CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

022445Orig1s000

NON-CLINICAL REVIEW(S)

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

FROM: Sushanta Chakder, Ph.D., Supervisory Pharmacologist

DATE: July 21, 2016

Application number: NDA 22445

Supporting Document: 078

Date of submission: July 17, 2015

Sponsor: Heron Therapeutics, Inc.

Drug Product: Sustol®/APF530

Comments:

Sustol (APF 530) has been developed for the prevention of chemotherapy-induced nausea and vomiting (CINV). Sustol is a new polymeric subcutaneous formulation of granisetron. APF 530 consists of granisetron and a polymer vehicle (b) (4). The primary component of the polymer is a triethylene glycol poly(ortho ester) polymer (TEG-POE (b) (4) (80%)) and a minor component (methoxy polyethylene glycol, MPEG, 20%). The polymer is designed for sustained release of granisetron following slow hydrolysis of the polymer after SC administration.

Carcinogenicity findings of Granisetron:

In a 24-month carcinogenicity study in rats statistically significant increases in the incidence of hepatocellular carcinomas and adenomas were observed in male rats at 5 mg/kg/day and above, and in female rats treated at 25 mg/kg/day. There was a no increase in liver tumors at a dose of 1 mg/kg/day, which is 16 times the recommended human dose based on body surface area in males and at 5 mg/kg/day (81 times the recommended human dose based on body surface area) in females. The hepatocellular tumor findings in male and female rats are not relevant to humans because hepatocellular tumors are common tumors in rodents, usually occur by modes of action

not relevant to humans, and the tumor incidences in male and female rats were observed at doses which provide ≥81 times exposure multiples for the oral clinical dose and ≥34 times the subcutaneous dose.

Granisetron was not mutagenic in an in vitro Ames test or the mouse lymphoma cell forward mutation assay, or genotoxic in the *in vivo* mouse micronucleus test and the in vitro and ex vivo rat hepatocyte UDS assays. However, it produced a significant increase in unscheduled DNA synthesis in HeLa cells in vitro and a significant increased incidence of cells with polyploidy in an in vitro human lymphocyte chromosomal aberration test. By a weight of evidence approach outlined in the ICH S2(R1) Guidance: Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use, granisetron is not considered genotoxic.

Since, granisetron had no genotoxic potential in a standard battery of genotoxicty assays which includes Ames test and the in vivo micronucleus test, and hepatocellular tumors were observed in rats at high multiples of human dose, there does not appear to be a human risk for carcinogenicity findings with granisetron in rats.

Safety of APF 530 (polymer vehicle):

Acute and subacute/subchronic (28- and 90-day studies in rats and dogs) toxicology, genotoxicity, and reproductive toxicology studies were conducted with the polymer, (b) (4). In toxicology studies, the major target organ was the injection site (inflammatory reactions). In the 90-day daily SC administration toxicology study in rats, lymphoid hyperplasia in the draining lymph nodes, splenic hematopoiesis and bone marrow myeloid hyperplasia were observed. However, similar histopathological changes were not observed following SC administration of the polymer once every 72 hours in the 28-day toxicology study in rats. In addition, similar findings were not observed in dogs in the 4-week or 3-month toxicology studies. Thus, the findings in rats in the 3-month toxicity study following daily SC administration are likely related to the injection site inflammatory reactions.

(b) (4) was negative in the Ames test, the mouse lymphoma assay, and the *in vivo* rat bone marrow micronucleus test. APF530 (Sustol) was also negative in the standard battery of genotoxicity studies.

In reproductive toxicology studies, (b) (4) had no adverse effects on fertility and early embryonic development in male and female rats and were not teratogenic in rats and rabbits. In a pre- and postnatal development study in rats, (b) (4) did not cause any significant adverse effect on pre- and postnatal developmental parameters.

Safety of the putative breakdown products of (b) (4)

Based on the metabolic fate study in humans, the putative breakdown products of TAG-POE (triethylene glycol poly(ortho ester)/ (b) (4) include: pentaerythitol, triethylene glycol, propionic acid and glycolic acid. Animals used in the toxicology studies would have been subject to similar metabolic fate and exposed to these putative breakdown products as well. A safety assessment of the breakdown products of (b) (4), based on available published literature, does not suggest that any of these breakdown products have a carcinogenic potential (See Pharmacology review of Tamal Chakraborti, Ph.D dated June 10, 2016).

Triethylene Glycol was not found to be genotoxic in the following genotoxicity assays: Ames test, Chinese hamster ovary (CHO) cell gene mutation assay, chromosomal aberration assay in CHO cells and sister chromatid exchange assays (Final Report on the Safety Assessment of Triethylene Glycol and PEG-4, Int J Toxicol, 25 (Suppl.2): 121-138, 2006; Reregistration Eligibility Decision (RED) for Triethylene Glycol, USEPA, September 26, 2003).
In addition, triethylene glycol had no carcinogenicity potential in studies conducted at doses up to the testing limit established by the US EPA (Reregistration Eligibility Decision (RED) for Triethylene Glycol, USEPA, 2003).

Pentaerythritol was not genotoxic in the Ames test and chromosomal aberration assay in Chinese hamster lung (CHL) cells [Organization for Economic Cooperation and Development (OECD), Screening Information DataSet (SIDS), Pentaerythritol, SIDS Initial Assessment Report for 8th SIAM, 1998]. It was not carcinogenic in rats and mice in 2-year carcinogenicity studies (J Appl Toxicol, 10(5):353-357; National Toxicology Program (NTP), U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Toxicology and Carcinogenesis Studies of Pentaerythritol Tetranitrate in F344/N Rats and B6C3F1 Mice, Technical Report Series, No. 365, August 1989, NIH Publication No. 89-2820).

Propionic acid was not genotoxic in the Ames test and an in vivo micronucleus test using male and female Chinese hamsters (OECD, SIDS, SIAM 25, 2007). In addition, the European Food Safety Authority (EFSA) Panel on Food Additives and Nutrient Sources added to Food (ANS) concluded that there is no concern with respect to genotoxicity and carcinogenicity for propionic acid (Scientific Opinion on the Re-Evaluation of Propionic Acid (E 280), Sodium Propionate (E 281), Calcium Propionate (E 282) and Potassium Propionate (E 283) as Food Additives, EFSA Journal, 12(7):3779, 2014).

Glycolic acid was not genotoxic in the Ames test (European Food Safety Authority (EFSA), Glycolic Acid, Scientific Opinion on the Safety Evaluation of the Substance Glycolic Acid for use in Food Contact Materials, EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), EFSA Journal 8(12):1927, 2010). In a chronic dietary carcinogenicity study in rats, there were no tumor findings in male and female rats [Cosmetic Ingredient Review; Final Report on the Safety Assessment of Glycolic Acid, Ammonium, Calcium, Potassium, and Sodium Glycolates, Methyl, Ethyl, Propyl, and Butyl Glycolates, and Lactic Acid, Ammonium, Calcium, Potassium, Sodium, and TEA-Lactates, Methyl, Ethyl, Isopropyl, and Butyl Lactate, and Lauryl, Myristyl, and Cetyl Lactates, Journal of American

College of Toxicology 17(Suppl 1):1-242, 1998). In addition, the US EPA has not classified glycolic acid as a human carcinogen, and the NTP has not listed glycolic acid as a human carcinogen. (National Library of Medicine Hazardous Substances Data Bank, Glycolic Acid)

Conclusions:

Based on the available information on the polymer vehicle (b) (4) and its putative breakdown products, the polymer vehicle does not appear to be a carcinogenic risk in subjects receiving Sustol. The API, granisetron, also does not pose a carcinogenic risk to humans. Granisetron, was not genotoxic in the Ames test ; the *in vivo* mouse micronucleus test and the in vitro and *ex vivo* rat hepatocyte UDS assays. The hepatocellular tumors observed in the 2-year daily oral doing carcinogenicity study of granisetron in rats were likely due to a mode of action /pathway not relevant to humans and just as important, the tumors were observed only at extremely high multiples of human exposure. Thus, a warning in the label for cancer risk for Sustol, (Granisetron in a polymeric vehicle formulation), in patients receiving the drug is not warranted.

