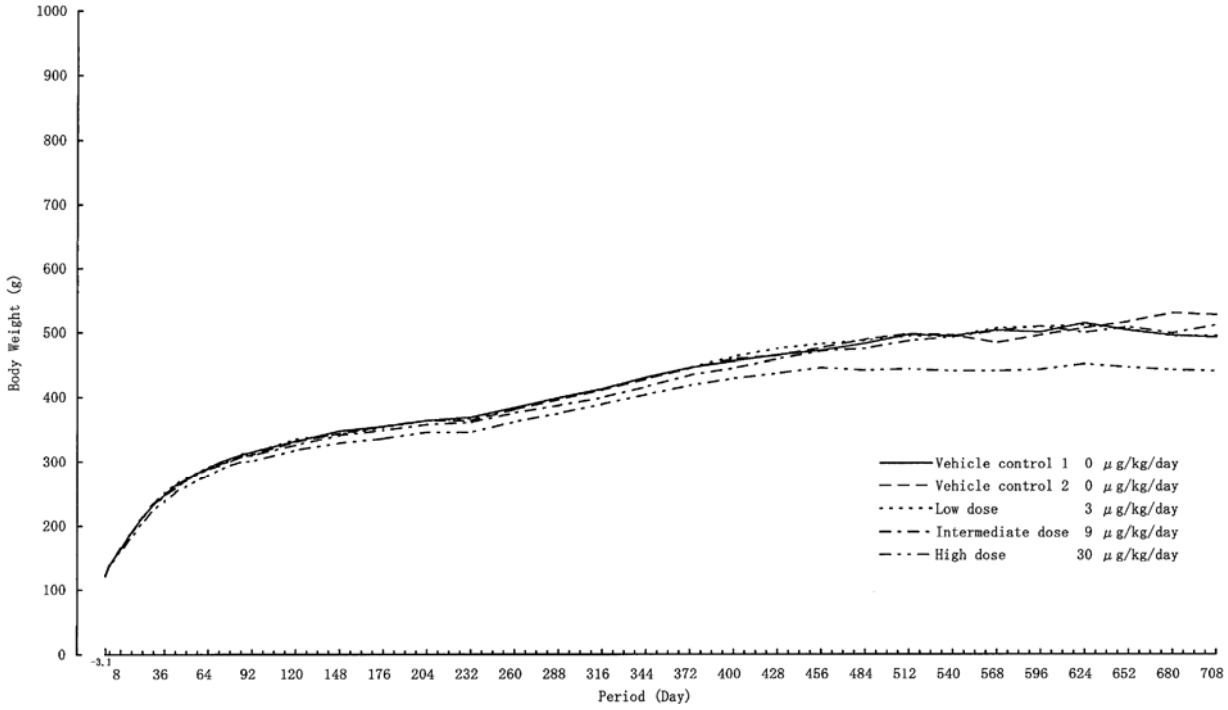


Figure 2: Group Mean Body Weights for Female Rats. (Sponsor's Figure)

Feed Consumption

The food consumption of all Main Study animals was measured once during the period from Day -3 to Day -1, once a week during the period from Day 1 to Week 13, and once a month during the period from Week 14 until the termination of dosing. Two days of cumulative food consumption was measured and mean daily food consumption was calculated.

A number of significant changes in food consumption relative to one or the other vehicle control groups including incidences of both increased and decreased food consumption occurred primarily in males in all of the AFP-168-treatment groups. However, because the changes occurred sporadically and at times comparable changes occurred in the vehicle control groups, the food consumption changes were not considered related to AFP-168 administration.

Hematology

Blood samples were collected from all Main Study animals (fasted for at least 16 hours prior to sampling) one day after the termination of dosing.

Significantly decreased red blood cell counts were noted in males in the 30 μg/kg group compared to the vehicle control groups. However, this change was considered to be related to hyperostosis and an associated decrease bone marrow cavity volume.

Increased white blood cell counts were noted in males in the 30 μg/kg group. This changed was driven by extremely high values in two males suffering from leukemia. However, overall, the incidence of leukemia determined through histopathology was not increased in the 30 μg/kg group compared to the vehicle control groups.

Gross Pathology

All animals were assessed for gross pathology on the termination day (1 day after the last dose). Animals were fasted for 16 hours before termination.

Generally similar incidences of gross lesions were observed in both the vehicle control and treated groups. These lesions included: decubitus ulcers and swelling of the skin, subcutaneous masses, discoloration of the liver, granular surfaces of the kidneys, cysts in the ovaries, thickening of the uterus, enlargement of the pituitary, enlargement and discoloration of the adrenals and impression of the bottom of the cerebrum

A few lesions were observed in the treated groups, but not in the vehicle control groups. These lesions included: nodules in the pancreas and seminal vescicles and masses in bone, cranial cavity, epididymides, ileum, kidneys, preputial glands, seminal vescicles, thymus, and Zymbal's glands. However, the lesions were observed only at low incidences (1 or 2 animals in each group of 34 to 45 surviving animals) and were not considered related to AFP-168 administration.

Organ Weights

Organ weights for the following organs were obtained at necropsy: adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes, and thyroids (with parathyroids). Right and left organs were weighed separately. In addition to

measurement of absolute weights, relative weights (organ to terminal body weight ratios) were calculated.

Absolute and relative spleen weights were increased in males in the 9 and 30 μg/kg groups compared to the vehicle control groups with a significant increase in the 30 μg/kg group. The increased spleen weights were influenced by high values due to leukemia in one male in each of the 9 and 30 μg/kg group and also more severe extramedullary hematopoeisis in males in the 30 μg/kg group.

Also significantly increased absolute and relative adrenal weights were noted in males in the 30 μg/kg group. However, the increased adrenal weights did not correlate with histopathological evidence of adrenal tumors in the 30 μg/kg group or any other AFP-168-treatment group.

The relative but not absolute weights of multiple other organs (kidney liver, lungs, heart, and brain were significantly increased in males and/or females in the AFP-168 high-dose group (30 μg/kg) compared to one or both of the vehicle control group values. These relative organ weight changes were considered attributable to decreased body weight in the high-dose group and not considered to be a direct effect of AFP-168. Other weight changes also thought to be related to decreased body weight in high-dose animals included: decreased absolute brain weight and increased relative brain weight in high-dose males compared to the values for vehicle control 2.

Histopathology

All Main Study animals in the vehicle control 1, vehicle control 2 and high-dose groups were assessed for histopathology. The extensive panel of organs and tissues listed in the Histopathology and Organ Weight Inventory Table were examined. In addition, tissues from moribund and dead animals and the organs and tissues with gross lesions from animals in the low and mid-dose groups were examined. Lesions suspected to be AFP-168-related were observed in the femur, spleen, sternum, uterus, and vagina in animals from the high-dose group, and these organs from animals in the low and mid-dose groups were also examined. Because one of the AFP-168 dose groups (Group 4: 9 μg/kg/day) demonstrated significantly lower cumulative survival for males than the vehicle control groups, Peto's test was used to statistically compare the incidence of neoplastic lesions. None of the AFP-168 dose groups experienced significantly different mortality or survival for females compared to the vehicle control groups and Fisher's exact test was used to compare the incidences of neoplastic lesions in females.
Peer Review
No
Neoplastic

None of the neoplastic lesions in any of the treatment groups were considered related to AFP-168 administration. The most frequently observed tumors in males were adenoma in the anterior lobe of the pituitary and adrenal pheochromocytoma. In females the most frequently observed tumors were adenoma or adenocarcinoma in the anterior lobe of the pituitary followed by mammary gland adenoma, fibroadenoma or adenocarcinoma.

Some statistically significant differences were noted between male animals in one or both of the vehicle control groups and one or more of the AFP-168 treatment groups for the incidence of adenocarcinoma and adenoma in the pars distalis of the pituitary. However, the differences were not considered toxicologically significant because in some instances the treated groups exhibited lower tumor incidences than the controls, and in all cases the differences did not follow a dose-related pattern (Table 70).

Table 70: Pituitary Tumors in Males and Females

Organ	Finding	Vehicle Control 1		Vehicle Control 2		3 μg/kg/day AFP-168		9 μg/kg/day AFP-168		30 μg/kg/day AFP-168	
		NP	P	NP	P	NP	P	NP	P	NP	P
Pituitary	**Males**										
	Adenocarcinoma in pars distalis	58	2	56	4	44	4 *	43	7 **##	59	0 #
	Adenoma in pars distalis	33	27	19	41	29	19	29	21 *	27	32 *
	Adenoma in pars intermedia	60	0	60	0	47	1	50	0	60	0
	Females										
	Adenocarcinoma in pars distalis	39	21	32	28	35	20	34	22	30	30
	Adenoma in pars distalis	33	27	32	28	28	27	24	32	34	26
	Adenoma in pars intermedia	59	1	60	0	54	1	56	0	59	1

Method of statistical analysis = Peto's test for males and Fisher's exact test for females
NP = Not present; P= present

Significantly different from Vehicle Control Group 1: * $p < 0.05$; ** $p < 0.01$
Significantly different from Vehicle Control Group 1: # $p < 0.05$; ## $p < 0.01$

The incidence of mammary adenoma seen as a subcutaneous mass was significantly higher in females in the 30 μg/kg/day group (4/26) compared to vehicle control 2 (0/31), but not vehicle control 1 (1/34) animals. The incidence of mammary adenoma was also significantly higher in females in the 9 μg/kg/day group (6/39) compared to vehicle control 1 (1/60) but not vehicle control 2 (12/60). In contrast, the incidence of adenoma in the mammary glands in females in the 3 μg/kg/day group, and the incidence of mammary fibroadenoma in a subcutaneous mass in females in the 9 and 30 μg/kg/day groups were significantly decreased compared to values for vehicle control 2. Endometrial stromal polyps in the uterus were increased in the 3 and 9 μg/kg/day groups, but not the 30 μg/kg/day group compared to vehicle control 1. The incidence of mammary gland and uterine neoplastic lesions are summarized in Table 71 below.

Generally the mammary gland and uterine tumors that were significantly increased in one or more AFP-168 treatment group were only significantly different with reference to one but not both vehicle control groups, and tumor incidences did not increase in a dose-dependent manner. As noted, female mammary adenomas associated with

subcutaneous mass were significantly increased in the AFP-168 high-dose group but only with reference to one of the vehicle-control groups. Also, all female mammary gland adenomas were not increased in the AFP-168 high-dose group, and the total of female mammary gland tumors (adenocarcinoma, adenoma, and fibroadenoma) associated with subcutaneous mass were not increased for any of the AFP-168 treatment groups. These comparisons suggest that none of the mammary gland or uterine tumor incidence changes were toxicologically significant.

Table 71: Female Mammary Gland and Uterine Neoplastic Lesions

Organ	Finding	Vehicle Control 1	Vehicle Control 2	3 µg/kg/day AFP-168	9 µg/kg/day AFP-168	30 µg/kg/day AFP-168
Mammary Gland	Adenocarcinoma	1/60	0/60	0/32	0/39	2/60
	Adenoma	1/60	12/60	##0/32	*6/39	4/60
	Fibroadenoma	0/60	1/60	0/32	0/39	2/60
	Total	2	13	0	6	8
Sub-cutaneous Mass (mammary gland)	Mammary adenocarcinoma	17/34	15/31	14/36	21/32	12/26
	Mammary adenoma	1/34	0/31	1/36	0/32	# 4/26
	Mammary fibroadenoma	20/34	23/31	24/36	# 14/32	## 10/26
	Total	38	38	39	35	26
Uterus	Endometrial stromal polyp	3/60	6/60	*11/60	*13/60	5/55

Method of statistical analysis = Fisher's exact test

Significantly different from Vehicle Control Group 1: * $p < 0.05$; ** $p < 0.01$

Significantly different from Vehicle Control Group 1: # $p < 0.05$; ## $p < 0.01$

Non Neoplastic

AFP-168 dose-related hyperostosis of the sternum and femur was evident with fibrosis of the bone marrow in some animals. The incidence of extramedullary hematopoiesis in the spleen was increased in males in all AFP-168-treated groups and this change was attributable to a decreased volume of the bone marrow cavity due to hyperostosis (Table 72).

Table 72: Non-Neoplastic Lesions in the Two-Year Rat Carcinogenesis Study. (Sponsor's Table)

Organs /tissues	Findings	Control 1		Control 2		3 µg/kg		9 µg/kg		30 µg/kg	
		M	F	M	F	M	F	M	F	M	F
Sternum	(Number evaluated)	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(60)
	Hyperostosis	1	13	11	16	27	38	50	52	60	57
Femur	(Number evaluated)	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(60)
	Hyperostosis	16	16	16	16	26	35	46	50	58	57
Spleen	(Number evaluated)	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(60)
	Extramedullary hematopoiesis	19	34	21	27	36	26	27	27	38	32

Toxicokinetics

Blood samples were obtained from the toxicokinetic animals in the AFP-168 treatment groups for toxicokinetic analysis on Day 1 and during Weeks 13 and 26 according to the schedule shown below in Table 73. Blood was processed to plasma and plasma concentrations of AFP-168 and AFP-172 were determined using a LC/MS/MS method.

Table 73: Schedule for Toxicokinetic Sampling in the Two-Year Rat Carcinogenesis Study.

Group	AFP-168 Dose (μg/kg/day)	Sampling Period	
		Day 1	Weeks 13 and 26
		Sampling Times	
Group 3	3	5, 15, and 30 minutes after dosing	Immediately prior to dosing, and 5, 15 and 30 minutes after dosing.
Group 4	9	5, 15, and 30 minutes and 1, 1.5 and 2 hours after dosing	Immediately prior to dosing, and 5, 15 and 30 minutes and 1, 1.5 and 2 hours after dosing.
Group 5	30		

AFP-168 was not detectable in any of the plasma samples; however, its active metabolite AFP-172, was detectable as noted below in Table 74. AFP-172 was consistently measureable in plasma from animals administered ≥9 μg/kg/day AFP-168 and less consistently in plasma from the low-dose animals (3 μg/kg/day). Substantial gender differences were not apparent at any time-point. T_{max} values ranged from 5 to 30 minutes. Day 1 C_{max} and AUC values were lower than those for Week 13 samples which were similar to those for Week 26 samples.

Table 74: AFP-172 Toxicokinetic Parameters for Day 1, Week 13, and Week 26 in the Two Year Rat Carcinogenesis Study. (Sponsor's Table)

Sex	Dose	Cmax (ng/mL)			$AUC_{(0\text{-}2h)}$ (ng·h/mL)			Tmax (h)		
	(μg/kg)	Day 1	Week 13	Week 26	Day 1	Week 13	Week 26	Day 1	Week 13	Week 26
Male	3	-	0.953	0.888	-	0.271	0.185	-	0.250	0.250
	9	2.40	3.96	3.79	1.20	2.83	3.12	0.083	0.250	0.250
	30	7.24	13.4	12.5	5.74	11.2	13.7	0.250	0.083	0.250
Female	3	0.373	-	0.573	0.0466	-	0.0716	0.083	-	0.083
	9	2.80	3.33	2.50	1.42	3.21	2.96	0.250	0.500	0.250
	30	8.08	11.1	8.03	5.84	13.1	10.1	0.083	0.083	0.083

-: Insufficient data to calculate

Dosing Solution Analysis

AFP-168 stock solutions were used within 2 days of preparation. AFP-168 dosing solutions were prepared at least once weekly and used within 11 days of preparation. AFP-168 stock solutions were assessed for AFP-168 concentration at each preparation. AFP-168 dosing solutions were assessed for the same parameters at the first preparation, 3, 6, 9, 12, 15, 18, and 21 months after initiation of dosing for males and at the last preparation for females.

The AFP-168 0.0015% stock solution was confirmed to be stable for 6 weeks at room temperature. In addition, the 0.00001% and 0.0015% solutions were confirmed to be stable for 3 weeks in a cold place (5°C) and at 30°C. The 0.0001% and 0.001% AFP-168 solutions were confirmed to be homogeneous for up to (b) (4) . The actual concentrations of the 0.0015% stock solution were (b) (4) of the nominal concentration. The actual concentrations of the 0.001%, 0.0003% and 0.0001% dosing formulations were (b) (4) of the nominal concentrations. Dosing formulation osmolarity ranged from 97 to 100 % of physiological osmolarity, and pH values ranged from 5.68 to 6.61.