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/s/

SUSHANTA K CHAKDER
07/21/2016

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	22445
Supporting document/s:	078
Type of Submission:	Resubmission/Class 2
Applicant's letter date:	July 17, 2015
CDER stamp date:	July 17, 2015
Product:	Sustol®/APF530
Indication:	Chemotherapy Induced Nausea and Vomiting (CINV)
Applicant:	Heron Therapeutics, Inc.
Review Division:	DGIEP
Reviewer:	Tamal Chakraborti, PhD
Supervisor/Team Leader:	Sushanta Chakder, PhD
Division Director:	Donna Griebel, MD
Project Manager:	Brian Strongin, RPh, MBA

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Introduction

In the polymer metabolic fate study (C2011-02) with APF530 in adult (18-50 years) healthy male and female subjects, putative breakdown products of the novel TEG-POE [triethylene glycol poly(ortho ester) (b) (4)] included pentaerythritol (PE), triethylene glycol (TEG), propionic acid (PA) and glycolic acid (GA). In the plasma, the concentrations of all the above putative polymeric breakdown products PE, TEG, PA and GA, were all below the limit of detection (clinical pharmacology review dated March 14, 2013 by Sandhya Apparaju, PhD). However, per the above clinical pharmacology review, detectable concentrations of PE, TEG and GA were observed in the urine samples. Propionic acid (PA) could not be detected in the urine samples possibly due to lack of adequate assay sensitivity.

We conducted an independent review of the available published literature to assess the genotoxic and carcinogenic potential of pentaerythritol (PE), triethylene glycol (TEG), propionic acid (PA) and glycolic acid (GA). This is discussed below.

Triethylene Glycol: Triethylene Glycol was not mutagenic or genotoxic in the Ames test, Chinese hamster ovary (CHO) mutation assay, chromosomal aberration assay using CHO cells and sister chromatid exchange assays [Final Report on the Safety Assessment of Triethylene Glycol and PEG-4, Int J Toxicol, 25 (Suppl.2): 121-138, 2006; Reregistration Eligibility Decision (RED) for Triethylene Glycol, USEPA, 2003]. The above United States Environmental Protection Agency (USEPA) document stated that a review of the available data has shown triethylene glycol to be negative for carcinogenicity in studies conducted up to the testing limit doses established by the USEPA [Reregistration Eligibility Decision (RED) for Triethylene Glycol, USEPA, 2003].

Pentaerythritol (PE): Pentaerythritol was negative in the Ames test and chromosomal aberration assay in Chinese hamster lung (CHL) cells [Organization for Economic Cooperation and Development (OECD), Screening Information DataSet (SIDS), Pentaerythritol, SIDS Initial Assessment Report for 8th SIAM, 1998]. There was no evidence of toxicity or carcinogenicity of pentaerythritol tetranitrate given (up to 10,000 ppm or approximately 500 mg/kg in male rats and male and female mice and up to 2500 ppm or approximately 125 mg/kg in female rats) in the diet to F344 rats and B6C3F1 mice for up to two years. In the above study, there were no significant treatment related neoplastic or non-neoplastic lesions in both species [Bucher JR, et al., 1990, No Evidence of Toxicity or Carcinogenicity of Pentaerythritol Tetranitrate given in the Diet to F344 rats and B6C3F1 Mice for up to two years, J Appl Toxicol, 10(5):353-357; National Toxicology Program (NTP), U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Toxicology and Carcinogenesis Studies of Pentaerythritol Tetranitrate in F344/N Rats and B6C3F1 Mice, Technical Report Series, No. 365, August 1989, NIH Publication No. 89-2820].

Propionic Acid (PA): Propionic acid was negative in the Ames test, in a DNA repair assay using *Escherichia coli* in the presence of metabolic activation, but displayed a non-dose-related positive response in the absence of metabolic activation. Propionic acid was also negative in an in vivo micronucleus test using male and female Chinese hamsters. Based on these results, propionic acid has shown no potential to induce gene mutations or chromosomal aberrations (OECD, SIDS, SIAM 25, 2007). The European Food Safety Authority (EFSA) Panel on Food additives and Nutrient Sources added to Food (ANS) considered that there is no concern with respect to genotoxicity and carcinogenicity for propionic acid [Scientific Opinion on the Re-Evaluation of Propionic Acid (E 280), Sodium Propionate (E 281), Calcium Propionate (E 282) and Potassium Propionate (E 283) as Food Additives, EFSA Journal, 12(7):3779, 2014].

Glycolic Acid: Glycolic acid was not found to be genotoxic based on negative Ames test with and without metabolic activation using *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and TA1538 (EFSA, Glycolic Acid, Scientific Opinion on the Safety Evaluation of the Substance Glycolic Acid for use in Food Contact Materials, EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), EFSA Journal 8(12):1927, 2010). In a chronic dietary exposure/carcinogenicity study in rats, animals were fed 1% (10,000 ppm or approximately 500 mg/kg/day) and 2% (20,000 ppm or approximately 1000 mg/kg/day) glycolic acid for 218-248 days. In the above study, there were no tumor findings in male and female rats [NIH PubChem, Glycolic acid, Compound Summary for CID 757; Hazardous Substances Data Bank (HSDB), Hydroxyacetic Acid; Cosmetic Ingredient Review: Final Report on the Safety Assessment of Glycolic Acid, Ammonium, Calcium, Potassium, and Sodium Glycolates, Methyl, Ethyl, Propyl, and Butyl Glycolates, and Lactic Acid, Ammonium, Calcium, Potassium, Sodium, and TEA-Lactates, Methyl, Ethyl, Isopropyl, and Butyl Lactate, and Lauryl, Myristyl, and Cetyl Lactates, Journal of American College of Toxicology 17(Suppl 1):1-242, 1998). The US EPA has not classified glycolic acid as a human carcinogen. The Department of Health and Human Services (DHHS), National Toxicology Program (NTP) has not listed glycolic acid as a human carcinogen (National Library of Medicine Hazardous Substances Data Bank, Glycolic Acid).

Recommendations

None

Additional Non Clinical Recommendations

None

Integrated Summary and Safety Evaluation

Based on the information available in the published literature, the putative breakdown products (pentaerythritol, triethylene glycol, propionic acid and glycolic acid) of the novel TEG-POE polymer used in Sustol do not appear to have genotoxic or carcinogenic potential.

Appendix/Attachments

None

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/s/

TAMAL K CHAKRABORTI
06/10/2016

SUSHANTA K CHAKDER
06/10/2016

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	22445
Supporting document/s:	124
Type of Submission:	Resubmission/Class 2
Applicant's letter date:	June 2, 2016
CDER stamp date:	June 2, 2016
Product:	Sustol®/APF530
Indication:	Chemotherapy Induced Nausea and Vomiting (CINV)
Applicant:	Heron Therapeutics, Inc.
Review Division:	DGIEP
Reviewer:	Tamal Chakraborti, PhD
Supervisor/Team Leader:	Sushanta Chakder, PhD
Division Director:	Donna Griebel, MD
Project Manager:	Brian Strongin, RPh, MBA

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Introduction

In this submission, the Applicant provided a safety assessment of pentaerythritol (PE) and propionic acid (PA) as requested by the Agency on May 25, 2016. The Division asked the Applicant to submit a safety assessment of pentaerythritol and propionic acid, as it relates to the polymer contained in the formulation, based on the information available in the literature.

Brief Discussion of Nonclinical Findings

The Applicant did not submit any nonclinical study report in this submission.