Study title: AFP-168: 78 Week Subcutaneous Administration Oncogenicity Study in the Mouse

Study no.:	MRL TT #04-5572
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 15, 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AFP-168, Lot # F01X010, Measured purity of 100.7% (HPLC)
CAC concurrence:	No

Key Study Findings

Daily subcutaneous administration of AFP-168 (10, 30, and 100 µg/kg/day) for 78 weeks to mice did not result in appreciable compound-related toxicity. No AFP-168-related changes were noted for mortality, body weights, clinical signs, hematology parameters, gross pathology, and non-neoplastic histopathology. In addition, incidence rates for neoplastic lesions were not significantly increased for the high-dose AFP-168 treatment group compared to the vehicle control groups.

Adequacy of Carcinogenicity Study

This mouse carcinogenicity study was of 78 weeks duration instead of the normal duration of two years. During this time, all groups experienced approximately 24 to 39% mortality with no significant differences between groups. This study is also limited by incomplete knowledge regarding mouse metabolism of AFP-168. The primary metabolite, AFP-172 was measured in mice, but it is unknown if additional mouse metabolites encompass the full range of human metabolites including the high percentage human metabolite, dinor-AFP-172.

Appropriateness of Test Models

The Crl:CD-1(ICR)BR strain of mice used in this study is commonly used in carcinogenicity studies and long-term mouse models are appropriate for carcinogenicity testing. However, as mentioned above, the study duration was 78 weeks instead of the usual two years.

Evaluation of Tumor Findings

The range of neoplasms in this study were generally consistent with those expected in aging mice of the Crl:CD-1(ICR)BR strain. In the AFP-168-treatment groups, no unusual tumors or significantly increased tumor incidence suggestive of AFP-168-related carcinogenesis was observed compared to the vehicle control groups.

Methods

Doses:	0, 10, 30, and 100 μg/kg/day
Frequency of dosing:	Single daily injections
Dose volume:	10 ml/kg
Route of administration:	subcutaneous
Formulation/Vehicle:	The stock AFP-168 solution was 0.0015% AFP-168 dissolved in the vehicle (0.9% saline).
Basis of dose selection:	The high dose (100 μg/kg/day) was chosen as a dose that provided an AUC exposure of over 25 fold higher than the exposures resulting from the clinical ocular dose in humans.
Species/Strain:	Crl:CD-1(ICR)BR strain
Number/Sex/Group:	Main Study: 51 mice/sex/group
Age:	Animals were approximately 7 weeks old at the time of dosing. At randomization Main Study males weighted 28.1 to 42.6 g and Main Study females weighed between 20.9 and 35.1 g.
Animal housing:	The mice were housed in groups of 3 (Main Study) or 2 (Toxicokinetic Study) in M2 cages measuring 330x150x130 mm. Animals received food and water *ad libitum* and cage bedding were clean Aspen wood chips.
Paradigm for dietary restriction:	none
Dual control employed:	Two vehicle control groups were included in the study
Interim sacrifice:	none
Satellite groups:	Mice designated as toxicokinetic animals included 18 animals/sex/group were in Group 3 (10 μg/kg/day) and 26 animals/sex/group for Groups 4 and 5 (30 and 100 μg/kg/group respectively)
Deviation from study protocol:	Multiple deviations from the study protocol were noted but the deviations were not considered to have altered the study results or compromised the integrity of the study.

Table 75: Study Design for the 78-Week Mouse Carcinogenicity Study. (Sponsor's Table)

Group Number	Description	Dose level (µg/kg/day)	Dose concentration (%)	Number of animals in group			
				Main study		Satellite study #	
				Male	Female	Male	Female
1	Control 1	0	0	51	51	-	-
2	Control 2	0	0	51	51	-	-
3	Low	10	0.0001	51	51	18	18
4	Intermediate	30	0.0003	51	51	26	26
5	High	100	0.001	51	51	26	26

for toxicokinetic investigations only; no other experimental observation data from these animals were reported.

Observations and Results

Mortality

All animals were observed at the beginning and end of the working day to ensure the animals were in good health. Moribund animals assigned to the Main Study were sacrificed and underwent full necropsy.

Four male animals (three from Group 5 and one from Group 2) were replaced with substitute animals during the first four weeks of treatment. Subsequently from Week 5 on, a total of 79 males and 87 females in the Main Study and 3 male and 2 female toxicokinetic animals died or were sacrificed. The distribution of animal deaths between groups did not differ significantly for either gender as shown in Table 76 below. Thus no AFP-168-dependent effects on mortality were observed.

Table 76: Mortality in the 78-Week Mouse Carcinogenicity Study. (Sponsor's Table).

Group	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Dosage (µg/kg/day)	0	0	10	30	100	0	0	10	30	100
Main study										
Group size:	51	51	51	51	51	51	51	51	51	51
Decedents (Week 78)	20	12	16	19	12	19	19	16	14	19
% Survival	61	76	69	63	76	63	63	69	73	63
Satellite study										
Group size:	-	-	18	26	26	-	-	18	26	26
Decedents (Week 25)	-	-	1	1	1	-	-	1	1	0

Clinical Signs

All animals were observed daily for signs of ill health or overt toxicity. In addition, each animal was given a detailed physical examination at weekly intervals including palpation for tissue masses.

Common clinical signs including hair loss, fur staining, fur thinning, swollen abdomen, and minor sores were observed in mice from all groups, but no AFP-168-dependent

effects on clinical signs were observed. APF-168 also had no apparent effect on the incidence or size of palpable masses.

Body Weights

Individual body weights were recorded pre-treatment, before treatment on the first day of dosing, at weekly intervals for the first 14 weeks, once every four weeks thereafter and before the terminal sacrifice.

Compared to the control groups, male mice in Group 3 (10 μg/kg/day) gained approximately 10% less weight, but males in Group 4 (30 μg/kg/day) and Group 5 (100 μg/kg/day) gained up to 5% more weight than control animals indicating an absence of AFP-168- related effects (Figure 3).

Test Article	Control		AFP-168		
Group	1	2	3	4	5
Level (ug/kg/day)	0	0	10	30	100

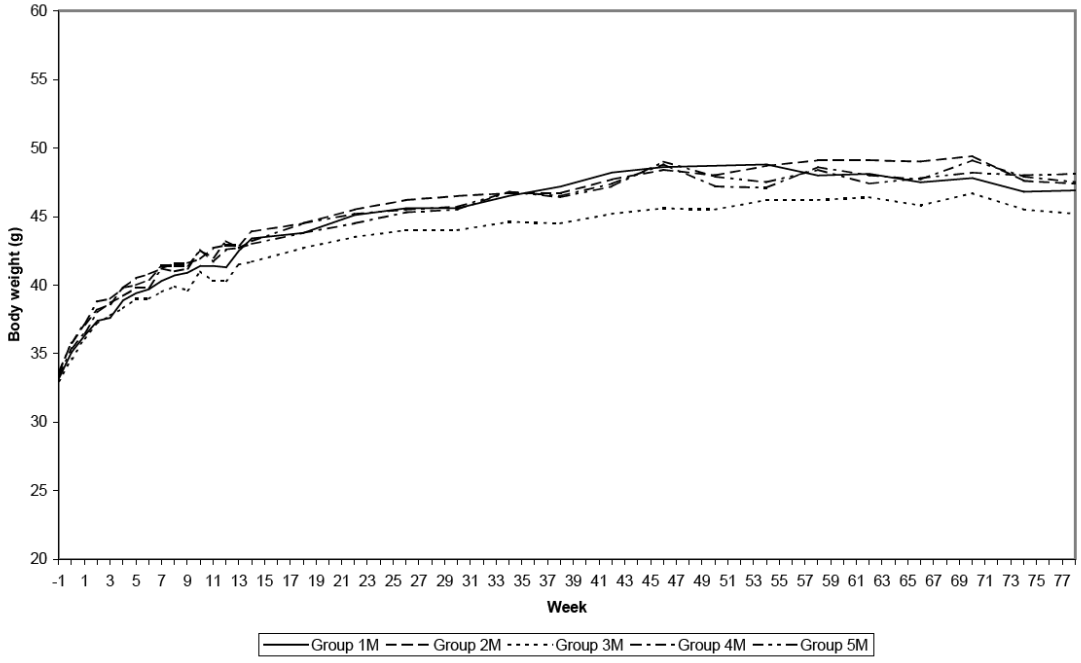


Figure 3: Male Group Mean Body Weights. (Sponsor's Figure)

Group 3 and Group 5 females receiving 10 or 100 μg/kg/day respectively gained 17 and 23% less weight respectively. However, female mice in Group 4 (30 μg/kg/day) gained about the same amount of weight as control animals suggesting changes were not related to AFP-168 administration (Figure 4).

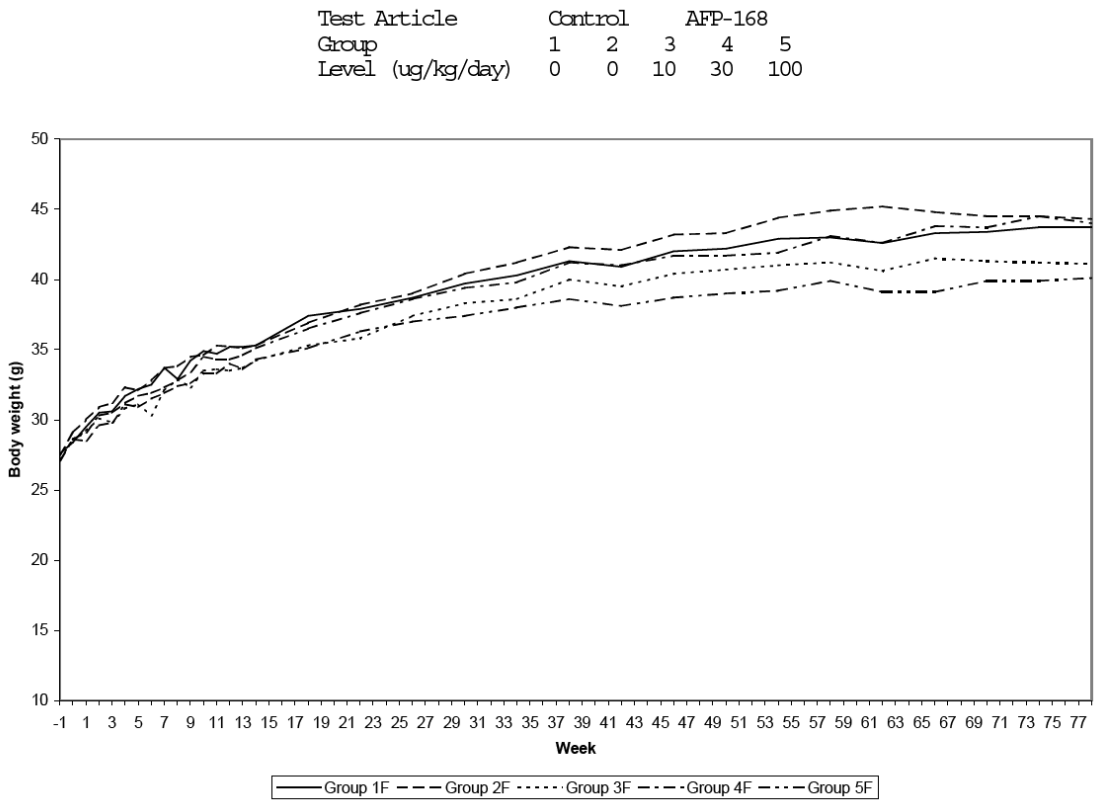


Figure 4: Female Group Mean Body Weights. (Sponsor's Figure)

Feed Consumption

Food consumption measured on a per cage basis was determined weekly for the first 14 weeks, then one week in every 4 weeks thereafter. Based on the per cage measurements, consumption was calculated as food g/animal/week.

Animals in all of the groups consumed approximately the same amount of food.

Hematology

Blood samples were collected for hematology analysis at the terminal sacrifice from unfasted animals. Red cell counts and total and differential white cell counts were assessed.

No AFP-168-related changes in red cell counts or total or differential white cell counts were observed.

Gross Pathology

All surviving Main Study animals were examined for gross pathology immediately following the terminal sacrifice. Animals found dead during the study and moribund animals sacrificed before the terminal sacrifice date were also necropsied and examined for gross pathology.

The gross pathology findings were generally consistent with those commonly found in aging mice. No gross pathology findings were considered AFP-168-related.

Histopathology

The full battery of organs and tissues listed in the Histopathology and Organ Weight Inventory Table from all (decedents and surviving animals) of the Main Study vehicle control (Groups 1 and 2) and high-dose animals (Group 5) were examined microscopically for histopathology. In addition, the same tissues and organs from the surviving animals in the low- (10 μg/kg/day) and mid- (30 μg/kg/day) dose groups were examined for histopathology. Tumor data was statistically evaluated using Peto analysis.
Peer Review
No
Neoplastic
The range of neoplasms in this study was generally consistent with those expected in aging mice of this strain. In the high-dose AFP-168-treatment group, no unusual tumors or significantly increased tumor incidence suggestive of AFP-168-related carcinogenesis was observed compared to the vehicle control groups. The tumor incidence for males and females are summarized in Table 77 and Table 78.

Table 77: Tumor Incidence in Male Mice

Tissue Code	Tumor type	Control Group #1	Control Group #2	10 μg/kg/day	30 μg/kg/day	100 μg/kg/day	P-value*
AD	Benign Subcapsular Cell Adenoma	5	1	1	1	2	0.778
HE	Lymphoid Tumor	3	2	3	6	3	0.541
LI	Hepatocellular Tumor	8	9	9	5	8	0.670
LU	Alveolar Epithelial Tumor	3	4	8	9	6	0.275
#	Skin/Appendage Fibroblastic Tumor	3	1	2	2	1	0.859

* statistical comparison of the mean control values versus the high-dose (100 μg/kg/day) group.
AD = Adrenal; HE =Hemolymphoreticular; LI =Liver; LU = Lung, # = combined tissues

Table 78: Tumor Incidence in Female Mice

Tissue Code	Tumor type	Control Group #1	Control Group #2	10 µg/kg/day	30 µg/kg/day	100 µg/kg/day	P-value*
MA	Malignant adenocarcinoma	1	4	3	2	3	0.507
HE	Lymphoid tumor	9	9	3	2	8	0.676
LU	Alveolar epithelial tumor	3	5	3	4	2	0.905
OV	Sex cord/stromal tumor	2	2	0	3	1	0.876
UT	Smooth muscle tumor	2	2	0	3	0	1.00
UT	Stomal tumor	5	0	5	1	5	0.217

* statistical comparison of the mean control values versus the high-dose (100 µg/kg/day) group.
MA = mammary gland; HE =Hemolymphoreticular; LU = Lung; OV = Ovary , UT = Uterus

Non Neoplastic
The spectrum of non-neoplastic microscopic lesions was generally consistent with that expected for mice of this strain. All of the non-neoplastic lesions occurring in the AFP-168 treatment groups also occurred in the vehicle control groups. There were no findings of an unusual nature or incidence suggestive of toxicity due to AFP-168.

Toxicokinetics

Blood samples were obtained by orbital sinus puncture under halothane anesthesia and processed to plasma on Day 1, and in Weeks 13 and 26 according to the timing shown in Table 79 below. Plasma AFP-168 and AFP-172 concentrations were measured using a LC/MS/MS method.

Table 79: Toxicokinetic Collection Schedule for the 78-Week Mouse Carcinogenicity Study

Group	Sample Time (minutes post-dose)					
	Pre-dose*	5 minutes	15 minutes	30 minutes	60 minutes	120 minutes
Group 3	4 males 4 females	4 males 4 females	4 males 4 females	4 males 4 females	----	----
Group 4	4 males 4 females	4 males 4 females	4 males 4 females	4 males 4 females	4 males 4 females	4 males 4 females
Group 5	4 males 4 females	4 males 4 females	4 males 4 females	4 males 4 females	4 males 4 females	4 males 4 females

* Predose samples were collected during Weeks 13 and 26 only

AFP-168 was not detected above the lower limit of quantification at any of the sampling time points. AFP-172, the major active metabolite of AFP-168, was detectable in plasma samples and selected toxicokinetic parameters for plasma AFP-172 are summarized below in Table 80. No major gender differences were apparent for the AFP-172 plasma C_{max} and $AUC_{(0-t)}$ values, and these values increased in a relatively dose-proportional

manner. Plasma T_{max} values varied from 0.083 to 0.25 hours indicating rapid absorption following subcutaneous administration.

Table 80: Selected Toxicokinetic Values for Plasma AFP-172 Associated with Subcutaneous AFP-168 Dosing in the Mouse Carcinogenicity Study. (Sponsor's Table).

Dose (µg/kg/day)	Sex (n=3)	C_{max} (ng/mL)			$AUC_{(0-t)}$ (ng.h/mL)			T_{max} (hour)		
		Day 1	Week 13	Week 26	Day 1	Week 13	Week 26	Day 1	Week 13	Week 26
10	Male	2.72	1.26	2.02	0.6032	0.5219	0.6741	0.083	0.25	0.083
	Female	3.84	0.97	1.45	1.0300	0.4194	0.5333	0.083	0.25	0.25
30	Male	7.48	4.67	4.86	2.6844	2.9764	2.2587	0.083	0.25	0.083
	Female	4.48	3.04	5.21	2.4163	2.3540	2.8868	0.083	0.25	0.083
100	Male	26.17	12.03	18.71	14.941	10.073	9.8043	0.083	0.25	0.083
	Female	17.95	13.51	15.76	8.9279	10.644	9.8201	0.25	0.25	0.083

Dosing Solution Analysis

All AFP-168 stock solutions were analyzed to determine the actual concentrations. The dosing solutions prepared for Day 1 and one dose each in Weeks 13, 26, 38, 51, 64, and 77 were analyzed to determine the achieved concentration. On the same days, osmotic pressure and pH were also measured for the dosing solutions.

Solutions were shown to be homogenous. The actual concentrations of the stock solutions were consistently 90-110% of the nominal concentrations. The dilution concentrations were consistently 100 to 130% of the nominal concentrations with the exception of the dosing solution for Group 5 in Week 64 which was 99% of the nominal concentration. The osmotic pressures for each tested dosing solution all fell within the range of 90% to 110% of the osmotic pressure of physiological saline, and dosing solution pH values ranged from 4.81 to 5.94.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

The reproductive and developmental toxicology associated with tafluprost administration was assessed in a fertility study in rats (Study Report No.: TT #01-5527), embryo fetal development studies in rats (Study Report No.: TT #01-5529) and rabbits (Study Report Nos.: TT #01-5528; TT #02-5551), and a prenatal and postnatal development study in rats (Study Report No.: TT #04-5573). These studies are reviewed below. In order to obtain data supporting the choice of the high doses for the definitive embryo fetal studies, range-finding studies were performed in pregnant rats (Study Report No.: TT

#01-5525) and rabbits (Study Report No.: TT #01-5525) and these studies are summarized in Table 82 below. In addition, a range-finding, prenatal and postnatal development study was first conducted in rats, and this study is summarized in Table 92 below.

Study title: AFP-168: Intravenous Study of Fertility and Early Embryonic Development in the Rat

Study report no.:	MRL TT #01-5527
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 18, 2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AFP-168, Batch # FP-0002, purity of 98.6%.

Key Study Findings

Male and female rats received intravenous doses of 0, 10, 30, and 100 µg/kg/day AFP-168 for two weeks before pairing, throughout the pairing period, and until Day 6 of gestation for the females or until necropsy in Week 9 of the treatment period for the males. No AFP-168 related effects on mating behavior were observed. The mating index was 100% for all treatment and control groups. The fertility and fecundity indexes were 100%, 91.7%, 91.7%, and 100% for both males and females in Groups 1, 2, 3, and 4 respectively. The regularity of the estrus cycle was unaffected by treatment. Also there were no effects on the outcome of pregnancy and all pregnant females had live embryos. The mean number of corpora lutea and the mean number of implantations per female were similar to the controls in all groups. The mean pre- and post-implantation loss in the treated animals was similar to or less than that of the controls.
Testis staging did not indicate any AFP-168-related abnormalities in any of the cell types present within the different stages of the spermatogenic cycle. The proportions of tubules at specific points in the cycle were consistent with the expected range.

Methods

Doses:	0, 10, 30, and 100 µg/kg/day
Frequency of dosing:	Once per day
Dose volume:	10 ml/kg
Route of administration:	Intravenous at a rate of ≈ 2 ml/minute
Formulation/Vehicle:	0.9% sodium chloride
Species/Strain:	Crl:CD(SD)IGSBR rats
Number/Sex/Group:	24/sex/group
Satellite groups:	none
Study design:	See Table 81 below. Male and female rats received intravenous doses of 0, 10, 30, and 100 µg/kg/day AFP-168 for two weeks before pairing, throughout the pairing period, and until

	Day 6 of gestation for the females or until necropsy in Week 9 of the treatment period for the males.
Deviation from study protocol:	Multiple deviations from the study protocol were noted but the deviations were not considered to have altered the study results or compromised the integrity of the study.

Table 81: Study Design for the Rat Fertility Study. (Sponsor's Table)

Group number	Group description	Dose level (µg/kg/day)	Dose concentration (%)	Dose volume (mL/kg)	Number of animals	
					Male	Female
1	Control	0	0	10	24	24
2	Low	10	0.0001	10	24	24
3	Intermediate	30	0.0003	10	24	24
4	High	100	0.001	10	24	24

Observations and Results

Mortality

Morbidity and mortality were assessed twice daily.

Two high-dose males were found dead on Treatment Days 10 and 15 respectively. These animals were found dead within one hour of dosing. Cause of death was uncertain, the animals appeared unremarkable at necropsy. No reproductive organ histopathology was observed.

Clinical Signs

All animals were examined daily for signs of ill health or reaction to treatment.

AFP-168-related clinical signs included pale extremities immediately after treatment in all AFP-168-treated animals. This effect was observed daily from the second day of treatment in the high-dose group and from the third day of treatment for the 10 and 30 µg/kg/day groups. The effect was transitory, disappearing after one hour, and appeared to be of approximately equal severity in all treatment groups.

Body Weight

Male body weights were recorded before treatment on the first day of dosing and at twice weekly intervals thereafter. Female body weights were recorded twice weekly from the start of estrous cycle monitoring until confirmation of mating, then daily from Day 0 to Day 13.

There were no AFP-168-related effects on body weight.

Feed Consumption

The food intake of each cage of animals was determined twice weekly during the pre-pairing periods. In addition, the individual food intake of mated females was recorded for Days 0 to 3, 3 to 6, 6 to 10, and 10 to 13 of gestation.

Food intake was not affected by AFP-168 treatment.

Toxicokinetics: Not performed

Dosing Solution Analysis

Thirteen 0.0015% stock solutions were analyzed to determine the achieved concentration. The dosing solutions were examined for achieved concentration, osmotic pressure, and pH on the first day and during the last week of treatment in each group. The target range for the stock solutions was 90-110% of the nominal concentration and the target range for the dosing solutions was 100-130%.

In a previous analysis, the Sponsor showed that a 0.0015% stock solution of AFP-168 in saline was stable for 6-weeks at room temperature and that 0.00001% to 0.0015% solutions were stable for 3 weeks at 5°C and 30°C. Two stock solutions out of the 13 were found to be outside the target range. One of these was discarded; the other which was only 1% outside the target range was used because a replacement was not available. The achieved concentration of the dosing formulations were within the target range with the exception of the 0.0001% formulation which was 1% above the target range on the first day of treatment. The pH values of the dosing solutions ranged from 4.90 to 6.30 and the osmotic pressures ranged from 98 to 100% of the osmotic pressure of saline.

Estrus cycle and Mating Data

The stage of estrous (assessed via daily vaginal washings) was recorded for each female from 15 days prior to treatment until mating confirmation. During the mating procedure, one male was housed with one female. Mating was confirmed by the presence of a vaginal plug in situ or sperm in the vaginal washing. The day on which mating was confirmed was designated Day 0 of gestation.

The regularity of the estrus cycle was unaffected by treatment. With the exception of one control pairing, all males mated within the initial four days of pairing.

Necropsy

Mated females were sacrificed on Day 13 of gestation and examined macroscopically. The ovaries and uteri were removed and examined. The following data was recorded: pregnancy status, number of corpora lutea, number and intrauterine position of implantations with recording of live embryos, early intrauterine deaths, and late intrauterine deaths. Early intrauterine deaths were classified as dead embryos composed of decidual or placental tissue only. Late intrauterine deaths were dead embryos composed of embryonic tissue in addition to placental tissue.

Males were sacrificed on the same days as the females and examined macroscopically for structural or pathological changes.

The following tissues from all adult animals were retained as appropriate for each gender: ovaries, uterus, cervix, vagina, pituitary, testes, epididymides, seminal vesicles, prostate, coagulation gland, lesions. The reproductive organs from all control and high-dose animals were examined for histopathology.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.): No AFP-168 related effects on mating behavior were observed. The mating index was 100% for all treatment and control groups. The fertility and fecundity indexes were 100%, 91.7%, 91.7%, and 100% for both males and females in Groups 1, 2, 3, and 4 respectively.

On Day 13 of gestation, there were no effects on the outcome of pregnancy and all pregnant females had live embryos. The mean number of corpora lutea and the mean number of implantations per female were similar to the controls in all groups. The mean pre- and post-implantation loss in the treated animals was similar to or less than that of the controls.

Histopathology: No AFP-168-related histopathology in the reproductive organs of AFP-168-treated animals was observed.

Testes staging: Testis staging did not indicate any AFP-168-related abnormalities in any of the cell types present within the different stages of the spermatogenic cycle. The proportions of tubules at specific points in the cycle were consistent with the expected range.

9.2 Embryonic Fetal Development

Table 82: Non-Pivotal Range-Finding Embryo-Fetal Development Studies

Study No./ Species/ type of study	Species/ Route/Dose/ Number per group	Results
MRL TT #01-5525/ Range-finding, embryo-fetal study in rats	Rat/ Intravenous/ 0, 10, 30, and 100 μg/kg/day/ 7 pre-mated females/group	1. There were no deaths. Pale extremities were seen immediately after dosing in intermediate and high-dose animals. 2. There was a dose-dependent increase in the incidence of post-implantation loss in the intermediate (12.1%) and high-dose (49.6%) groups compared to controls (6.0%) with two high-dose animals having litters with no live fetuses. 3. There were no dose-related fetal malformations. 4. On the basis of this study, 30 μg/kg/day was chosen as the high dose for the definitive rat embryo-fetal study.
MRL TT #01-5523/ Range-finding, embryo-fetal study in rabbits	Rabbit/ intravenous/ 0, 1, 3, and 10 μg/kg/day/ 7 pre-mated females /group	1. Clinical signs including tremors, splayed legs and excessive licking were observed immediately after dosing in the high-dose group. 2. All control females had litters with viable fetuses. Two low-dose females had only intrauterine deaths with no live fetuses, and no live fetuses occurred for all the intermediate- or high-dose females. 3. No maternal NOAEL was determined for this study.

Study title: AFP-168: Intravenous Study of Embryo-Foetal Development in the rat.

Study no.:	MRL TT #01-5529
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 31, 2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AFP-168, batch # FP-0002, Purity of 98.6%.

Key Study Findings

Intravenous administration of AFP-168 to pre-mated female rats from Gestation Days 6 to 17 at doses of 0, 3, 10, and 30 µg/kg/day produced no maternal toxicity. AFP-168 produced dose-dependent teratogenicity. The 30 µg/kg/day dose of AFP-168 produced an increased number of intrauterine deaths, reduced fetal maturity, and a low incidence of defects of the vertebral column. Similar events, but of lower frequency and severity occurred with the intermediate 10 µg/kg/day dose, but no effects occurred at the low dose (3 µg/kg/day).

Methods

Doses:	0, 3, 10, and 30 µg/kg/day
Frequency of dosing:	Once per day
Dose volume:	10 ml/kg
Route of administration:	intravenous
Formulation/Vehicle:	0.9% sodium chloride
Species/Strain:	Crl:CD(SD)IGSBR rats
Number/Sex/Group:	24 females/group
Satellite groups:	none
Study design:	See Table 83 below. Premated female rats were treated intravenously with 0, 3, 10, and 30 µg/kg/day AFP-168 from Gestation Days (GD) 6 to 17. All animals were euthanized on GD 20 and pregnancy and fetal outcome parameters were assessed.
Deviation from study protocol:	Multiple deviations from the study protocol were noted but the deviations were not considered to have altered the study results or compromised the integrity of the study.

Table 83: Study Design for the Rat Embryo-Fetal Study. (Sponsor's Table)

Group number	Group description	Dose level (μg/kg/day)	Dose concentration (%)	Dose volume (mL/kg)	Number of females
1	Control	0	0	10	24
2	Low	3	0.00003	10	24
3	Intermediate	10	0.0001	10	24
4	High	30	0.0003	10	24

Observations and Results

Mortality

All animals were examined twice daily for morbidity and mortality.

One female receiving 10 μg/kg/day AFP-168 died during blood sampling procedures on GD 17, but this death was considered unrelated to AFP-168 administration.

Clinical Signs

All animals were examined at least once daily for signs of ill health or overt toxicity from Gestation Day (GD) 4 to GD 20. In addition, animals were examined immediately after dosing and one hour after dosing for signs of reaction to treatment.

No clinical signs considered to be related to AFP-168 administration were observed.

Body Weight

The body weight of each female was recorded on GD 4 and daily from GD 6 to GD 20.

Mean body weights did not differ between groups.

Feed Consumption

Individual food consumption was recorded on the same days as the body weight recordings.

There were no apparent effects on food intake.

Toxicokinetics

Blood samples were obtained and processed to plasma for toxicokinetic analysis on Days 6 and 17 of gestation. On both of these days, blood samples from three animals in each group were obtained predose, and 1, 5, 15, 30, 60, and 120 minutes after dosing. Plasma samples were analyzed for AFP-168 and AFP-172 using a validated LC/MS/MS method.

Neither AFP-168 nor AFP-172 was detectable in plasma obtained from control females. Also AFP-168 plasma levels above the lower limit of quantification were detectable only in a few animals in the AFP-168-dose groups. Plasma AFP-172 was generally

detectable for AFP-168-treatment animals, but only above the lower limit of quantification for 1 to 15 minutes after dosing. Consequently plasma AUC exposure values could only be consistently calculated for Group 4 (Table 84). Plasma C_{max} increased in a dose-dependent manner and appeared to increase with repeated-dosing. The T_{max} value for all the treatment groups was 1 minute after dosing.

Table 84: Toxicokinetics Parameters for AFP-172 Following AFP-168 Administration to Pregnant Rats. (Sponsor's Table)

Group	Dose (µg/kg/day)	AUC (0-120min) (ng.min/mL)		Mean C_{max} (ng/mL) (range)		Mean T_{max} (min)	
		Day 6	Day 17	Day 6	Day 17	Day 6	Day 17
2	3	-	-	6.67 (5.90 - 7.85)	9.13 (6.39 -11.42)	1	1
3	10	-	327.05	23.35 (14.39 - 30.50)	62.36 (20.34 - 104.37)	1	1
4	30	217.23	335.42	46.69 (35.12 - 57.56)	136.86 (74.53 - 168.58)	1	1

- unable to calculate AUC value, due to insufficient data

Dosing Solution Analysis

Two stock solutions of 0.0015% concentration were prepared daily and 0.00003%, 0.0001%, and 0.0003% formulations were prepared from the stock solutions. The stock solutions were analyzed for concentration on Day 1. The dosing formulations were analyzed for achieved concentration, osmotic pressure, and pH in the first and last weeks of treatment in each group. Solutions were sampled on the first and last days of treatment and an additional analysis was conducted on Day 4 of treatment.