Recommendations

None

Additional Non Clinical Recommendations

None

Studies Submitted

Studies Reviewed

The Applicant did not submit any nonclinical study report in this submission. The Applicant provided safety assessment of pentaerythritol and propionic acid as requested by the Agency on May 25, 2016.

Studies Not Reviewed

N/A

Integrated Summary and Safety Evaluation

Assuming complete hydrolysis, a 500 mg dose of Sustol will produce 132 mg of pentaerythritol (approximately 19 mg/day or 0.32 mg/kg/day based on once weekly dosing) and 143 mg of propionic acid (approximately 20 mg/day or 0.33 mg/kg/day based on once weekly dosing).

Pentaerythritol: Pentaerythritol (CAS # 115-77-5) is a polyol, highly soluble in water, and used as a building block for numerous industrial applications. The structure of pentaerythritol is shown below (from page 3 of Section 1.11.2 of the submission).

Figure 1.11.2-1. Structure of pentaerythritol

The oral LD_{50} value for pentaerythritol in rats ranged from >2 g/kg to >5 g/kg. Pentaerythritol was not mutagenic in the Ames test and chromosomal aberration assays. In mice, pentaerythritol was absorbed and excreted rapidly following oral administration. Approximately, 68% of the orally administered pentaerythritol appeared in the urine of mice. In dogs, approximately 70% of the administered pentaerythritol was excreted in the urine within 24 hours following oral administration. Feeding studies in human volunteers revealed that about 85% of pentaerythritol fed was eliminated unchanged in the urine. In a 90-day oral toxicity study (Organization for Economic Co-operation and Development (OECD), Screening Information DataSet (SIDS), Pentaerythritol, SIDS Initial Assessment Report For 8th SIAM, 1998) in rats with a mixture of mono- (88%) and dipentaerythritol (12%), the Lowest Observed Effect Level (LOEL) was 5.0% (50,000 ppm, equivalent to 2500 mg/kg/day). The Permitted Daily Exposure (PDE) for pentaerythritol was calculated to be approximately 20 mg/day using the LOEL of 2500 mg/kg/day as follows.

PDE = (2500x50) ÷ (5x10x5x2.5x10) = 20 mg/day (F1 = 5 for rat to human extrapolation; F2 = 10 for individual variation; F3 = 5 for 90-day study; F4 = 2.5 for no severe toxicity findings such as carcinogenicity, neurotoxicity or teratogenicity; F5 = 10 for oral to subcutaneous route extrapolation)

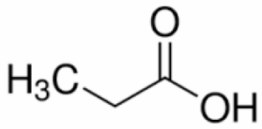
The estimated worst case scenario daily exposure (approximately 19 mg/day) of pentaerythritol from Sustol is lower than the above PDE (20 mg/day) and does not appear to raise a safety concern.

In addition, the safety of the polymer has been established in repeat dose subcutaneous toxicity studies in rats in which pentaerythritol exposure was about 140 times higher than the estimated exposure in humans.

Propionic Acid:

Propionic acid (CAS # 79-09-4) is a naturally-occurring carboxylic acid found in foods and is a normal intermediary metabolite. The structure of propionic acid is shown below (from page 4 of the Section 1.11.2 of the submission).

Figure 1.11.2-2. Structure of propionic acid



Propionic acid was not mutagenic the Ames test and in vivo micronucleus assay. Calcium propionate was not teratogenic in rats and rabbits. In a 100-day oral toxicity study with propionic acid in dogs, the Lowest Observed Adverse Effect Level (LOAEL) was 1848 mg/kg/day in males and 1832 mg/kg/day in females (OECD SIDS Initial Assessment Profile, Propionic acid, SIAM 25, 2007). The PDE for propionic was calculated to be approximately 37 mg/day using the average LOAEL of 1840 mg/kg/day as follows.

PDE = (1840x50) ÷ (2x10x5x2.5x10) = 36.8 mg/day (F1 = 2 for dog to human extrapolation; F2 = 10 for individual variation; F3 = 5 for 100-day study; F4 = 2.5 for no severe toxicity findings; F5 = 10 for oral to subcutaneous route extrapolation)

The estimated worst case scenario daily exposure (approximately 20 mg/day) of propionic acid from Sustol is lower than the above PDE (approximately 37 mg/day) and does not appear to raise a safety concern.

In addition, the safety of the polymer has been established in repeat dose subcutaneous toxicity studies in rats in which propionic acid exposure was about 140 times higher than the estimated exposure in humans.

Appendix/Attachments

None

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/s/

TAMAL K CHAKRABORTI
06/07/2016

SUSHANTA K CHAKDER
06/07/2016

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PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	22445
Supporting document/s:	078
Type of Submission:	Resubmission/Class 2
Applicant's letter date:	July 17, 2015
CDER stamp date:	July 17, 2015
Product:	Sustol®/APF530
Indication:	Chemotherapy Induced Nausea and Vomiting (CINV)
Applicant:	Heron Therapeutics, Inc.
Review Division:	DGIEP
Reviewer:	Tamal Chakraborti, PhD
Supervisor/Team Leader:	Sushanta Chakder, PhD
Division Director:	Donna Griebel, MD
Project Manager:	James B Carr, MPAS, PA-C

This addendum is to make a correction/addition to the following statements in the pharmacology review dated December 21, 2015.

- The following statement made on page 14-15 of the pharmacology review dated December 21, 2015 is not correct.

> The (b) (4) tip cap inserts are made from different elastomers; however, the extractable profiles were similar as shown in the table (from page 20 of 3.2.P.2.4 of SDN 081 dated September 25, 2015) below.

(b) (4)

Upon re-examination of the information in the above table, we have concluded that the theoretical potential extractable profile of (b) (4) and water extractable profile of (b) (4) tip caps are not similar. However, the error made in the pharmacology review would not affect the safety assessment, since the Applicant has conducted leachable studies with the (b) (4) syringes with (b) (4) tip cap inserts and the 6-month data at 2-8°C and 25°C/60% RH (Relative Humidity) showed no leachables above the Method Detection Limits (MDLs). Moreover, the values presented for the (b) (4) are theoretical potential extractables and not actual extractables data.

- The following was stated on page 6 of the pharmacology review dated December 21, 2015.

> The Applicant intends to market the (b) (4) syringe barrel with either the (b) (4) or (b) (4) tip cap. The following table (from page 6 of Section 3.2.P.2.4 of SDN 81 dated September 25, 2015) shows the components of the (b) (4) and (b) (4) container closure system.

(b) (4)

In an email communication, the Division asked (email dated 1/28/2016) the Applicant to clarify which syringe (b) (4)) and tip cap (b) (4) they intend to market. The Applicant confirmed (email communication dated 1/28/2016) that

they intend to market the (b) (4) syringe with the (b) (4) tip cap (b) (4) as shown below (from the Applicant's email dated 1/28/2016).

(b) (4)

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/s/

TAMAL K CHAKRABORTI
02/18/2016

SUSHANTA K CHAKDER
02/18/2016

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PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

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Executive Summary

Introduction

This resubmission is to addresses the deficiencies in the Agency's Complete Response (CR) letter dated March 27, 2013. In this submission, the Applicant submitted extractable and leachable profiles of the container closure system for APF530.

Brief Discussion of Nonclinical Findings

The Applicant did not submit any nonclinical study report in this submission. As mentioned above, the Applicant submitted extractable and leachable profiles of the container closure system.

Leachables/extractables (L/E) studies were conducted with (b) (4) syringes. (b) (4) were detected in leachable studies. The levels of these leachables did not appear to raise a safety concern and are acceptable. Extractable studies were conducted with (b) (4) tip cap. Based on the results of the extractable studies, a toxicological evaluation was performed to identify the target leachables based on a maximum daily dose of 0.52 g/day of APF530. A total of 14 compounds were identified as target leachables based on the toxicological evaluation of the results of extractable studies. Leachable studies were conducted with APF530 final drug product placed in (b) (4) syringes with (b) (4) tip cap inserts. No leachables were detected above the Method Detection Limits (MDLs) in the leachable studies. Overall, daily exposures to leachables from the container closure system for APF530 appear to be reasonably safe.