Previously the Sponsor showed that AFP-168 solutions of 0.0015% were stable for 6-weeks at room temperature. The 0.0001% and 0.0015% solutions were shown to be stable for 3 weeks at 5°C and 30°C. The two stock solutions had actual concentrations that were (b) (4) of the nominal concentrations on Day 1. The actual concentrations of the AFP-168 dosing solutions were as shown in Table 85 below. The actual concentrations generally fell within the target ranges of 90-110% and 100-130% of the nominal concentrations for the stock solutions and dosing formulations respectively.

Table 85: Actual Concentrations of the AFP-168 Formulations. (Sponsor's Table)

	Results as % nominal		
Day	0.00003%	0.0001%	0.0003%
1 4 16	(b) (4)		

The pH of the AFP-168 formulations fell within the range of 5.04 to 5.17 and the osmotic pressure of the formulations was consistently within the target range of 90 to 110% of the osmotic pressure of saline.

Necropsy

Surviving females were sacrificed on Day 20 after confirmation of mating, and examined for gross pathology.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

The ovaries and uteri of each female were removed and the following parameters were assessed: pregnancy status, gravid uterus weight, and the number of corpora lutea. Also the number and intrauterine position of implantations were assessed and categorized as live fetuses, early intrauterine deaths, late intrauterine deaths, and dead fetuses. Early intrauterine deaths were classified as those with decidual or placental tissue, and late intrauterine deaths were classified as those with fetal tissue in addition to placental tissue. Fully formed fetuses that appeared to have died shortly before necropsy were classified as dead fetuses.

The 20% mean post-implantation loss was greater in the high-dose group compared to the 8.6% loss in the vehicle control group. Two high-dose females had total litter loss as did one female in the vehicle control group due mainly to late intrauterine deaths for all three females. High-dose females also demonstrated an increased number of early and late intrauterine deaths, increased post-implantation loss, and decreased mean numbers of fetuses compared to control females. However, the differences were not statistically significant except for the number of high-dose dams having late intrauterine deaths (Table 86). The mean number of corpora lutea and the mean number of implantations were similar to those of the controls for all the AFP-168-treatment groups.

Table 86: Maternal Caesarean Data

Test article	Control	AFP-168		
Group	1	2	3	4
Level (µg/kg/day)	0	3	10	30

6.1.1 Uterine/implantation data - all surviving pregnant females including total embryo/foetal loss

	Group 1	Group 2	Group 3	Group 4	Statistics
Number of surviving pregnant females	23	23	18	24	
Number of females with total embryo/foetal loss	1	0	0	2	
Number of females with live foetuses on Day 20 gestation	22	23	18	22	
Mean number of corpora lutea per female	14.9	13.6	14.2	13.6	J
Mean number of implantations per female	11.7	12.1	13.2	11.8	J
Pre-implantation loss:					
mean%	18.6	11.4	7.0	13.0	
number of dams affected	15	13	7	14	F+
Early intrauterine deaths:					
mean number	0.6	0.4	0.4	1.1	
number of dams affected	9	7	6	11	F+
Late intrauterine deaths:					
mean number	0.0	0.0	0.0	0.5	
number of dams affected	0	0	0	5*	F+
Dead foetuses:					
mean number	0.0	0.0	0.0	0.0	
number of dams affected	0	0	0	0	X
Post-implantation loss:					
mean%	8.6	3.2	2.9	20.0	
number of dams affected	9	7	6	13	F+
Mean number of foetuses per female	11.2	11.7	12.8	10.1	J

F+ = Cochran-Armitage and Fisher's Exact (upper tail) * P<0.05
J = Kruskal-Wallis, Terpstra-Jonckheere, Wilcoxon ** P<0.01
X = not analysed *** P<0.001

Offspring (Malformations, Variations, etc.)

Individual fetal and placental weights were recorded and fetuses were categorized as to gender and examined externally. Approximately half of the fetuses in each litter were dissected and the viscera examined, followed by skeletal staining. The remaining fetuses were fixed, dissected and examined for gross pathology

Fetal weights were significantly decreased in the intermediate and high-dose AFP-168 groups compared to controls with mean fetal weights of 3.70, 3.59 and 3.89 g respectively. The mean placental weight was slightly but not significantly higher for the high-dose group (0.61 g) compared to the control group (0.55 g). This difference was largely driven by a very high placental weight for one high-dose animal.

Visceral malformations occurred in all of the AFP-168-treatment groups, but not in control animals (Table 87). The visceral malformations were not affected by AFP-168 dose in terms of the number of fetuses or litters affected. Visceral malformations included but were not limited to: additional structure in the eye, severe renal pelvic cavitation, severely distended left ureter, umbilical hernia, absent kidneys and ureters, and abnormal lung lobulation. However, specific visceral malformations occurred in only one litter per dose group, different visceral malformations occurred with the different

AFP-168 doses, and the least number of malformations occurred in the high-dose group. These factors suggest the visceral malformations were not AFP-168-related.

Skeletal malformations occurred in the intermediate and high dose AFP-168 groups. In the high dose group, there were a greater number of variations of the lumbar centra, thoracic arches, and thoracic centra. Also there was a dose-related significant increase in the numbers of intermediate and high-dose litters with unossified 5th sternebra.

Table 87: Rat Fetal Malformations and Variations

Test article	Control		AFP-168	
Group	1	2	3	4
Level (µg/kg/day)	0	3	10	30

6.3 Foetal defect data

	Group 1	Group 2	Group 3	Group 4	Statistics
EXTERNAL/VISCERAL DEFECTS					
Number of foetuses examined	257	270	231	243	
Number of litters examined	22	23	18	22	
Number showing malformations	0	5	7	2	
Mean % of foetuses examined	0.0	5.8	3.0	1.0	
Number of litters affected	0	4	3	2	F+
Number showing variations	38	47	30	45	
Mean % of foetuses examined	14.2	20.7	13.6	22.0	
Number of litters affected	15	19	13	14	F+
SKELETAL DEFECTS					
Number of foetuses examined	126	138	116	122	
Number of litters examined	22	23	18	22	
Number showing malformations	0	0	3	3	
Mean % of foetuses examined	0.0	0.0	2.4	2.4	
Number of litters affected	0	0	1	2	F+
Number showing variations	120	121	107	117	
Mean % of foetuses examined	95.4	84.2	93.5	96.3	
Number of litters affected	22	22	18	22	F+
Total number of foetuses showing malformations	0	5	7	5	
% of foetuses examined	0.0	1.9	3.0	2.1	
Number of litters affected	0	4	3	3	F+

F+ = Cochran-Armitage and Fisher's Exact (upper tail)

Study title: AFP-168: Intravenous Study of Embryo-Foetal Development in the Rabbit

Study no.:	MRL TT #01-5528
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 12, 2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AFP-168, Batch # FP-0002, Purity of 98.6%.

Key Study Findings

In female rabbits receiving intravenous doses of 0, 0.03, 0.1, and 0.3 μg/kg/day AFP-168, early post-implantation loss occurred to a significantly elevated degree in the intermediate- and high-dose groups. In the high-dose group there were no live fetuses, and in the intermediate-dose group only two litters contained live fetuses. Abortions occurred in eleven and three of the intermediate-dose and high-dose females compared to one control female. In the low-dose group, the majority of litters were viable, but three fetuses in separate litters had abdominal wall malformations, and six fetuses in five litters had cranial and/or spinal malformations. An NOAEL was not established.

Methods

Doses:	0, 0.03, 0.1, and 0.3 μg/kg/day
Frequency of dosing:	Once per day
Dose volume:	1 ml/kg for Groups 1, 3, and 4 and 0.3 mg/kg for Group 2
Route of administration:	intravenous
Formulation/Vehicle:	0.9% sodium chloride
Species/Strain:	Crl.NZW/Kbl BR rabbits
Number/Sex/Group:	24 female rabbits/group
Satellite groups:	none
Study design:	See Table 88 below. Premated rabbits were treated intravenously with 0, 0.03, 0.1, and 0.3 μg/kg/day AFP-168 from Gestation Day (GD) 1 until GD 19. All animals were euthanized on Day 29 and pregnancy and fetal outcome parameters were assessed.
Deviation from study protocol:	Multiple deviations from the study protocol were noted but the deviations were not considered to have altered the study results or compromised the integrity of the study.

Table 88: Study Design for the First Rabbit Embryo-Fetal Study. (Sponsor's Table)

Group number	Group description	Dose level (μg/kg/day)	Dose concentration (%)	Dose volume (mL/kg)	Dose rate (mL/min)	Number of females+
1	control	0	0	1.0	2	24
2	low	0.03	0.00001	0.3	0.6	24
3	intermediate	0.1	0.00001	1.0	2	24
4	high	0.3	0.00003	1.0	2	24

+ to allow for at least 20 pregnant animals per group

Observations and Results

Mortality

All animals were inspected twice daily for morbidity or mortality.

No animals were found dead during the experiment. However, single animals in the vehicle control and the low dose (0.03 μg/kg/day) groups, and eleven and three mothers in the intermediate dose (0.1 μg/kg/day) and high-dose (0.3 μg/kg/day) groups aborted their pregnancies between GD 17 and GD 23 and were euthanized prior to GD29.

Clinical Signs

All animals were examined at least once per day for signs of ill health or overt toxicity. In addition, the animals were observed immediately after dosing and at 0.5 and 1 hour after dosing for signs of reaction to treatment.

No clinical signs occurred after dosing. The only clinical signs observed were red tissue and fluid under the cages of the mothers that aborted their pregnancies.

Body Weight

Body weights were recorded on GD 4 and GD 7 to GD 29.

A slight weight loss relative to the control animals occurred in the intermediate and high-dose animals between GD 7 and GD 10 and between GD 10 and GD 15. The difference was significant only for the intermediate dose between GD 12 and GD 15. Thereafter the growth rate was similar to those of the controls.

The mean gravid uterus weight in the two intermediate-dose females with viable fetuses was significantly less than that of the controls.

Feed Consumption

Individual food intake was assessed on the same days as the body weight measurements.

Food intake was not reduced by AFP-168 treatment.

Toxicokinetics

Blood samples were obtained and processed to plasma on Day 7 and Day 19 of gestation. On both of these days, blood samples were obtained from three animals predose, and at 1, 5, 15, 30, 60, and 120 minutes after dosing. Plasma concentrations of AFP-168 and AFP-172 were measured using a validated LC-MS/MS method.

AFP-168 was not detected in plasma from the high-dose and control animals and the other two groups were not examined for plasma AFP-168 concentrations. AFP-172 was detectable in the plasma of females in the 0.1 and 0.3 μg/kg/day groups on GD 7 and GD 19, but only in the 1 and 5 minute samples. Plasma AFP-172 concentrations were only detectable in the 0.03 mg/kg/day group in the 1 minute sample on GD 7. The AFP-172 toxicokinetic parameters, C_{max}, T_{max}, and $AUC_{(0\text{-}120\ min)}$ could not be calculated due to insufficient data.

Dosing Solution Analysis

Two AFP-168 stock solutions of 0.0015% concentration were prepared and used within their established stability period. The dosing formulations were prepared daily from the

stock solutions. The stock solutions were assessed once for concentration and the dosing formulations were assessed for achieved concentration, osmotic pressure and pH on the first dosing day and during the last week of treatment. In addition, the 0.00001% dosing solution was assessed on the eighth day of dosing.

Previously AFP-168 solutions of 0.0015% concentration were shown to be stable for 6-weeks at room temperature. Also 0.0001% and 0.0015% solutions were shown to be stable for 3 weeks at 5°C and 30°C. In this experiment, the actual concentrations of the two 0.0015% AFP-168 stock solutions were shown to be (b) (4) of the nominal concentrations. The actual concentrations of the dosing solutions were as shown in Table 89. The target ranges for the stock solutions was 90-110% and for the dosing solutions was 100 to 130% of the nominal concentations. As shown in the table below, the actual concentrations of the 0.00001% dosing solutions sometimes exceeded the target range.

Table 89: Actual Concentrations of the AFP-168 Dosing Solutions in the Rat Embryo-Fetal Study. (Sponsor's Table)

Stock used	Sample taken	Results as % nominal Group 2 (0.00001%)	Group 3 (0.00001%)	Group 4 (0.00003%)
1	First day	(b) (4)		
1	Day 8#			
2	Last week			

+ Analysis of single dilution before dividing into separate aliquots for Group 2 and 3.

The measured pH values for the dosing solutions ranged from 5.02 to 5.25 and the actual osmotic pressure measurements for the dosing solutions ranged from 99 to 100% of the osmotic pressure of normal saline.

Necropsy

Surviving females were sacrificed on Day 29 after mating and examined for gross pathology.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

The overies and uteri were removed and assessed for the following parameters: pregnancy status, gravid uterus weight, number of corpora lutea, the number and intrauterine position of implantations subdivided into live fetuses, early intrauterine deaths, late intrauterine deaths, and dead fetuses.

Dose-related effects on pregnancy outcome parameters occurred in all three treatment groups. In the 10 intermediate dose and 13 high-dose females that had evidence of pregnancy on GD 29, the mean number of implantations were significantly less than in control females. The mean implantation values for the control, intermediate, and high-dose groups were 10.5, 7.9, and 7.8 respectively. In addition, in 8/10 intermediate-dose,

and 13/13 high-dose females, all implantations died early in gestation leaving no live fetuses. The respective values of 91.6% and 100% for the mean incidences of post-implantation loss for the intermediate- and high-dose groups were significantly higher than the mean incidence of post-implantation loss for control females (13.1%). Also in the intermediate and high-dose groups the mean number of corpora lutea (3.6 for both groups) was significantly lower compared to control animals (11.5).

In the low-dose group, the mean post-implantation loss was 29.2% compared to 13.1% in control females with three low-dose females and one control female having total intreauterine death with no live fetuses. In low-dose females that had some live fetuses, the percentage of post-implantation loss (18.0%) was slightly higher than that of control females (8.7%) with the majority of intrauterine deaths occurring late in gestation.

Offspring (Malformations, Variations, etc.)

Live fetuses were sacrificed following delivery by caesarian section, and individual fetal and placental weights were recorded and fetuses were examined externally. The heads and hearts of approximately one half of the fetuses in each litter were fixed for later examination. All the fetal carcasses were dissected, categorized as to gender, and their viscera was examined. Subsequently the fetal carcasses were eviscerated, and the skeletons were fixed and stained.

In the high-dose group there were no live fetuses. In the two litters with surviving fetuses in the intermediate-dose group, the mean fetal weight was similar to that of the controls. Placental weight was slightly but not significantly higher than for the controls and both values fell within the historical control range. Also the mean litter weight was reduced compared to controls due to the reduced number of live births and one litter contained only females and one litter was approximately equal for each gender. In the low-dose group, the mean fetal, and placental weights, and the litter gender proportionality were similar to those of the controls. The mean litter weight reflected the reduced number of live fetuses.

One control fetus had a severe brain malformation, exposed meninges through a skull opening. In the low-dose group, fetal malformations were observed in nine fetuses from six mothers. Six of the affected fetuses had defects of the skull, brain, and/or spine and three had abdominal wall defects. The overall number of fetal variations in the low-dose group was similar to that of the controls, but the skeletal variations in the low-dose group were more varied than in the control group and often associated with the malformations. No fetal malformations were observed in the two intermediate-dose litters with surviving fetuses, and visceral and/or skeletal variations were similar in incidence and type compared to the control litters.

Study title: AFP-168: Intravenous Study of Embryo-Foetal Development in the Rabbit – Second Study

Study no.:	MRL TT #02-5551
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 19, 2002
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AFP-168, Batch No.: FP-0002, purity of 98.6%.