Recommendations

Approvability

From the nonclinical standpoint, this NDA is recommended for approval.

Additional Non Clinical Recommendations

None

Comments on Impurities/Degradants of Concern

The specification for the final drug product (FDP) is shown below (from page 1-2 of Section 3.2.P.5.1 of the submission).

Table 1: Finished Final Granisetron Injection, extended release (APF530 FFDP)

Attribute	Acceptance Criteria	Test Method
Description	(b) (4)	LWI-0000355
Product Description		LWI-0000352
Identity (^{1}H-NMR)[1]		LWI-0000444 USP <761>
Identity (^{13}C-NMR)[1]		
Identity (IR)		LWI-0000253 USP <197>
Identity (HPLC)[1]		LWI-0000343
Granisetron Assay (HPLC) (mg)[1]		LWI-0000343
Delivered Dose Uniformity		LWI-0000347 USP <905>
Granisetron Related Substances (HPLC) (%)[1]		LWI-0000344
Dynamic Viscosity @ 37°C (cP)[1]		LWI-0000011 USP <912>
Molecular Mass Distribution (GPC)[1]		LWI-0000342
In Vitro Release @ 37°C/50°C (%)[1,2]		LWI-0000449 LWI-0000345
(b) (4)		(b) (4)
Particulate Matter (Light Obscuration)[1]		LWI-0000359 USP <788>
Endotoxin (LAL)		LWI-0000003 USP <85>

Attribute	Acceptance Criteria		Test Method
Sterility[1]	Complies		LWI-0000024 USP <71>
Container Closure Integrity	Visual	Pass	LWI-0000346
	Ingress	Pass	

[1] Results taken from final drug product (FDP) in-process testing

[2] Level 1, see Table 2 for tiered Acceptance Criteria

The above specification of the drug product is acceptable from nonclinical standpoint.

Container Closure System

1.25 mL (b) (4) Syringe: The container closure system used for clinical trial material and the initial (b) (4) registration batches was a 1.25 mL (b) (4)

(b) (4) syringe with (b) (4) Tip Cap and a (b) (4) Gray Stopper.

(b) (4) 1.0 mL Long Barrel Syringe: A (b) (4) 1.0 mL long barrel syringe with a (b) (4) Tip Cap and a (b) (4) Gray Stopper was used for the first terminally irradiated registration batches as shown in the table below (from page 3 of Section 3.2.P.2.4 of SDN 81 dated September 25, 2015).

(b) (4)

The 1.0 mL (b) (4) long barrel syringe and (b) (4) rubber tip caps and stoppers were made of the same material composition as the 1.25 mL (b) (4) syringe. In addition, the syringe stoppers and tip caps were made by the same supplier and the drug contacting surfaces between the two syringe designs was the same.

1.0 mL (b) (4) Long Barrel Syringe: The Applicant qualified a second glass syringe vendor (b) (4) and a second tip cap (b) (4) as shown in the following table (from page 3 of Section 3.2.P.2.4 of SDN 81 dated September 25, 2015).

(b) (4)

The Applicant intends to market the (b) (4) syringe barrel with either the (b) (4) tip cap. The following table (from page 6 of Section 3.2.P.2.4 of SDN 81 dated September 25, 2015) shows the components of the (b) (4) container closure system.

(b) (4)

Extractables/Leachables

Extractable Studies

1.25 mL (b) (4) Syringe

Leachables/extractables (L/E) studies on non-irradiated and irradiated 1.25 mL (b) (4) syringes were performed at (b) (4) In this study, (b) (4) APF530 (Lot #146-005-009) was (b) (4) filled into 1.25 mL (b) (4) syringes and samples were tested for leachables and extractables. Samples were evaluated for compounds that were identified from a previous L/E study performed on non-irradiated syringes containing APF530. Extraction studies were conducted under the following conditions: 2-8^{0}C

storage (long-term storage condition, 3 years) and 40^0C/75%RH (relative humidity; accelerated storage condition, 3 and 6 months).

The L/E profile of APF530 in the primary container closure system stored at 2-8^0C for 3 years (long term storage condition) is shown in the following table (from page 9 of Section 3.2.P.2.4 of SDN 081 dated September 25, 2015).

(b) (4)

The L/E profile of APF530 in the primary container closure system stored at 40^0C/75%RH (accelerated storage condition) at 3 and 6 months is shown in the following table (from page 10 of Section 3.2.P.2.4 of SDN 081 dated September 25, 2015).

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(b) (4)

1.0 mL (b) (4) Long Barrel Syringe

The product contact surfaces of the 1.0 mL (b) (4) long barrel glass syringe were the same as the contact surfaces of the (b) (4) syringe used in Phase 3 studies. The (b) (4) materials in the 1.0 mL (b) (4) long barrel syringe were the same as for the 1.25 mL (b) (4) syringe. Both the 1.25 mL and 1.0 mL (b) (4) glass syringes were comprised of the same components. The L/E data was collected on the 1.25 mL (b) (4) syringe was considered relevant, since syringe stoppers and tip cap were the same composition and made by the same supplier and the drug product contacting surfaces between the two syringes were not changed. The stopper surface area of the 1.25 mL (b) (4) syringe was greater than the surface area of the 1.0 mL (b) (4) long barrel syringe, and therefore the 1.25 mL syringe size represented a worst case scenario for leachables and extractables.