Key Study Findings

Pregnant New Zealand White rabbits (24 females/group) were treated intravenously once daily with tafluprost at dose levels of 0.001, 0.003, and 0.01 µg/kg/day from Days 7 to 19 of gestation inclusive. The high-dose was based on results from the previous study, and all animals were euthanized on Day 29 of gestation. During the treatment period, there were no dose-related deaths or abortions, and there were no effects on clinical signs, body weights and food intake. At cesarean section, no effects on reproductive parameters including post-implantation loss were observed. Mean fetal, litter, and placental weights were unaffected by treatment, no individual visceral or skeletal malformation was statistically increased in the AFP-168 treatment groups.

Methods

Doses:	0, 0.001, 0.003, and 0.01 µg/kg/day
Frequency of dosing:	Once per day
Dose volume:	0.1 ml/kg for Groups 1 and 4, 0.01 ml/kg for Group 2, and 0.03 ml/kg for Group 3.
Route of administration:	intravenous
Formulation/Vehicle:	0.9% sodium chloride
Species/Strain:	Crl.NZW/Kbl BR rabbits
Number/Sex/Group:	24 pregnant females/group
Satellite groups:	3 pregnant females/group for Groups 2-4
Study design:	See Table 90 below. Vehicle or AFP-168 was administered intravenously to mated female rabbits from Gestation Day (GD) 7 to GD 19. The females were maintained until GD 29 when they were euthanized, the fetuses removed and euthanized and mothers and fetuses were examined.
Deviation from study protocol:	Multiple deviations from the study protocol were noted but the deviations were not considered to have altered the study results or compromised the integrity of the study.

Table 90: Study Design for the Second Rabbit Embryo-Fetal Study. (Sponsor's Table)

Group number	Group description	Dose level (µg/kg/day)	Dose concentration (%)	Dose volume (mL/kg)	Number of animals	
					Main study	Satellites#
1	control	0	0	0.1	24	0
2	low	0.001	0.00001	0.01	24	3
3	intermediate	0.003	0.00001	0.03	24	3
4	high	0.01	0.00001	0.1	24	3

satellite animals were used to provide blood samples for plasma analysis

Observations and Results

Mortality

All animals were examined for morbidity or mortality twice daily.

No morbidity or deaths were attributed to treatment with AFP-168. Three control females and four intermediate-dose females were killed on the advice of the veterinary surgeon due to very low food consumption at the start of the study.

Clinical Signs

All animals including satellite animals were examined twice daily for signs of ill health or overt toxicity from GD 3 to GD 29. In addition, the animals were observed immediately after dosing and for 0.5 hours after dosing.

All of the animals that were euthanized due to very low food consumption appeared thin, and of these animals one control female and three intermediate-dose females exhibited distended urinary bladders upon euthanasia and necropsy.

In the animals surviving until GD 29, one high-dose animal appeared thin between GD 25 and GD 29 coinciding with weight loss and reduced food intake during this period. Also at necropsy for this animal, the liver was pale and mottled.

Body Weight

The body weight of each female including satellites was recorded on GD 4 and daily from GD 7 to GD 29.

There were no changes in mean body weight that were considered related to AFP-168 administration. Also there were no AFP-168-related changes in mean gravid uterus weight.

Feed Consumption

Individual food intake was recorded daily including the satellite animals and reported on the same days as the body weights.

As noted above, the food intake of three control animals and four intermediate-dose animals was very low from GD 4 and these animals were euthanized. Also low food intakes was observed for two high-dose females and two vehicle control females between GD 23 and 29, but these changes were not considered related to AFP-168 administration.

Toxicokinetics

Blood samples were obtained and converted to plasma from the satellite animals on GD 7 to GD 19 of gestation. On both days, each animal was bled predose, and 0.33, 1, 5, 15, 30, and 60 minutes after dosing. Samples were analyzed for plasma AFP-168 and AFP-172 concentrations using a validated LC-MS/MS technique with a lower limit of quantification (LLOQ) of 20 pg/ml.

No quantifiable levels of AFP-168 or AFP-172 were observed in any of the treatment samples. All plasma concentrations were below the lower limit of LLOQ.

Dosing Solution Analysis

Four AFP-168 stock solutions of 0.0015% concentration were prepared and used within their established stability period. The dosing formulations (0.00001%) were prepared daily from the stock solutions. The stock solutions were assessed once for concentration and the dosing formulations were assessed for achieved concentration, osmotic pressure and pH on the first dosing day and during the last week of treatment. In addition, the 0.00001% dosing solution was assessed on the eighth day of dosing.

Previously AFP-168 solutions of 0.0015% concentration were shown to be stable for 6-weeks at room temperature. Also 0.00001% and 0.0015% solutions were shown to be stable for 3 weeks at 5°C and 30°C. In this experiment, the actual concentrations of the 0.0015% AFP-168 stock solutions were shown to be (b) (4) of the nominal concentrations. Stock solution #3 (b) (4) of nominal) was not used as it was outside the target range of 90-110% of nominal. The actual concentrations of the dosing solutions were (b) (4) of nominal respectively on the first day and in the last week of dosing. Actual pH values for the dosing solutions ranged from 4.91 to 5.12, and osmotic pressure fell within 90 to 110% of saline.

Necropsy

The Main Study females were killed on Day 29 after mating in random group order and examined macroscopically. Satellite animals were euthanized on Day 19 after the final blood collection, and their pregnancy status was recorded.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

The ovaries and uteri were removed, and the following maternal parameters were assessed: pregnancy status, gravid uterus weight, number of corpora lutea, number and intrauterine position of implantations, live fetuses, early uterine deaths, late intrauterine deaths, and dead fetuses.

The mean number of fetuses, and the group mean pre- and post-implantation loss was not affected by AFP-168 treatment.

Offspring (Malformations, Variations, etc.)

Live fetuses were humanely euthanized. Individual fetal and placental weights were recorded and fetuses were examined externally. Approximately one half of the fetuses in each litter were decapitated, and the heads were fixed and sectioned serially for later analysis. The hearts of approximately one half of the fetuses in each litter were also fixed and sectioned into several coronal slices for later examination. All fetuses were categorized as to gender, dissected and their viscera examined. The fetuses were then eviscerated and the carcasses fixed, then processed to stain the ossified skeleton before skeletal examination. Fetal abnormalities were classified as malformations (rare and/or potentially lethal) or variations (commonly occurring non-lethal abnormalites).

Mean fetal, litter, and placental weights were unaffected by treatment. External and/or visceral malformations were seen in 3, 4, 4, and 6 fetuses from the control, low, intermediate, and high-dose groups respectively. In addition, skeletal malformations were seen in 2, 8, 6, and 8 fetuses from the same respective groups. However, for each individual malformation the incidence was 0 or 1 per AFP-168 treatment group with a mean percent ≤ 2% of the fetuses in each group. No individual malformation was significantly increased in any of the AFP-168-treatment groups compared to the vehicle control group.

The number of fetuses with external, visceral, and skeletal variations in all the AFP-168-treatment groups was generally similar to that of the controls (Table 91). The number of fetuses in the treatment groups with abnormally pale contents in the gall bladder and/or non-eruption of the incisors were higher than in the controls; however, the differences were not statistically significant.

Table 91: Incidence of Select Variations in the Second Rabbit Embryo-Fetal Study.

Observed Defect	Class	Incidence (mean %)/ Number of litters affected			
		Group 1	Group 2	Group 3	Group 4
Pale gall bladder contents	V	4 (4.7)/ 4	15 (7.7)/ 9	11 (6.4)/ 9	21 (10.8)/ 11
Blood vessel branches from the left subclavian artery arising directly from the aorta	V	6 (4.6)/ 5	17 (7.5)/ 7	14 (6.9)/ 7	9 (4.5)/ 8
Head – Buccal cavity incisor, not erupted	V	2 (1.4)/ 2	10 (4.2)/ 4	14 (7.1)/ 7	14 (7.4)/ 7
Fifth middle phalanx, ossification incomplete	V	9 (4.5)/ 6	11 (5.5)/ 6	11 (6.3)/ 9	12 (5.2)/ 8
Fifth middle phalanx, unossified	V	45 (28.1)/ 14	50 (26.6)/ 18	56 (31.5)/ 14	62 (27.3)/ 17
Pelvic Girdle, Iliac alignment, caudal shift, 27 pre-pelvic vertebrae	V	38 (23.5)/ 11	58 (29.2)/ 17	53 (28.9)/ 14	69 (34.1)/ 20
First lumbar vertebrae – left long/right short	V	10 (5.2)/ 8	12 (5.4)/ 9	10 (5.6)/ 8	19 (9.7)/ 14
First lumbar vertebrae – left short/right long	V	6 (3.6)/ 4	12 (5.4)/ 8	3 (1.7)/ 3	11 (4.9)/ 8
First lumbar vertebrae – long	V	70 (43.3)/ 14	95 (47.5)/ 20	85 (47.8)/ 18	103 (51.8)/ 22
First lumbar vertebrae – short	V	26 (15.8)/ 13	33 (15.3)/ 15	35 (19.7)/ 17	25 (11.1)/ 15
Sternebra – ossification incomplete	V	3 (1.2)/ 3	4 (1.9)/ 4	12 (6.0)/ 4	11 (6.1)/ 7
Caudal vertebrae – terminal, misshapen/misaligned/fused/absent	V	2 (2.6)/ 4	4 (1.8)/ 4	5 (2.5)/ 5	7 (3.5)/ 6
V = variation					

9.3 Prenatal and Postnatal Development

Table 92: Non-Pivotal Range-Finding Prenatal and Postnatal Development Studies

Study No./ Species/ type of study	Species/ Route/Dose/ Number per group	Results
MRL TT #04-5573/ Dose Range-Finding, Pre- and Postnatal Development study in rats	Rats/ intravenous/ 0, 0.3, 1, 3, and 10 µg/kg/day AFP-168/ 8 pregnant females/group	1. No AFP-168-related effects on general condition, food consumption, or gross pathology of the F_0 dams. 2. Body weight gain was reduced in the high-dose group on Days 16-20 of gestation. 3. No AFP-168-related effects on duration of gestation, gestation index, or delivery index. 4. Poor nursing and total litter loss was evident in one dam in both the intermediate- and high-dose groups. 5. In F_1 offspring, there were no differences between the control and AFP-168 treated groups in the number of implantation sites, total number of newborns or delivery index. Also the weaning index, body weights, physical development or early behavior of offspring did not change. 6. However, in the intermediate- and high-dose groups, the number of dead fetuses tended to increase and the birth index and viability index on Day 4 of lactation tended to decrease. 7. Based on the results of this study, the top dose for the definitive study was determined to be 10 µg/kg/day.
Gestation Index = percent of dams with live newborns/pregnant dams. Delivery Index = percent of newborns/ implantation sites. Birth Index = percent of live newborns/ implantation sites. Viability Index = percent of viable fetuses/ total fetuses.		

Study title: A study for effects of AFP-168 on pre- and post-natal development including maternal function, in rats via intravenous administration.

Study no.:	MRL TT #04-5574
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	October 4, 2004
GLP compliance:	Yes
QA statement:	A QA statement was not included in the study report
Drug, lot #, and % purity:	AFP-168, Lot # 037010, purity of 101.5%

Key Study Findings

Female Sprague-Dawley rats (22 rats/group) were treated intravenously once daily with tafluprost at dose levels of 0.3, 1, 3, and 10 µg/kg/day from Gestation Day 6 to Day 20 of lactation. No treatment-related maternal deaths, and no effects on body weight, food intake, and necropsy. Some dams receiving 1 µg/kg/day AFP-168 and higher exhibited poor nursing behavior resulting in decreased viability indices on Day 4 of lactation to

offspring. Increased mortality of newborns, decreased body weights, and delayed pinna unfolding at Day 3 of lactation were noted in offspring in the 10 μg/kg/day group. No other treatment-related effects were evident in physical development, sensory functions, genital development and fertility of F_1 offspring in any treatment group. The NOAEL values were considered to be 10 μg/kg/day in dams (F_0) and 0.3 μg/kg/day for development of the F_1 generation. For the F_2 generation, there were no significant differences between the control and AFP-168 treatment groups in the number of corpora lutea, implantations, live F_2 embryos, pre-implantation loss or embryonic mortality. However, other F_2 parameters, including F_2 body weights, external appearance, and male/female ratio were not reported.

Methods

Doses:	0, 0.3, 1, 3, and 10 μg/kg/day
Frequency of dosing:	Once per day
Dose volume:	3 ml/kg at a rate of 0.6 ml/min.
Route of administration:	intravenous
Formulation/Vehicle:	Isotonic sodium chloride solution.
Species/Strain:	Crj:CD(SD)IGS rats
Number/Sex/Group:	22 mated females/group
Satellite groups:	none
Study design:	See Table 93 below. Pregnant female rats received daily intravenous doses of vehicle or AFP-168 from gestation day (GD) 6 until lactation day (LD) 20 which is roughly the period from implantation to weaning in rats.
Deviation from study protocol:	Minimal deviations from the study protocol were noted. These deviations were not considered to have altered the study results or compromised the integrity of the study.

Table 93: Study Design for the Pre- and Post-Natal Development Study in Rats. (Sponsor's Table)

Group	Test substance	Dose level (μg/kg/day)	Concentration (w/v%)	Dose volume (mL/kg)	Rate of dosing (mL/min)	No. of mated females	Animal Nos.
Control	Saline	–	–	3	0.6	22	001-022
Low	AFP-168	0.3	0.00001	3	0.6	22	101-122
Low-mid	AFP-168	1	0.000033	3	0.6	22	201-222
Mid-high	AFP-168	3	0.0001	3	0.6	22	301-322
High	AFP-168	10	0.00033	3	0.6	22	401-422

Observations and Results

F_0 Dams

Survival:	One high-dose female died during the course of the study due to unknown causes.

Clinical signs:	Only loss of fur was noted in one animal. No AFP-168-related clinical signs were noted.
Body weight:	No AFP-168-related changes in body weight were noted.
Feed consumption:	The females receiving 3 μg/kg/day AFP-168 demonstrated a significant, transient decrease in food consumption from LD 4 – LD 7, but due to a lack of a similar effect in the high-dose group, this decrease did not appear to be treatment-related.
Uterine content:	There were no significant differences between the control and AFP-168 treatment animals with regard to the number of implantation sites, total number of newborns, delivery index, or proportion of male live newborns.
Necropsy observation:	Dams that were necropsied on Day 21 did not exhibit any AFP-168-related or abnormal gross pathology findings.
Toxicokinetics:	Toxicokinetics were not performed.
Dosing Solution Analysis	The actual concentrations of the first and last preparations of each dosing solution ranged from (b) (4) of the nominal concentrations. The dosing solution osmolarity was 98-102% of the osmolarity of saline. The dosing solution pH values ranged from 5.98 to 6.19.
Other:	Delivery and Gestation Parameters: One 3 μg/kg/day female delivered her babies late on GD 25. However, no abnormal delivery was evident in any dam, and there were no significant differences between any of the AFP-168-treatment groups in the duration of gestation, gestation index, or delivery index. Nursing Parameters: One dam in each of the 0.3, 3, and 10 μg/kg groups and 3 dams in the 1 μg/kg group exhibited poor nursing behavior on LD 0, 1, or 2. Poor nursing behavior resulted in the deaths of all offspring from 2 dams in each of the 1, 3, and 10 μg/kg/day groups within 2 days after birth.