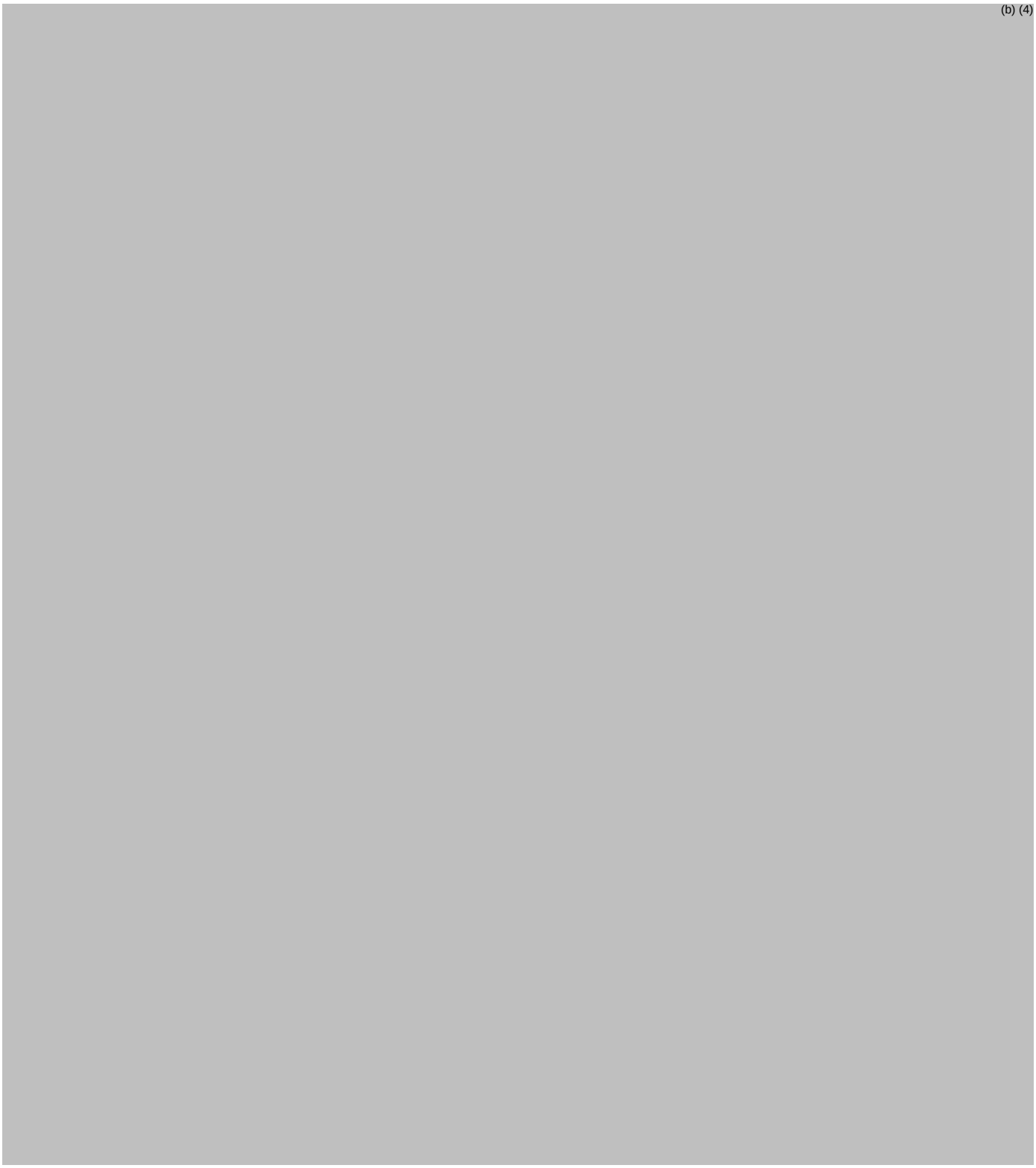
1.0 mL (b) (4) Long Barrel Syringe

***Syringe Barrel*:**

The transition from (b) (4) to (b) (4) syringe barrel did not introduce any new material. Extractable studies on both glass barrels showed similar results. Extractables from the inner surface of (b) (4) and (b) (4) syringe barrels were evaluated in a side-by-side comparison. Extraction of the glass syringes was performed using the following four solvents: (b) (4)

(b) (4)

(b) (4)



Overall, the side-by-side extractables comparison between (b) (4) syringes showed no significant differences. There was no glass extractable from (b) (4) syringes that warranted further leachables analysis.

Stopper

The (b) (4) Stopper remained unchanged between the 1.0 mL (b) (4) and the 1.0 mL (b) (4) prefilled syringe configurations. Leachable studies (XP-1078-169), discussed above, performed with the 1.25 mL (b) (4) syringe were considered representative.

Tip Cap

The Applicant intends to market the (b) (4) syringe barrel with either the (b) (4) tip cap. The (b) (4) syringe system uses the (b) (4) as the sole drug product contacting material within the (b) (4) Closure (b) (4) met the requirements for Type I closures specified in USP <381> *Elastomeric Closures for Injection* and also met the requirements as a non-cytotoxic material according to USP <87> *Biological Reactivity Tests, In Vitro*. It is to be mentioned here that (b) (4) tip cap has been used in other approved products in European Union (EU) as shown in the table below (from page 15 of 3.2.P.2.4 of SDN 081 dated September 25, 2015).

(b) (4)

The (b) (4) tip cap inserts are made from different elastomers; however, the extractable profiles were similar as shown in the table (from page 20 of 3.2.P.2.4 of SDN 081 dated September 25, 2015) below.

(b) (4)

Extractable studies were conducted with (b) (4) tip cap as described below. Extractables were isolated and analyzed using different methods from the (b) (4) tip cap under different conditions.

(b) (4)

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Overall, no leachables were detected above the MDLs in the leachable studies with (b) (4) tip cap insert.

Studies Submitted

Studies Reviewed

The applicant did not submit any nonclinical study report in this submission. The applicant provided extractables and leachables profiles of the container closure system for APF530 along with toxicological evaluation of extractable data of (b) (4) rubber tip cap (b) (4) ((b) (4) Report No. (b) (4) 140069).

Studies Not Reviewed

N/A

Integrated Summary and Safety Evaluation

This resubmission is to addresses the deficiencies in the Agency's Complete Response (CR) letter dated March 27, 2013. This resubmission contains information regarding the extractables and leachables profiles for container closure system for APF530.

Granisetron Injection, extended release (APF530), is filled into a 1.0 mL (b) (4) long barrel glass syringe with (b) (4) Luer-Lock Closure (b) (4) gray (b) (4) plunger stopper and either (b) (4) tip cap insert.

The transition from (b) (4) to (b) (4) syringe barrel did not introduce any new material. The results of the extractable studies and the side-by-side extractable comparison between (b) (4) and (b) (4) syringes showed no significant differences. There was no glass extractable from (b) (4) syringes detected that warranted further leachable analysis. The (b) (4) Stopper remained unchanged between the 1.0 mL (b) (4) and the 1.0 mL (b) (4) syringe. Leachable and extractable analysis was performed on the (b) (4) Plunger and (b) (4) Tip Cap. Leachable studies were also conducted with (b) (4) tip cap using different methods under different conditions. Interim 3 and 6-month data from the leachable studies with (b) (4) tip cap showed no leachables above the method detection limits (MDLs). Overall, daily exposures to leachables from the syringe, stopper and tip cap appear to be reasonably safe. From the nonclinical standpoint, this NDA is recommended for approval.

Appendix/Attachments

None

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/s/

TAMAL K CHAKRABORTI
12/21/2015

SUSHANTA K CHAKDER
12/21/2015

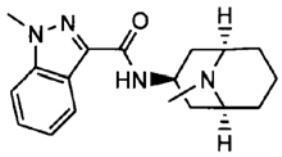
DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	22445
Supporting document number (SDN):	063, 064
Sequence No.:	058, 059
Applicant's letter date:	SDN 063 (Sequence No. 058): March 6, 2013 SDN 064 (Sequence No. 059): March 14, 2013
CDER stamp date:	SDN 063 (Sequence No. 058): March 7, 2013 SDN 064 (Sequence No. 059): March 14, 2013
Product:	Sustol®/APF530
Indication:	Chemotherapy induced nausea and vomiting (CINV)
Applicant:	A.P. Pharma
Review Division:	DGIEP
Reviewer:	Tamal K. Chakraborti, Ph.D.
Supervisor:	Sushanta K. Chakder, Ph.D.
Division Director:	Donna Griebel, M.D.
Project Manager:	Jagjit Grewal, M.P.H.

Drug: Sustol®/APF530

Structure:



Pharmacological Category: Serotonin-3 (5-Hydroxytryptamine 3/5-HT3) receptor antagonist

Indication: APF530 is recommended for the following indications in cancer patients:

- Moderately emetogenic cancer chemotherapy-prevention of acute and delayed nausea and vomiting associated with initial and repeat courses
- Highly emetogenic cancer chemotherapy-prevention of acute nausea and vomiting associated with initial and repeat courses.

Submission Contents: In response to the FDA's information request (email dated February 28, 2013), the applicant provided clarifications on the ADME studies in rats and information regarding the *in vivo* degradation of MPEG (b) (4) (b) (4) a component of (b) (4) (the vehicle for Sustol injection).

Background: Drug product APF530 has three components: Granisetron (API, 2%), MPEG (b) (4) (~19.6%) and AP135 [Tri(ethylene glycol) poly(ortho ester)/TEG-POE, a novel excipient, ~78.4%). The composition is shown below (from Section 2.3.P.1 of the original submission).

Table 1 : Composition of APF530, 500 mg.