F_1 Generation

Survival:	At birth: The high-dose (10 μg/kg/day) group demonstrated a greater number of dead newborns, and a lower birth index compared to controls. This effect was not statistically significant, but it was considered AFP-168-related. There were no significant differences between any of the AFP-168-treatment groups and the control group in the number of implantation sites, total number of

	newborns, delivery index, or male proportion of live newborns. During Lactation: The viability index on LD 4 was lower in the 1, 3, and 10 μg/kg/day groups compared to the control group with a significant reduction for the high-dose group. This effect was considered to be AFP-168 related. The weaning index was comparable between all the AFP-168 treatment groups and the control group.
Clinical signs:	Pre-weaning: Some of the newborns in the 1, 3, and 10 μg/kg/day groups exhibited no milk in the stomach at 0, 1, or 2 days of age. All of the newborns from 2 dams in each of these groups died within 2 days of birth. Some of the offspring from 2 or 3 dams in the control and 0.3, 1, and 3 μg/kg/day groups died during the lactation period as did some of the offspring from 10 dams in the 10 μg/kg/day group. Post-weaning: No abnormal clinical signs were evident in any offspring after weaning.
Body weight:	Pre-weaning: Body weights of male and female newborns in the 10 μg/kg/day group were significantly lower than control offspring and this difference was considered AFP-168-related. However, the offspring in all groups demonstrated similar body weights after ≥ 4 days of age. Post-weaning: Body weights of female offspring in the 10 μg/kg group were significantly but transiently lower than control group animals at 28 days of age. Because of its transient nature, this effect was not considered to be AFP-168-related.
feed consumption:	Not assessed.
Physical development:	Pre-weaning: The incidence of pinna unfolding at 3 days of age was statistically lower in the high-dose group (56.5%) compared to the control group (95.5%).This effect was considered related to growth retardation associated with the low birth weights in the high-dose group. At ≥ 4 days of age, all of the groups demonstrated approximately 100% incidence of pinna unfolding. Post-weaning: All F_1 offspring exhibited positive visual placing responses, pupillary reflexes, Preyer's reflexes and pain responses.
Neurological assessment:	Pre-weaning: There was no significant difference between the control and AFP-168 treatment groups for the incidence of back righting or negative

	geotaxis before weaning. Post-weaning: All F_1 offspring exhibited positive visual responses, papillary reflexes, Preyer's reflexes, and pain responses.
Gross Pathology	Post-weaning: No gross pathology considered related to AFP-168 treatment was observed in any male or female offspring culled at four days of age or in offspring at 21 days of age. A few AFP-168-treated animals at both ages demonstrated dilation of the renal pelvis, but the incidence was low in each group and the effect was not dose-related. No gross pathology was evident at 10 weeks of age.
Reproduction:	No AFP-168-related changes in preputial separation or vaginal opening were observed. Also there were no significant differences between any of the AFP-168-treatment groups and the control group for mating or the fertility index.
Other:	No AFP-168-related gross pathology was noted in males after mating. Also no abnormal clinical signs or gross pathology were evident in any female, including non-pregnant females. No significant differences in F_1 female body weights were noted during gestation for the AFP-168 treatment groups versus the control group.

F_2 Generation

Survival:	There were no significant differences between the control and AFP-168 treatment groups for embryonic mortality.
Body weight:	Not assessed
External evaluation:	Not assessed
Male/Female ratio:	Not assessed
Other:	There were no significant differences between the control and AFP-168 treatment groups in the number of corpora lutea, implantations, live F_2 embryos, or pre-implantation loss.

10 Special Toxicology Studies

Study Title: Skin sensitization study of AFP-168 ophthalmic solution by adjuvant and patch test in the guinea pig (Santen Study No.: 996305; Study No.: MRL TT #99-5549).

Key Findings

Under the conditions of the study, neither concentration of AFP-168 (0.005% and 0.05%) demonstrated a potential for skin sensitization.

Objective
To evaluate the skin sensitization potential of AFP-168 ophthalmic solution in the guinea pig by adjuvant and patch tests.

Methods

The Adjuvant and Patch test was used to determine the skin sensitization potential of 0.005% and 0.05% AFP-168 ophthalmic solutions in the guinea pig. The negative control was the vehicle for the 0.05% AFP-168 ophthalmic solution and the positive control was 2,4-dinitrochlorobenzene (DNCB). Each group of 8 guinea pigs was injected with emulsified Freund's complete adjuvant intradermally into the shoulder skin in 4 corner areas, and a scratch mark in the shape of # was made using injection needles at the site of injection. At the same time, a closed patch of each test preparation was applied occlusively for 24 hours. The same abrasions and applications were repeated on each of the next two days such that the initial sensitization consisted of 3 days of treatment. The second sensitization was performed at the same area and consisted of application of each test preparation at the same areas for 48 hours. Two weeks later, each test preparation was applied openly to the flank as the challenge procedure.

Results

Animals in the vehicle control group, and the 0.005% and 0.05% AFP-168 ophthalmic solution sensitization groups did not demonstrate skin reactions in response to the challenge procedure. In contrast, all of the animals in the positive control group demonstrated positive skin reactions after challenge with DNCB.

11 Integrated Summary and Safety Evaluation

Tafluprost (AFP-168) ophthalmic solutions are intended for the topical ocular treatment of elevated intraocular pressure in open angle glaucoma or ocular hypertension. Tafluprost acid (AFP-172) the active metabolite of tafluprost, is a fluorinated analogue of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) with high affinity and selectivity for the FP prostanoid receptor.

Three other PGF_{2a} analogues are currently approved for the treatment of elevated intraocular pressure in patients with open angle glaucoma or ocular hypertension. These are latanoprost (Xalatan®), travoprost (Travatan®), and bimatoprost (Lumigan®). According to their product labels, all of the approved PGF_{2a} analogues are associated with specific ocular and eye anexa adverse effects. These include increased pigmentation of the iris, periorbital tissue (eyelid) and eyelashes, and macular edema.
`

The presumed mechanism of tafluprost and other $PGF_{2\alpha}$ analogues with regard to glaucoma is reduction of intraocular pressure by increasing uveoscleral outflow of aqueous humor. Further activity may be mediated by tafluprost-induced relaxation of the ciliary muscle and changes in ciliary muscle extracellular matrix thus facilitating increased outflow from aqueous humor.

In primary pharmacology studies, AFP-172 was shown to bind to the FP prostanoid receptor with subnanomolar affinity. For a large panel of receptors, the IC_{50} for AFP-172 binding was greater than 1 μM with the exception of the guinea pig EP3 receptor where the IC_{50} value was 67 nM. In experiments designed to assess the ocular hypotensive effects of tafluprost and AFP-172 in ocular normotensive and hypertensive monkeys, both tafluprost and AFP-172 at topical ocular doses ≥ 0.0005% were shown to significantly reduce IOP. Tafluprost was also shown to have an additive effect with timolol (a non-selective β-adrenergic receptor blocker for treatment of open angle glaucoma) in the reduction of IOP in ocular normotensive monkeys. In an aqueous humor dynamics study in monkeys, tafluprost was shown to significantly increase uveoscleral outflow and significantly increase total outflow facility.

Tafluprost was extensively tested in safety pharmacology studies designed to assess central nervous system, cardiovascular and respiratory system function. In addition, because $PGF_{2\alpha}$ is known to increase the force of uterine contractions, tafluprost was assessed for its ability to affect uterine contractions in non-pregnant rats and rabbits.

Single intravenous doses of 10, 30, and 100 μg/kg tafluprost did not affect general locomotor activity in mice. In an Irwin test in mice receiving intravenous tafluprost, doses of 10 and 30 μg/kg did not significantly alter any physiological or behavioral Irwin parameters compared to vehicle. Irwin parameters were also largely unaltered in high-dose mice receiving 100 μg/kg tafluprost with the exception of 1/6 mice in this group. Within five minutes after dosing, the affected mouse demonstrated an ataxic gate, decreased spatial locomotor activity and limb tone, decreased pupillary constriction in response to light, and increased piloerection, startle response, toe pinch response, and corneal and pinna reflexes. The results suggest that in a small percentage of mice, high μg doses of tafluprost can alter central nervous system responses. Susceptibility to this effect appears to differ with species. In the IV toxicology studies, dogs generally demonstrated tafluprost-related clinical signs including emesis, panting, vocalization, and miosis while rats did not.

The ability of tafluprost to alter cardiovascular function was assessed *in vitro* in a hERG assay, and in dog isolated cardiac purkinje fibers, and *in vivo* in anesthetized dogs. In the hERG assay, AFP-172 at a concentration of 100 ng/ml did not alter hERG potassium tail currents compared to vehicle. In dog purkinje fibers, AFP-172 at concentrations of 1, 10, and 100 ng/ml did not alter the resting potential, maximum rate of depolarization, upstroke amplitude, or action potential duration in a consistent manner at 0.5 or 1 Hz stimulation frequencies.

Intravenous AFP-168 at doses of 0.1 and 1 μg/kg, but not at lower doses of 0.003 and 0.01 μg/kg altered several parameters of cardiac function in anesthetized dogs. Affected parameters included dose-dependent increases in arterial pressure, heart rate, and dP/dTmax. Also femoral blood flow was significantly but transiently decreased in dogs receiving the high dose of 1 μg/kg. ECG waveforms were also affected by the 0.1 and 1 μg/kg AFP-168 doses. Dose-dependent changes included decreased RR interval and increased QTc interval as well as increased QT interval for the high dose. The changes

were only significant for the high dose. Other dose-dependent trends included decreased PR interval, T-wave height, and ST interval. While all of the waveform changes fell within normal limits, they are indicative of a dose-dependent potential for AFP-168 to alter ECG parameters. Also, in agreement with its greater affinity for the FP prostanoid receptor, AFP-168 appears to be more potent than latanoprost (Xalatan®) and $PGF_{2\alpha}$ in altering cardiac function parameters. However, cardiovascular effects occurred only minimally in repeated-intravenous dose toxicology studies in dogs, and did not occur in repeated-ocular dose studies in monkeys. Because clinical exposures are expected to be on the order of 100 fold lower than the exposures corresponding to the high doses in the monkey studies, cardiovascular toxicity is not expected to be a clinical concern.

Respiratory function was also assessed in the anesthetized dog studies, and the higher doses of 0.1 and 1 μg/kg but not the lower doses of 0.003 and 0.01 μg/kg were shown to stimulate a dose-dependent increase in the rate of respiration as well as increased minute volume, and peak inspiratory flow and decreased peak expiratory flow and tidal volume. With the exception of peak inspiratory flow, these changes returned to near baseline 40 minutes after dosing. As was the case for its effects on cardiac function parameters, AFP-168 was more potent in stimulating increased respiratory rate than latanoprost and $PGF_{2\alpha}$.

In isolated uteri from non-pregnant rats, AFP-172 increased the maximum tension, resting tension, and frequency of spontaneous contractions and was roughly 10X more potent than $PGF_{2\alpha}$ in producing the same effects. In contrast, in isolated uteri from non-pregnant rabbits, AFP-172 increased the maximum tension of spontaneous contractions with roughly the same potency as $PGF_{2\alpha}$. Also $PGF_{2\alpha}$ had more of an effect on uterine resting tension at a high concentration of 10^{-7} g/ml than the same concentration of AFP-172.

The Sponsor developed multiple validated LC-MS/MS assays for the detection of tafluprost and tafluprost acid in plasma from mice, rats, rabbits, dogs, and monkeys.

A monkey study evaluating the pharmacokinetics of tafluprost dosed via topical ocular or intravenous administration revealed that tafluprost was very rapidly absorbed from the eye and that ocular bioavailability was high. Similar T_{max} and C_{max} values were obtained when the same total dose of radioactive tafluprost (^{3}H-AFP-168) was administered by the topical ocular route or intravenously. The AUC values associated with ocular dosing were approximately half those obtained after intravenous dosing. Plasma $T_{1/2}$ values for tafluprost acid following ocular and intravenous dosing of tafluprost in monkeys were on the order of 10 hours. Toxicokinetic analysis following 39 weeks of intravenous dosing in dogs and 52 weeks of ocular dosing in monkeys indicated that tafluprost acid C_{max} and AUC values increased in a roughly dose-proportional manner, and tafluprost acid did not accumulate in plasma following tafluprost administration by both routes.

Removal of benzalkonium chloride from the tafluprost formulation did not significantly alter corneal penetration and aqueous humor concentrations of tafluprost in rabbits.

Topical ocular administration of tafluprost in rats resulted in widespread ocular distribution. Thirty minutes after single-ocular doses of ^{3}H-AFP-168, the highest ocular concentrations occurred in the cornea, followed by aqueous humor, the iris/ciliary body, sclera, conjunctiva, retina choroid, vitreous humor and lens. Repeated daily dosing of ^{3}H-AFP-168 produced a similar pattern of ocular tissue distribution. Generally radioactivity did not accumulate in any of the ocular tissues with the exception of the lens where concentrations increased by approximately 50% after 21 days of dosing.

Tafluprost was also rapidly absorbed in monkey eyes after a single topical ocular administration of ^{3}H-AFP-168. The highest concentrations of tafluprost acid were observed in the cornea, and the bulbar and palpebral conjunctiva 5 minutes after dosing. Overall radioactive concentrations in ocular tissues decreased with time, but low concentrations were still detectable in most ocular tissues 24 hours after dosing possibly in the form of tritiated water.

Studies examining the binding of tafluprost acid to serum albumin from rat, rabbit, dog, and human serum indicated that 500 ng/ml tabluprost acid was greater than 90% bound by serum albumin in all species.

In rats following topical ocular administration of ^{3}H-AFP-168, radioactivity rapidly appeared in systemic tissues but the levels were much lower than those in ocular tissues. The highest concentrations of systemic radioactivity generally occurred 30 minutes after a single ^{3}H-AFP-168 dose with the highest concentrations in liver, duodenum wall, ileum wall, Harderian gland, stomach wall, and kidney. Much lower tissue concentrations were found in the brain, spinal cord, heart, bone marrow, testis and ovaries. This pattern of absorption suggests that tafluprost administered to the eye may have drained into the oral cavity via the nasolacrimal duct with subsequent excretion via renal and hepatobiliary routes. The results indicate that tafluprost and its metabolites did not readily cross the blood brain barrier. Tissue levels in the primary excretory organs declined slightly slower than plasma levels. However tissue distribution results following 7, 14, and 21 days of dosing were similar to those following a single dose indicating systemic tissue accumulation did not occur.

In monkeys after a single ocular dose, ^{3}H-AFP-168 was rapidly absorbed into systemic tissues with the highest concentrations in the lacrimal glands, kidney and liver. Concentrations of radioactivity in plasma were higher than those in whole blood indicating that ^{3}H-AFP-168-derived radioactivity was distributed mainly in the extracellular fraction of blood.

To assess milk secretion, a single topical ocular dose of ^{3}H-AFP-168 was administered to female rats at a single timepoint following pregnancy and between 10 and 13 days after parturition. Tafluprost effectively transferred into milk with C_{max} values similar to those measured in plasma and blood. The maximal concentrations of total radioactivity in milk occurred two hours after dosing and concentrations declined steadily thereafter but low concentrations were still measureable 96 hours after dosing. The $T_{1/2}$ for radioactivity in milk was estimated to be 45.1 hours.

In another experiment, placental transfer of ^{3}H-AFP-168 was evaluated after topical ocular administration to female rats on Day 12 or Day 18 of gestation. Tafluprost readily crossed the placenta. Following Day 12 dosing and Day 18 dosing the highest concentrations of radioactivity associated with amniotic fluid and fetuses were measured 4 hours after dosing. The highest concentrations in most other maternal tissues occurred 15 minutes after dosing indicating slower deposition of tafluprost into fetal tissues. For the Day 12 and Day 18 administrations, the fetus to plasma ratios were 0.62 and 0.71 respectively based on AUC_{24}, indicating fetal exposure to radioactivity was approximately two thirds that of plasma exposure.

Comparative metabolism by rat, dog, monkey and human hepatocyte suspensions was studied. In all species, tafluprost was quickly hydrolyzed to tafluprost acid. In rat hepatocytes, 62% of the resulting tafluprost acid was further metabolized and corresponding values in monkeys and human hepatocytes were 72% and 50% respectively. Substantially less metabolism of tafluprost acid occurred in dog hepatocytes. As shown in Table 94, all of the putative major metabolites occurring above 10% in human hepatocytes were also present in hepatocyte suspensions from rat and monkey.