Component	Concentration (% w/w)	To deliver Concentration (mg/unit)	To fill Concentration (mg/unit)	Function	Reference to Quality Standard
AP135	78.4	392	(b) (4)	Excipient	Manufacturer[1]
MPEG (b) (4)	19.6	98		Excipient	NF
Granisetron[4]	2	10		Active	Manufacturer[2]
Totals	100	500			

[1] Acceptance Criteria developed by manufacturer and A.P. Pharma
[2]Acceptance Criteria based on manufacturer specifications
[3]Polyethylene Glycol Monomethyl Ether, A.P. Pharma compositional code: (b) (4)
[4]A.P. Pharma compositional code: RM36

MPEG (b) (4)(CAS No. 9004-74-4) is a compendial (NF) excipient and was used to (b) (4)

At the Division's internal meeting on February 26, 2013, several questions were raised on the safety of the degradation products of MPEG (b) (4) and based on the discussion of the above internal meeting, the following queries were sent to the applicant for further clarification. The MPEG (b) (4) degradation issue was also mentioned in the subsequent Sponsor's meeting on March 1, 2013. In these submissions (Sequence # 058 and 059), the applicant provided responses and clarifications to the Agency's queries and provided information regarding MPEG (b) (4) degradation. The FDA queries and the sponsor's responses are discussed below.

Query #1:

We have the following comments and information requests. We request that you provide a response by March 1, 2013, in order to facilitate our evaluation of your NDA.

1. What is the position of 14C-radiolabel (which carbon atom of the test material was radio-labeled) in the following ADME studies in rats:

   a. Study AP24-21/7436-123 (Distribution and excretion study in rats using 14C-(b) (4))

   b. Study APF27-01/7436-140 (Distribution and excretion study in rats using 14C-APF530)

   c. Study AP25-01/7436-127 (Metabolic profiling study in rats using 14C-(b) (4))

The applicant clarified that the position of the ^{14}C-radiolabel in the test articles for all the above three ADME studies in rats (b) (4)

.

(b) (4)

Query # 2:

In study AP25-01/7436-127 (Metabolic profiling study in rats using 14C-(b) (4)), the results showed two peaks (a major and a minor peak). The major peak showed chromatographic characteristics consistent with triethylene glycol (TEG). The minor peak was not identified in the study report. Did you identify the minor peak in this study? If so, what was the minor peak?

The applicant clarified that in study AP25-01/7436-127, the unidentified minor peak that was observed as a shoulder on the major peak was also observed in the control urine,

spiked with ^{14}C-(b) (4), and was therefore assumed to be present in the starting material.

Query # 3

Do you have available any nonclinical data for in vivo degradation of MPEG(b) (4)?

The applicant stated that they did not conduct nonclinical studies on the metabolism of MPEG(b) (4) The applicant, however, provided a summary of the information available from the published literature, which is discussed below.

MPEG Metabolism Is Analogous to Triethylene Glycol (TEG) Metabolism

The applicant has conducted a review of the potential for TEG to degrade, either chemically or metabolically, into ethylene glycol (EG) and diethylene glycol (DEG). The degradation and metabolic breakdown pathways for TEG have been well characterized and published in the literature. The predominant *in vivo* metabolic product of TEG is TEG monocarboxylic acid, resulting from the oxidation of the alcohol groups of TEG. It was concluded that the likelihood of TEG breakdown to lower glycols (EG and DEG) is remote and may occur only in trace amounts after metabolism of TEG. The subsequent metabolites (glyoxylic acid, glycoaldehyde, oxalic acid, 2-hydroxyethoxyacetic acid, and diglycolic acid) of EG and DEG would be present at undetectable levels.

MPEG(b) (4)is a low molecular weight polyethylene glycol (PEG) wherein one of the two terminal hydroxyl groups of PEG has been capped with a methyl group, rendering it resistant to further reaction. Thus, MPEG is analogous to PEG/TEG with the exception of capping of one hydroxyl group which renders it less vulnerable to further metabolism to lower glycols. The methoxy capping of PEGs reduces the toxicity of PEG as the hydroxyl groups required for metabolism/toxicity is not free for reaction (capped) or it would require O-demethylation to yield a free OH group prior to metabolism by alcohol dehydrogenase (Webster R, et al. 2009. PEG and PEG Conjugates Toxicity: Towards an understanding of the toxicity of PEG and its relevance to PEGylated biologicals, Pegylated Protein Drugs: Basic Science and Clinical Applications, Ed. F.M. Veronese, p.127-146). Toxicity, metabolism, and excretion data for PEGs have been extensively reviewed in the literature (Webster et al. 2007. PEGylated proteins: evaluation of their safety in the absence of definitive metabolism studies, Drug Metabol Dispos 35:9-16; Webster R, et al. 2009. PEG and PEG conjugates toxicity: Towards an understanding of the toxicity of PEG and its relevance to PEGylated biologicals, Pegylated Protein Drugs: Basic Science and Clinical Applications, Ed. F.M. Veronese, p.127-146; Working PK, et al. 2007. Safety of Poly(ethylene glycol) and Polyethylene Glycol Derivatives, In Poly(ethylene glycol). ACS Symposium Series, Washington DC, 1997).

Metabolism of Polyethylene Glycols Does Not Lead to the Production of Ethylene Glycol

As with TEG, metabolism of PEGs involves oxidation of the alcohol groups to carboxylic acids. Diacid and hydroxyl acid metabolites of PEG have been observed in the plasma and urine of burn patients (Hunt DF et al. 1982. Mixture analysis by triple-quadrupole mass spectrometry: metabolic profiling of urinary carboxylic acids. Clin Chem 28:2387-2392). In humans, after oral and IV exposure, PEGs were excreted mainly unchanged in the urine and feces. Part of the absorbed PEGs was metabolized to lower oligomers, glycolic acid, hydroxyglycolic acids and the diglycolic acids homologs, carbon dioxide (CO_2, detected in exhaled air), and in very minor quantities of oxalic acid. In repeat-dose animal toxicity studies with high exposure levels of PEGs, the lack of a toxicity profile typical for EG indicated that EG was not formed following exposure to PEGs or their derivatives in significant quantities *in vivo* [Fruijtier-Polloth, C. 2005. Safety assessment of polyethylene glycols (PEGs) and their derivatives as used in cosmetic products. Toxicology 214:1-38].

Metabolism studies with PEG have demonstrated that there is no evidence of EG formation in animals and humans (Webster R, et al. 2009. PEG and PEG conjugates toxicity: Towards an understanding of the toxicity of PEG and its relevance to PEGylated biologicals, Pegylated Protein Drugs: Basic Science and Clinical Applications, Ed. F.M. Veronese, p.127-146; Shaffer CB, et al. 1950. The absorption and excretion of a liquid polyethylene glycol. J Am Pharm Assoc Sci Ed 39:340-344). In a study in humans (n = 3), more than 75% of MPEG-400 was excreted intact in the urine following IV administration. No EG metabolite was detected in the urine following exposure to MPEG-400. More recent studies also reported that the majority of injected PEG molecules were excreted intact in the urine (Webster et al. 2007. PEGylated proteins: evaluation of their safety in the absence of definitive metabolism studies, Drug Metabol Dispos 35:9-16). The major metabolic pathway reported in rats, cats, and humans were oxidation of the alcohol groups of PEGs to carboxylic acids followed by urinary excretion (Friman S et al. 1990. Biliary excretion of different sized polyethylene glycols in the cat. J Hepatol 11:215-220; Hunt DF et al. 1982. Mixture analysis by triple quadrupole mass spectrometry: metabolic profiling of urinary carboxylic acids. Clin Chem 28:2387-2392).

Toxicity Data for MPEG-350 Demonstrated that Lower Glycols Are Not Formed In Vivo

The United Nations OECD-SIDS report (SIDS Initial Assessment Report. 2002. High Boiling Ethylene Glycol Ethers Category, UNEP Publications) on high boiling ethylene glycol ethers cited a number of toxicology studies using MPEG-350. (b) (4) The above document reported an intravenous 14-day, repeat-dose toxicity study [North American Science Associates, Inc (NAMSA). 1997. Subchronic intravenous toxicity study in the rat. Carbowax Sentry M-PEG 350-NF and Carbowax Sentry PEG 400 NF and FCC. Report TS064-900/S) in rats in which MPEG-350 was

administered intravenously at 1,000 mg/kg/day. There were no treatment-related effects on body or organ weights, hematology, serum chemistry and histopathology. The results indicated that, even at high doses, no significant amount of lower glycols or their metabolites were formed. The NOAEL was identified as 1000 mg/kg/day. The tested IV dose of 1000 mg/kg of MPEG-350 in this study in rats was about 588 times the maximum anticipated weekly exposure (~ 1.7 mg/kg) to MPEG (b) (4) from 500 mg of Sustol based on mg/kg basis or about 95 times the maximum anticipated weekly exposure (~ 1.7 mg/kg) to MPEG (b) (4) from 500 mg of Sustol based on body surface area.

Comments: (b) (4) the novel vehicle for Sustol (APF530), containing 80% APF135 and 20% MPEG (b) (4), has been tested adequately in a number of toxicology studies including ADME studies, repeated dose subcutaneous toxicology studies in rats and dogs, genotoxicity and reproductive toxicology studies. Toxicology studies were conducted in both rats and dogs using subcutaneous (SC) route of administration, the intended route of administration in humans using the maximum feasible dose (MFD).

In the 28-day SC toxicology studies in rats and dogs, the major target organ appeared to be restricted to the injection site (inflammatory reactions). (b) (4) (vehicle) was negative in the Ames test, mouse lymphoma assay, and the *in vivo* rat bone marrow micronucleus test. In reproductive toxicology studies, (b) (4) did not appear to have adverse effects on fertility and early embryonic development in male and female rats and did not show any adverse effects of embryofetal development in rats and rabbits. In addition, in pre- and postnatal development study in rats, (b) (4) did not cause any significant adverse effect on pre- and postnatal developmental parameters. Overall, the results of nonclinical toxicology studies with the novel polymeric vehicle, (b) (4) containing 20% MPEG (b) (4) of Sustol did not reveal any significant organ toxicity except the injection site reactions.

There were concerns regarding the *in vivo* breakdown of MPEG (b) (4) and subsequent production of EG and DEG. From the information available in the literature, it appears that the MPEG metabolism is analogous to the metabolism of PEGs/TEG. As mentioned above, MPEG (b) (4) is a low molecular weight PEG wherein one of the two terminal hydroxyl groups of PEG has been capped with a methyl group, rendering it less vulnerable to further metabolism to lower glycols. Metabolism studies with PEGs have indicated that there is no evidence of formation of EG in animals and humans. The predominant *in vivo* metabolic product of TEG is TEG mono-carboxylic acid, resulting from oxidation of the alcohol groups. The likelihood of TEG breakdown to lower glycols (EG and DEG) was considered to be remote and may occur only in trace amounts following metabolism of TEG.

To summarize, based on the available information, it appears that MPEG metabolism is analogous to the metabolism of PEGs, which involves oxidation of the alcohol groups to carboxylic acids. Metabolic studies in animals and humans indicated that metabolism of PEGs does not lead to the formation of EG. In addition, the available information and results of the animal toxicity studies conducted with the novel vehicle polymer (b) (4)

containing 20% MPEG (b) (4) did not reveal any organ toxicity that are typical for toxicity profile for EG or DEG indicating EG or DEG was not formed *in vivo* following exposure to MPEG (b) (4) Overall, based on the available information, the likelihood of MPEG (b) (4) breakdown to lower glycols (EG and DEG) appears to be remote. In addition, in the clinical trials with APF530, there was no evidence for EG and DEG related toxicities including renal injury or metabolic disturbances such as acidosis (Office of Clinical Pharmacology review dated March 14, 2013).

RECOMMENDATION: None

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/s/

TAMAL K CHAKRABORTI
03/18/2013

SUSHANTA K CHAKDER
03/18/2013

MEMORANDUM

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

FROM: Sushanta Chakder, Ph.D.
Supervisory Pharmacologist, DGIEP

DATE: March 15, 2013

Application number: NDA 22-445

Date of submission: May 16, 2009 (Original Submission)
September 27, 2012 (Class 2 Resubmission)

Sponsor: A.P. Pharma

Drug Product: Sustol®/APF530 (Granisetron Injection, Extended Release)

Indication: Prevention of acute and delayed nausea and vomiting associated with initial and repeat courses of moderately emetogenic chemotherapy (MEC); Prevention of acute nausea and vomiting associated with initial and repeat courses of highly emetogenic chemotherapy (HEC)

Comments:

Under NDA 22445, the Applicant, A.P. Pharma is seeking approval of an extended release formulation of granisetron (Sustol) to be administered by the subcutaneous route.
Sustol (APF530) formulation contains 2% granisetron in a polymer vehicle (b) (4).
The polymer vehicle (b) (4) consists of two components – tri-ethylene glycol poly ortho ester (TEG-POE or AP135, 78.4%), and polyethylene glycol monomethyl ether (MPEG (b) (4) 19.6%). (b) (4)

Following injection, APF135 undergoes hydrolysis and the granisetron present in the formulation is released slowly (extended release). A single dose of Sustol (500 mg) contains 10 mg of granisetron which is expected to be released over a period of 120 hours. In clinical trials, Sustol was administered for four consecutive cycles of chemotherapy with a minimal interval of 7 days and a maximum interval of 28 days between doses.
The formulation of Sustol Injection (APF530) is shown in the Table below.

Component	Concentration (% w/w)	To deliver Concentration (mg/unit)	To fill Concentration (mg/unit)	Function
AP135	78.4	392	(b) (4)	Excipient
MPEG (b) (4)	19.6	98		Excipient
Granisetron[1]	2	10		Active
Totals	100	500		

(b) (4)

Methoxy polyethylene glycol (b) (4) is an NF-compliant excipient which represents approximately 20% of the vehicle in Sustol. There is a theoretical possibility that MPEG (b) (4) may be broken down to diethylene glycol (DEG) and monoethylene glycol (EG) following SC administration. However, in a metabolism study conducted in rats following SC administration of ^{14}C (b) (4) (polymeric vehicle of APF530), triethylene glycol was identified as the major metabolic peak in the urine, and a minor peak that was observed at the shoulder of the major chromatographic peak, was also observed in control urine spiked with ^{14}C-(b) (4) leading to the assumption that this peak came from the starting material. In this study, about 80% of the administered radioactivity was excreted in the urine by Day 6.

In 90 day repeat dose toxicity studies in rats and dogs, in which the animals received daily SC doses of APF530 at 73 and 43 times higher doses, respectively, than the recommended human dose (based on body surface area) did not show any toxicity relevant to ethylene glycol or diethylene glycol (kidney or liver toxicity, metabolic acidosis). The primary target organ of toxicity was the site of injection, and the injection site reactions consisted of inflammation, edema, hemorrhage, fibrosis, erosion, ulceration, degeneration and necrosis.

MPEG (b) (4) used in Sustol, is a low molecular weight polyethylene glycol (PEG) in which one of the two terminal hydroxyl groups of PEG has been capped with a methyl group, rendering it resistant to further reaction. In a recent submission in response to the nonclinical IR, the applicant referred to a 14-day repeat dose IV toxicity study in rats in which administration of MPEG-350 at an IV dose of 1,000 mg/kg/day, was not associated with any organ system toxicity [North American Science Associates, Inc (NAMSA). 1997. Subchronic intravenous toxicity study in the rat. Carbowax Sentry M-PEG 350-NF and Carbowax Sentry PEG 400 NF and FCC. Report TS064-900/S).

The NOAEL dose of 1000 mg/kg IV dose of MPEG-350 used in this study in rats is more than 500-fold higher than the anticipated weekly exposure of MPEG (b) (4) from the Sustol formulation. However, this study is of only 2 weeks duration.

Based on published literature, the sponsor contends that the metabolism of triethylene glycol (TEG) involves oxidation of the alocohol groups to carboxylic acids. In rats and rabbits, following oral administration of radiolabeled TEG, majority of the metabolites were identified as TEG, TEG-monocarboxylic acid and TEG-dicarboxylic acid.