Table 94: Comparative Metabolism of Tafluprost in Rat, Dog, Monkey, and Human Hepatocytes. (Sponsor's Table)

(b) (4)

Study System		*In vitro* using hepatocytes						
Test Method		Sample was added with ^{3}H-tafluprost and was incubated for 4 hours at 37°C, and then radioactivity concentrations in sample for each metabolite were measured						
Analyte		Radioactivity (Radionuclide : ^{3}H)						
Specific Activity		814 GBq/mmol						
Assay		HPLC- radiochemical detector						
		% of injected radioactivity						
Species	Concentration tested (µM)	Hydroxy-tetranor AFP-172	Hydroxy-dinor AFP-172	Tetranor AFP-172	Dinor-AFP-172	AFP-172 GLU	AFP-172 (tafluprost acid)	Tafluprost
Rat	2.5	0.3	ND	35.8	12.1	ND	37.5	0.3
	25	0.2	ND	15.1	7.4	ND	65.1	0.2
Dog	2.5	ND	ND	ND	0.9	ND	96.3	0.1
	25	ND	ND	ND	0.6	ND	97.5	0.1
Monkey	2.5	0.4[b]	ND[b]	1.9[b]	22.0[b]	ND[b]	27.7[b]	0.1[b]
	25	0.6	ND	1.9	21.9	ND	45.3	0.1
Human	2.5	4.1	2.8	5.1	25.9	3.3	49.7	0.1
	25	1.2	1.3	2.5	19.2	3.2	66.5	0.0

Mean (n = 2).
ND: no peak appeared on chromatograms.
a: glucuronide.
b: n=1.

Tafluprost was shown to be metabolized by rabbit eye carboxyesterases to tafluprost acid *in vitro*. Little or no metabolism of tafluprost acid was found to occur in incubations with 12 different recombinant CYP-450 isozymes, thus suggesting tafluprost acid was not a substrate for human CYP-450.

The cumulative study data indicate that tafluprost whether administered topically to the eye or intravenously undergoes enterhepatic circulation with substantial excretion in bile with final deposition in feces as well as urinary excretion. In monkeys following bilateral

topical ocular dosing, approximately 38% and 48% of the administered dose was recovered in urine in males and females respectively. A further 36% in males and 29% in females was recovered in feces. Slightly more urinary excretion occurred following intravenous elimination. In a rat study examining biliary excretion and enterhepatic circulation of ^{3}H-AFP-168 administered by the topical ocular route, approximately 50% (male) and 33% (female) of the excreted radioactivity was excreted in bile, 25% (male) and 41% (female) was excreted in urine, and 3% (male) and 5% (female) was excreted in feces.

Tafluprost was administered intravenously in several repeated-dose toxicology studies including in 28-day, and 26-week studies in rats, and 28-day and 39-week studies in dogs. Several specific forms of systemic toxicity were identified in each species. In the 26-week study in rats, histopathology was noted in the femur, sternum, spleen, liver, and female kidney. Toxicity included dose-related mortality, and hyperostosis and myelofibrosis in bone marrow of the femur and sternum at all the administered doses (10, 30, and 100 μg/kg/day). In addition at all doses there was increased hematopoesis in the spleen, liver, and male femoral bone marrow and an increase in corticomedullary mineralization in the kidney of females. Changes in the hematological composition of the blood (≥ 10 μg/kg/day) and bone marrow (100 μg/kg/day) were also observed. Many of these effects may be attributable to known pharmacological effects associated with activation of the FP prostanoid receptor. However, in this study a NOAEL dose was not established.

Less systemic toxicity was apparent in dogs. In the 39-week repeated-dose study in dogs employing intravenous doses of 0.1, 1, and 10 μg/kg/day, the most pronounced effects occurring primarily in the high-dose animals was transient clinical signs including salivation, emesis, loose feces, increased respiration, increased heart rate, and increased blood pressure. These effects occurred in a dose-dependent fashion, and in the 4-week intravenous dose study in dogs the 10 μg/kg/day dose was also associated with a prolonged QTc interval. Other toxicity included increased salivary gland weights and minor hypertrophy of the submandibular gland acini of animals receiving the 1 and 10 μg/kg/day doses. These effects may have been secondary to salivation. In high dose animals increased eosinophilia in the adrenal cortex was observed. Also 1/8 high dose animals experienced severe hepatic failure and had to be sacrificed in Week 19. The cause for this occurrence was not elucidated, and while none of the surviving high-dose animals demonstrated liver histopathology, at the end of the experiment high dose males demonstrated a slight but significant elevation in serum ALT. Increased ALT activity was also noted in the 28-day dog toxicology study although the change was reversible after two weeks recovery. Excluding acute clinical signs (salivation, emesis, miosis) following dosing, the NOAEL values for both the 28-day and 39-week intravenous dose toxicology studies in dogs was 1 μg/kg/day.

Because NOAEL values could not be assigned in the 26-week rat intravenous-dose study, it is difficult to determine safe tafluprost exposure levels with regard to systemic toxicity. As shown in Table 95, the dog NOAEL values are associated with C_{max} and AUC values that were substantially higher than those associated with the C_{max} and AUC

exposures obtained in humans after 8 days of daily topical ocular dosing with the tafluprost dose intended for marketing (0.0015%).

Table 95: Safety Analysis for Tafluprost-Related Systemic Toxicity Based on the Intravenous Dose Toxicology Studies in Dogs

Study No./ Description	NOAEL Dose (µg/kg/day)	NOAEL Dose C_{max} (ng/ml) for AFP-172	NOAEL Dose AUC (ng x h/ml) for AFP-172	Safety Factor Based on C_{max} [a]	Safety Factor Based on AUC [b]
TT #99-5550/ 28-Day Intravenous Dose Toxicology Study in Dogs	1	2.33	0.385	74-95	40-68
TT #01-5530/ 39-week Intravenous Dose Toxicology Study in Dogs	1	1.44	0.319	46-59	33-56

[a] The Range of C_{max} values obtained in humans after 8 days of once daily topical ocular dosing with 0.0015% tafluprost (the intended dose for marketing) = 24.42 - 31.43 pg/ml

[b] The Range of $AUC_{0\text{-}last}$ values obtained in humans after 8 days of once daily topical ocular dosing with 0.0015% tafluprost (the intended dose for marketing) = 5.67–9.69 pg x h/ml

Tafluprost was also administered by the topical ocular route in several repeated-dose topical ocular studies in monkeys, and the results from these studies most clearly delineate the potential for ocular tafluprost to cause systemic toxicity. In all of repeated-ocular dose toxicology studies, tafluprost administration did not produce systemic toxicity and the systemic exposure values for tafluprost acid in these studies greatly exceeded the expected clinical exposure (Table 96). These data strongly suggest that clinical administration of tafluprost by the topical ocular route is unlikely to cause systemic toxicity.

Table 96: Safety Analysis for Tafluprost-Related Systemic Toxicity Based on the Ocular Dose Toxicology Studies in Monkeys

Study No./ Description	NOAEL Dose (μg/kg/day)	NOAEL Dose C_{max} (ng/ml) for AFP-172	NOAEL Dose AUC (ng x h/ml) for AFP-172	Safety Factor Based on C_{max} [a]	Safety Factor Based on AUC [b]
TT #99-5553/ 28 day ocular toxicology study in monkeys	30 μg/day	7.51	1.53	239-308	158-270
TT #00-5537/ 13-week ocular toxicology study in monkeys	30 μg/day	5.81	1.39	185-238	143-245
TT #01-5531/ 52-week ocular toxicology study in monkeys	30 μg/day	4.38	1.23	139-179	127-217

[a] The range of C_{max} values obtained in humans after 8 days of once daily topical ocular dosing with 0.0015% tafluprost (the intended dose for marketing) = 24.42 -31.43 pg/ml

[b] The range of $AUC_{0\text{-}last}$ values obtained in humans after 8 days of once daily topical ocular dosing with 0.0015% tafluprost (the intended dose for marketing) = 5.67–9.69 pg x h/ml

Specific ocular toxicity did occur with ocular administration of tafluprost in the repeated-ocular dose toxicology studies. While the tafluprost-related ocular changes represent the main toxicological concern for clinical tafluprost administration, all of the ocular changes are consistent with ocular changes observed with other marketed $PGF_{2\alpha}$ analogues and appear to be related to the pharmacological effect of $PGF_{2\alpha}$. In the monkey study where tafluprost was administered alone and in combination with timolol, iridial darkening was associated with tafluprost but not timolol dosing. In the 52-week repeated ocular-dose study in monkeys, iridial darkening was observed in all tafluprost-treatment groups at tafluprost doses as low as 0.0005% administered twice daily. Other toxicities included sunken eyelids and blue gray discoloration of the lower eyelid at BID doses of ≥ 0.005% tafluprost. All of the ocular changes persisted after their initial appearance in the 52-week study. Sunken eyes and iridial darkening were also observed in a 13-week topical ocular study in monkeys, but in this study, the sunken eyelids were reversible, suggesting the possibility of recovery for this change with treatment of limited duration. Other ocular toxicities including pronounced inflammation or alterations in electroretinography did not occur with topical ocular administration of tafluprost at any of the administered doses.

All of the ocular effects noted to occur with ocular administration of tafluprost have also been observed with other marketed $PGF_{2\alpha}$ analogues including Xalatan® (latanoprost), Lumigan® (bimatoprost), and Travatan ® (travoprost). Two effects, iridial and lower eyelid darkening may be irreversible but not associated with loss of function. These

effects are noted in Section 5, Warnings and Precautions of the product labels for Xalatan®, Lumigan® and Travatan®.

Similarly, the other tafluprost-related ocular effect in the monkey studies, sunken upper eyelid was not associated with loss of function. This effect was reversible in the 13-week topical ocular monkey study. The product labels for Xalatan®, Lumigan® and Travatan® do not include warnings about sunken upper eyelid, but this effect is reported to occur in monkeys dosed with ocular Travatan®, and Lumigan® and is reported in the literature for patients treated with Travatan®, and Lumigan® (Nakakura et al., 2011). These effects along with the other ocular effect associated with the $PGF_{2\alpha}$ analogues, eyelash darkening and thickening, are considered to be mainly cosmetic, not associated with loss of function, and not toxicologically significant. With this in mind the high doses of tafluprost in the repeated-ocular toxicology studies can be considered the NOAEL doses and these doses can be used to determine the ocular human equivalent doses and margins of safety for the clinical dose intended for marketing (Table 97). Based on the ocular human equivalent dose of 30 μg/eye/day, the safety margin for ocular toxicity for the clinical dose intended for marketing is a substantial 67 fold value.

Table 97: Safety Analysis for Tafluprost-Related Ocular Toxicity Based on the Ocular-Dose Toxicology Studies in Monkeys

Study Nos./ Description	NOAEL dose	Ocular HED [a]	Human Ocular Dose [b]	Ocular Safety Margin
TT #99-5553, TT #00-5537, TT #01-5531/ 28-day, 13-week and 52-week ocular toxicology studies in monkeys	30 μg/day/eye	30 μg/day/eye	0.45 μg/eye/day	67
[a] Assumes approximately equal corneal surface areas for monkeys and humans. [b] Assumes a single ocular dose of 0.0015% tafluprost in a 30 μl drop size.				

Tafluprost was tested for genetic toxicity in three GLP-compliant assays. These included an *in vitro* bacterial reverse mutation assay (Ames assay), an *in vitro* chromosome aberration assay in cultured Chinese Hamster lung cells, and an *in vivo* mouse bone marrow micronucleus assay. For the *in vitro* assays, metabolic activation was carried out using S9 rat liver metabolic activation. Because all human tafluprost metabolites in hepatocyte assays were also found in rat hepatocyte incubations, it is assumed that the rat S9 activations generated metabolites in the *in vitro* genetic toxicology studies that were relevant to humans. However, metabolic analysis for tafluprost was not performed in mice. Thus it is uncertain if all of the human metabolites were present in the mouse micronucleus assay. Tafluprost was negative for genetic toxicity in all three assays. In the Ames assay, tafluprost at concentrations up to 5000 μg/plate did not induce mutation in *Salmonella typhimurium* (TA98, TA100, TA 1535,

and TA 1537) and *Escherichia coli* (WP2 uvrA) strains in the absence and presence of S9 metabolic activation. In the chromosome aberration assay, tafluprost at concentrations extending to cytotoxicity did not induce chromosome aberrations in the absence and presence of S9. In the mouse micronucleus assay, mice were dosed with a single intraperitoneal injections of 175, 350 and 700 mg/kg tafluprost with the high-dose based on range-finding data indicating that substantial mortality at a dose of ≥ 1000 mg/kg. Tafluprost did not induce micronuclei in the polychromatic erythrocytes of mouse bone marrow at any of the tested doses.

Two carcinogenicity studies were performed, a 24-month study in rats and a 78-week study in mice. Both studies employed subcutaneous dosing and AFP-172 exposure was confirmed with toxicokinetic analysis. In addition, 13-week, subcutaneous dosing, range-finding assays were performed in both species to aide in selection of the high doses for the definitive carcinogenicity studies.

One consideration in determining the suitability of the species used in the carcinogenicity studies is the comparability of the human metabolic profile and that of the test species. The primary metabolite of tafluprost in rats, and humans was shown to be AFP-172. In addition, in human and rat hepatocyte incubation experiments, all of putative human metabolites were observed in rat hepatocyte incubations. Thus the tafluprost metabolites assessed in the rat carcinogenicity study are expected to encompass those seen in humans. AFP-172 was also formed in mice but hepatocyte metabolic profiling of tafluprost was not performed in mice. Thus it is possible that unlike rats, mouse tafluprost metabolites may not encompass all of the major human metabolites, and this is a potential limitation for the mouse carcinogenicity study.

The rat carcinogenicity study employed doses of 3, 9, and 30 μg/kg/day. The high dose selected for this study was based on the comparative exposure levels for AFP-172 in rats and humans. According to the ICH Guidance for Industry “SIC(R2) Dose Selection for Carcinogenicity Studies” “a selection of a high dose for carcinogenicity studies that represents a 25 to 1 exposure ratio of rodent to human AUC of parent compound and/or metabolites is considered pragmatic.” As shown in Table 98 below, initially in the 13-week range-finding assay, with later confirmation in the definitive 24 month rat cancer study, the AUC exposure in rats was shown to be more than 500 fold greater than the AUC exposure in humans after 8 days of once daily topical ocular dosing with the intended dose for marketing (0.0015% tafluprost).

Table 98: The AUC Exposure Ratio for AFP-172 Following Subcutaneous Dosing in Rats Versus Topical Ocular Dosing in Humans.

Study No./ Description	Species/ High Dose	High Dose $AUC_{(0-2h)}$ (ng x h/ml) for AFP-172	Human $AUC_{(0-t)}$ (pg x h/ml) for AFP-172[c]	Ratio of Rat to Human AUC
TT #03-5574/ 13-Week Rat Range-Finding Study	Rat/ 30 μg/kg/day (Subcutaneous)	5.97[a]	5.67–9.69	616-1053
TT #03-5575/ 24-Month Rat Carcinogenicity Study	Rat/ 30 μg/kg/day (Subcutaneous)	11.9[b]		1228-2099

[a] The mean male and female $AUC_{(0-2h)}$ value in Week 13.
[b] The mean male and female $AUC_{(0-2h)}$ value in Week 26.
[c] Range of AUC values obtained in humans after 8 days of once daily topical ocular dosing with 0.0015% tafluprost (the intended dose for marketing).

Also, in serum albumin binding studies, AFP-172 was shown to be approximately 94% bound to rat serum albumin, and 99% bound to human serum albumin. These results suggest that the percentage of free AFP-172 in plasma is similar for both rats and humans and that the rat/human ratio of AUC exposure for total AFP-172 is representative of the same ratio for free AFP-172. Based on the AUC exposure ratio and the similar serum albumin binding patterns in rats and humans, the high dose of tafluprost used in the rat carcinogenicity study is considered justified.

In addition to the justification for the dose selection, the rat carcinogenicity study was adequately conducted in terms of duration, the number of male and female animals in each study group (60/sex), dosing schedule, and route of administration, In the rat carcinogenicity study, no AFP-168-related increase in the incidence of tumors was considered to have occurred indicating a negative potential for carcinogenicity in this study.

The mouse carcinogenicity study employed doses of 10, 30, and 100 μg/kg/day. The criteria used in the selection of the high dose for the mouse carcinogenicity study was the same as that used for the rat study. As with the rat, the AUC exposures for AFP-172 in the 13-week mouse range-finding study and the mouse carcinogenicity study were shown to be more than 500 fold greater than the AUC exposure in humans after 8 days of once daily topical ocular dosing with the intended dose for marketing (0.0015% tafluprost). These results are summarized below in Table 99.

Table 99: The AUC Exposure Ratio for AFP-172 Following Subcutaneous Dosing in Mice Versus Topical Ocular Dosing in Humans.

Study No./ Description	Species/ High Dose	High Dose AUC (ng x h/ml) for AFP-172	Human AUC (pg x h/ml) for AFP-172	Ratio of Mouse to Human AUC
TT #03-5576/ 13-Week Mouse Range-Finding Study	Mouse/ 100 µg/kg/day (subcutaneous)	6.44 [a]	5.67–9.69 [c]	663-1136
TT #04-5572/ 78-Week Carcinogenicity Study	Mouse/ 100 µg/kg/day (subcutaneous)	9.81 [b]		1228-1730

[a] The mean male and female $AUC_{(0\text{-}2h)}$ value in Week 13.
[b] The mean male and female $AUC_{(0\text{-}t)}$ value in Week 26.
[c] Range of $AUC_{0\text{-}last}$ values obtained in humans after 8 days of once daily topical ocular dosing with 0.0015% tafluprost (the dose intended for marketing).

Analysis of AFP-172 binding to mouse plasma proteins was not performed. However, 99% of AFP-172 was shown to bind to human serum albumin, indicating that a higher percentage of AFP-172 could not practically bind to mouse serum albumin. Note 8 of the ICH Guidance "S1C(R2) Dose Selection for Carcinogenicity Studies" indicates that "…when protein binding is high and the unbound fraction of drug is greater in rodents than in humans, the comparison of total plasma concentration of drug is appropriate." This Guidance statement indicates that the mouse/human ratio of AUC exposure for total AFP-172 can be considered to be representative of the same ratio for free AFP-172.

As in the rat study, in the mouse carcinogenicity study, no unusual tumors or significantly increased tumor incidence suggestive of AFP-168-related carcinogenesis was observed compared to the vehicle control groups. However, unlike the rat carcinogenicity study, the mouse carcinogenicity study was considered deficient in two respects. The mouse carcinogenicity study was of 78-weeks duration instead of the normal duration of two years. Also the primary metabolite, AFP-172 was measured in mice, but the identity of additional mouse metabolites and whether they encompassed the full range of human metabolites including the high percentage human metabolite, dinor-AFP-172, is unknown.

The CDER Executive Carcinogenicity Assessment Committee reviewed the carcinogenicity studies and findings for NDA 202514 on May 3rd, 2011. The Committee recommendations and conclusions included the following. For the rat carcinogenicity study, the committee concurred that the study was adequate and that there were no tafluprost-related neoplasms. For the mouse carcinogenicity study, the committee concurred that there were no tafluprost-related neoplasms but that the study was not

adequate due to its non-standard duration of only 1.5 years, and incomplete evaluation of the low and mid-dose group for many tissues.

Despite the inadequate design of the mouse carcinogenicity study, the Committee concurred that sufficient carcinogenicity testing had been conducted for tafluprost administered in the clinical setting by the topical ocular route at the maximum recommended human dose (MRHD). Factors influencing this decision were the very low systemic exposure associated with the expected MRHD, negative genetic toxicology findings, and the lack of neoplasm findings in carcinogenicity studies for three other marketed drugs in the same pharmacological class.

The Committee recommended that due to the inadequate design of the mouse carcinogenicity study, the drug label for tafluprost ophthalmic solution should not include mention of the mouse carcinogenicity study in Section 13.1, Carcinogenesis, Mutagenesis, Impairment of Fertility.

The reproductive and developmental toxicology associated with tafluprost administration was assessed in a fertility study in rats, embryo fetal development studies in rats and rabbits, and a pre- post-natal development study in rats.

AFP-168 administered intravenously in doses of up to 100 μg/kg/day did not affect the fertility of male and female rats. Dosing occurred for 2 weeks before pairing, throughout the pairing period, and until Gestation Day 6 in females or sacrifice in Week 9 of the treatment period in males. No AFP-168 effects on mating behavior were observed, and the mating index was 100% for all groups. Similarly the fertility and fecundity indexes were between 90 and 100% for all groups. In females the estrus cycle regularity was not affected by treatment, and there were no effects on the outcome of pregnancy. All pregnant females had live embryos, and the mean number or corpora lutea, implantations per female, and pre- and post-impantation loss in treated animals was similar to or less than that of the controls. In males testes staging did not indicate any AFP-168-related abnormalities in any cell cycle types present within the different stages of the spermatogenic cycle and the proportion of tubules at specific points in the cycle were consistent with the expected range.

Embryo-fetal development studies were conducted in rats and rabbits. In a preliminary range-finding assay in rats, a dose of 100 μg/kg/day was associated with early and late resorptions, post-implantation loss and decreased numbers of live fetuses. In the main study, intravenous doses of 0, 3, 10 and 30 μg/kg/day were administered from Gestation Days 6 to 17. At AFP-168 doses of ≥ 10 μg/kg/day, lower mean fetal body weights and reduced ossification of the fifth sternebra were observed. At the high dose of 30 μg/kg/day, skeletal malformations included malformations in the vertebral column, and a greater number of variations of the lumbar and thoracic vertebrae. The NOAEL for fetal toxicity was considered to be 3 μg/kg/day and 30 μg/kg/day for maternal toxicity.

In a preliminary dose-range finding study in rabbits increased intrauterine deaths were observed in pregnant females administered ≥ 1 μg/kg/day AFP-168, and a dose range of 0, 0.03, 0.1, and 0.3 μg/kg/day, administered from Gestation Days 7 to 19, was chosen for the main study. In this study, the intermediate and high doses of 0.1 and 0.3 μg/kg/day respectively experienced significantly more post-implantation loss compared to controls. In the intermediate dose group only two litters contained live fetuses, and in the high-dose group there were no live fetuses. In the low-dose group, the majority of litters were viable, but three fetuses in separate litters had abdominal wall malformations, and six fetuses in five litters had cranial and/or spinal malformations. While no maternal toxicity occurred, the NOAEL value for fetal toxicity was considered to be less than the low dose of 0.03 μg/kg/day.

In a second embryo-fetal study in rabbits, no individual fetal malformation was significantly increased by any of the intravenous doses of AFP-168 (0.001, 0.003, and 0.01 μg/kg/day) compared to the control group. Also the number of fetuses with external, visceral, and skeletal variations in all the AFP-168 treatment groups was generally similar to controls and no maternal toxicity was observed. The NOAEL dose was considered to be the high dose (0.01 μg/kg/day). At this dose, plasma concentrations of tafluprost acid (AFP-172) were consistently below the limit of detection (20 pg/ml).

A preliminary pre-post-natal development study in rats was conducted to determine the doses for the main study. In the range-finding study, intravenous AFP-168 suppressed maternal body weight gains and reduced F_1 generation viability at ≥ 3 μg/kg/day. In the definitive study, tafluprost was administered intravenously to female rats at dose levels of 0.3, 1, 3, and 10 μg/kg/day from Day 6 of gestation to Day 20 of lactation. At tafluprost doses of ≥ 1 μg/kg/day, some F_0 females exhibited poor nursing behavior resulting in decreased F_1 offspring viability on Day 4 of lactation. In the high-dose group, delayed pinna unfolding on Lactation Day 3 as well as increased F_1 newborn mortality and decreased body weights were observed at a tafluprost dose of 10 μg/kg/day. However, no tafluprost-related effects on physical development, sensory function, genital development, or fertility were noted for the F_1 generation. The NOAEL was considered to be 0.3 mg/kg/day for nursing and development of the F_1 generation. In the F_2 generation, no significant differences in embryonic mortality or the number of corpora lutea, implantation,s, live F_2 embryos, or pre-implantation loss were noted for any of the tafluprost treatment groups. However, F_2 generation body weights, male/female ratio were not assessed, and F_2 fetuses were not externally evaluated for malformations.

In conclusion, 0.0015% topical ocular tafluprost is approvable from a Pharmacology/Toxicology perspective for topical ocular administration in a preservative-free formulation. Tafluprost-related systemic toxicity is not expected to be a clinical concern because of the very low systemic exposure associated with the expected clinical dose. Specific ocular effects (iridial darkening, sunken eyelids, eyelid and eyelash darkening) are expected to accompany clinical dosing but these effects are considered to be mainly cosmetic and have also been noted with three other marketed

drugs from the same pharmacologic class. Tafluprost was not mutagenic in genetic toxicology studies and was negative for carcinogenicity in rat and mouse studies. Tafluprost is not expected to alter male or female fertility, but it was teratogenic in rat and rabbit embryo-fetal studies at low multiples of the human dose and the product label will include this information.

Citations

1. Nakakura S*. et al.*: Latanoprost therapy after sunken eyes caused by travoprost or bimatoprost. Optom Vis Sci, epub ahead of print, 2011.

12 Appendix/Attachments

Table 100: Hematology and Coagulation Parameter Table

Study No.	TT #01-5526	TT #01-5530	TT #01-5531
Species	**rat**	**rat**	**monkey**
Hemoglobin concentration	X	X	X
Packed cell volume	X	X	X
Mean cell volume	X	X	
Mean cell hemoglobin concentration	X	X	
Hematocrit			
Erythrocyte count	X	X	X
Platelet count	X	X	X
Platelet crit	X	X	
Platelet distribution width	X	X	
Mean platelet volume	X	X	
Mean corpuscular volume			X
Mean corpuscular hemoglobin			X
Mean cell hemoglobin	X	X	
Hemoglobin distribution width	X	X	
Mean corpuscular hemoglobin concentration			X
Red cell distribution width	X	X	
Total leukocyte count	X		X
Reticulocyte count	X	X	X
Reticulocyte hemoglobin content			
Differential leukocyte count (Absolute neutrophil, lymphocyte, monocyte, eosinophil, basophil counts)	X	X	

Blood smear for cell morphology (if necessary for interpretation)	X		
Prothrombin time	X	X	X
Activated partial thromboplastin time	X	X	X

Table 101: Clinical Chemistry Parameter Table

Study No.	**TT #01-5526**	**TT #01-5530**	**TT #01-5531**
Species	**rat**	**rat**	**monkey**
Aspartate aminotransferase	X	X	X
Alanine aminotransferase	X	X	X
Alkaline phosphatase	X	X	X
Blood urea nitrogen			
Creatinine	X	X	X
Glucose	X	X	X
Cholesterol	X	X	X
Triglycerides	X	X	X
Total protein	X	X	X
Protein electrophoresis	X	X	X
Albumin		X	X
Total bilirubin	X	X	X
Sodium	X	X	X
Potassium	X	X	X
Chloride	X	X	X
Calcium	X	X	X
Inorganic phosphorus	X	X	X
Gamma-glutamyl transferase		X	X
Glutamate dehydrogenase			
Globulin		X	X
Albumin/globulin ratio	X	X	X
Urea	X	X	X

Table 102: Histopathology and Organ Weight Inventory Table

Study	**TT #11-7800**	**TT #01-5526**	**TT #01-5530**	**TT #01-5531**
Species	**monkey**	**rat**	**rat**	**monkey**
Adrenals	*	X*	X*	X*
Aorta			X	X
Bone Marrow			X	X
Bone (femur)		X	X	X
Bone (rib)			X	

Bone (tibia and femorotibial joint)				
Brain	*	X*	X*	X*
Cecum		X	X	X
Cervix	*			
Colon		X	X	X
Duodenum		X	X	X
Epididymis	*	X*	X*	X*
Epiglottis			X	
Esophagus		X	X	X
Extraorbital lacrimal gland				
Eye	X	X	X	X
Eyelash	X			X
Eyelids (upper and lower)	X		X	X
Third eyelid			X	
External ear				
Fallopian tube				
Gall bladder	*		X	X
Gross lesions	X	X	X	X
Harderian gland		X		
Heart	*	X*	X*	X*
Hypophysis				
Ileum		X	X	X
Injection site		X	X	
Jejunum		X	X	X
Kidneys	*	X*	X*	X*
Lacrimal gland	X	X	X	X
Larynx		X	X	
Liver	*	X*	X*	X*
Lungs		X*	X	X*
Lymph nodes, bronchial				
Lymph nodes mandibular		X	X	X
Lymph nodes, mesenteric		X	X	X
Lymph nodes, superficial inguinal				
Mammary Gland		X		X
Nasal cavity		X		
Nasal turbinates				
Optic nerves	X	X	X	X

Ovaries	*	X*	X*	X*
Palpebral conjunctiva				X
Pancreas		X	X	X
Parathyroid	*	X*	X*	X*
Parotid Glands				
Peripheral nerve				
Peyer's Patch				
Pharynx		X		
Pituitary	*	X*	X*	X*
Prostate	*	X*	X*	X*
Rectum			X	X
Salivary gland		X*	X*	X*
Sciatic nerve		X	X	X
Seminal vesicles	*	X*		X
Skeletal muscle			X	X
Skin		X	X	X
Spinal cord		X	X	X
Spleen	*	X*	X*	X*
Sternum		X	X	X
Stomach		X	X	X
Testes	*	X*	X*	X*
Thymus	*	X*	X*	X*
Thyroid	*	X*	X*	X*
Tongue		X*	X	X
Trachea		X*	X	X
Ureter				
Urinary bladder		X*	X	X
Uterus	*	X*	X*	X*
Vagina		X	X	X
Zymbal gland		X		

X, histopathology performed
*, organ weight obtained

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

JAMES S WILD
07/19/2011

WENDELYN J SCHMIDT
07/20/2011

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 202514 **Applicant: Merck Sharp & Dohme Corp.** **Stamp Date: January 7, 2011**

Drug Name: Tafluprost (Saflutan™) **NDA/BLA Type: NDA**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?		X	The following studies are missing: 1. An *in vitro* plasma protein binding study using plasma from appropriate nonclinical species and humans. An *in vitro* albumin binding study was submitted for rat, rabbit, dog and human serum albumin and this may suffice. However, mouse albumin binding was not determined and mouse plasma exposure is used to determine the high dose in the mouse cancer study. 2. Repeated-dose ocular toxicology studies of sufficient duration in two species. However, 1-, 3-, and 12 month ocular studies were conducted in monkeys and 6 and 9 month intravenous studies were conducted in rats and dogs.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		1. The new formulation to be marketed is without the preservative, benzalkonium chloride. Some, but not all of the pivotal nonclinical studies used a benzlkonium chloride containing solution, but at the End of Phase II meeting on 8/3/2009, the FDA indicated that the studies conducted with the benzalkonium chloride

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
				containing solution would support the new formulation without benzalkonium chloride. 2. Many of the pivotal toxicology studies (general toxicology, in vivo genetic toxicology, carcinogenicity, and reproductive toxicology) did not use the formulation to be marketed. However, all of the excipients in the formulation to be marketed have been previously used in marketed ophthalmic drugs at equal or greater concentrations and the excipients are further qualified by their use in the clinical studies supporting NDA 202514.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		1. No special studies were requested
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?		X	1. Dose multiple comparisons are absent or based on plasma C_{max} levels.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?		X	1. There is no apparent abuse potential.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			NA

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? __Yes_____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

1. A potential review issue concerns the fact that mouse plasma exposure is used to justify the high dose in the mouse cancer study, but mouse plasma protein binding and mouse metabolic profiles have not been determined.

James S. Wild	3/22/2011
Reviewing Pharmacologist	Date
Wendelyn J. Schmidt	3/22/2011
Team Leader/Supervisor	Date

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

JAMES S WILD
03/22/2011

WENDELYN J SCHMIDT
03/22/2011