Triethylene glycol is not considered a toxic substance, and in animal studies very high doses were unable to show any remarkable toxicity.

In humans, more than 75% of the intravenously injected MPEG400 was excreted unchanged in the urine (Webster et al, 2007, Drug Metabol Dispos 35:9-16). No ethylene glycol was detected in this study.

In summary, the nonclinical toxicology and metabolism studies conducted with the polymer vehicle containing MPEG (b) (4) does not indicate that the metabolism/breakdown of MPEG (b) (4) is associated with the generation of toxic levels of ethylene glycol and diethylene glycol. In addition, available information on the metabolism of MPEG and polyethylene glycol (PEG) do not suggest that the toxicity of ethylene glycol and diethylene glycol generated from MPEG is a safety concern for the new vehicle used in Sustol.

Recommendation:

I agree with the primary reviewer, Dr. Tamal Chakraborti that from a nonclinical standpoint, the NDA application is approvable with the recommended changes in the labeling.

Sushanta Chakder, Ph. D. Date
Supervisory Pharmacologist, HDF-180

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/s/

SUSHANTA K CHAKDER
03/18/2013

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	22445
Supporting document/s:	037
Sequence No.:	033
Type of Submission:	Resubmission/Class 2
Applicant's letter date:	September 27, 2012
CDER stamp date:	September 27, 2012
Product:	Sustol®/APF530
Indication:	Chemotherapy induced nausea and vomiting (CINV)
Applicant:	A.P. Pharma
Review Division:	DGIEP
Reviewer:	Tamal K. Chakraborti, Ph.D.
Supervisor/Team Leader:	Sushanta K. Chakder, Ph.D.
Division Director:	Donna Griebel, M.D.
Project Manager:	Jagjit Grewal, M.P.H.

Disclaimer

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TABLE OF CONTENTS

1 Executive Summary

1.1 Introduction

APF530 (Sustol®) is a new formulation containing 2% (w/w) granisetron and a polymer vehicle (b) (4)). This polymer vehicle (b) (4) has two components: the polymer, tri(ethylene glycol) poly(ortho ester) (TEG-POE or APF135, 78.4%), and the compendial (NF) excipient polyethylene glycol monomethyl ether (MPEG (b) (4) 19.6%). (b) (4) Upon injection, the TEG-POE (APF135) polymer in the formulation comes in contact with water and undergoes hydrolysis resulting in extended release (ER) of granisetron. A single dose of Sustol delivers 10 mg of granisetron over 120 hours. Sustol is intended to be used for the prevention of acute and delayed nausea and vomiting associated with initial and repeat courses of moderately and highly emetogenic cancer chemotherapy. The recommended dose of Sustol is a single subcutaneous (SC) injection administered approximately 30 minutes before the initiation of chemotherapy. Sustol has been administered as frequently as once every 7 days and in up to 4 consecutive cycles of chemotherapy. SUSTOL is for subcutaneous administration only.

1.2 Brief Discussion of Nonclinical Findings

N/A

1.3 Recommendations

1.3.1 Approvability

From the nonclinical standpoint, this NDA is recommended for approval.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The draft labeling of Sustol® conforms to the content and format of labeling for human prescription drugs under 21CFR201.57(b)(1). However, the following changes are recommended.

8.1 Pregnancy

Applicant's Version:

8.1 Pregnancy

(b) (4)

Evaluation: The text should be modified as recommended below. Results of the embryofetal development studies in rats and rabbits with the polymer vehicle (b) (4) should be included in the label.

Recommended Version:

8.1 Pregnancy

(b) (4)

Reproduction studies with granisetron hydrochloride have been performed in pregnant rats at intravenous doses up to 9 mg/kg/day ((b) (4) times the recommended (b) (4) human dose based on body surface area) and oral doses up to 125 mg/kg/day ((b) (4) times the recommended (b) (4) human dose based on body surface area). Reproduction studies have been performed in pregnant rabbits at intravenous doses up to 3 mg/kg/day (b) (4) times the recommended (b) (4) human dose based on body surface area) and at oral doses up to 32 mg/kg/day ((b) (4) times the recommended (b) (4) human dose based on body surface area). These studies did not reveal any evidence of impaired fertility or harm to the fetus due to granisetron.

Reproduction studies with the polymer vehicle for Sustol have been performed in pregnant rats at subcutaneous doses up to 0.295 g/day ((b) (4) times the recommended (b) (4) human dose (b) (4) based on body surface area) and in pregnant rabbits at subcutaneous doses up to 1.18 g/day ((b) (4) times the recommended (b) (4) human dose (b) (4) based on body surface area). These studies did not reveal any evidence of impaired fertility or harm to the fetus due to the polymer vehicle.

(b) (4)

8.3. Nursing Mothers

(b) (4)

Evaluation: The text is in accordance with 21CFR 201.57(c)(9)(iii) 8.3 and no revision is needed.

Recommended Version:

None

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Applicant's Version:

13.1 Carcinogenesis, Mutagenesis and Impairment of Fertility

(b) (4)

Evaluation: The format is in accordance with 21CFR 201.57(c)(14)(i) 13.1. However, the text should be modified as proposed below and the results of the genotoxicity studies with Sustol and the polymer vehicle should be added. In addition, the results of the fertility and early embryonic development study in rats with the polymer vehicle (b) (4) should be incorporated. Moreover, it should be stated that long-term studies in animals with Sustol have not been performed to evaluate the carcinogenic potential.

Recommended Version:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term studies in animals with Sustol have not been performed to evaluate the carcinogenic potential. In a 24-month carcinogenicity study, rats were treated orally with granisetron at 1, 5 or 50 mg/kg/day. The 50 mg/kg/day dose was reduced to 25 mg/kg/day during week 59 due to toxicity. [redacted] doses represent about [redacted] times the recommended [redacted] (10 mg/week [redacted] mg/m /week) based on body surface area. There was a statistically significant increase in the incidence of hepatocellular carcinomas and adenomas in males treated with 5 mg/kg/day ([redacted] times the recommended [redacted] human dose based on body surface area) and above, and in females treated with 25 mg/kg/day ([redacted] times the recommended [redacted] human dose based on body surface area). No increase in liver tumors was observed at a dose of 1 mg/kg/day ([redacted] time the recommended [redacted] human dose based on body surface area) in males and 5 mg/kg/day ([redacted] times the recommended [redacted] human dose, based on body surface area) in females.

In a 12-month oral toxicity study, treatment with granisetron 100 mg/kg/day ([redacted] times the recommended [redacted] human dose based on body surface area) produced hepatocellular adenomas in male and female rats while no such tumors were found in the control rats. A 24-month mouse carcinogenicity study of granisetron did not show a statistically significant increase in tumor incidence, but the study was not conclusive. Because of the tumor findings in rat studies, SUSTOL should be prescribed only at the dose and for the indication recommended *[see Indications and Usage (1), and Dosage and Administration (2)]*.

Granisetron was not mutagenic in an *in vitro* Ames test and mouse lymphoma cell forward mutation assay, and *in vivo* mouse micronucleus test and *in vitro* and *ex vivo* rat hepatocyte UDS assays. It, however, was positive in a mouse lymphoma assay with metabolic activation and produced a significant increase in UDS in HeLa cells *in vitro* and a significant increased incidence of cells with polyploidy in an *in vitro* human lymphocyte chromosomal aberration test.

Sustol and the polymer vehicle were not mutagenic in the Ames test, mouse lymphoma assay, and the *in vivo* rat bone marrow micronucleus test.

Granisetron at subcutaneous doses up to 6 mg/kg/day ([redacted] based on body surface area), and oral doses up to 100 mg/kg/day ([redacted] based on body surface area) was found to have no effect on fertility and reproductive performance of male and female rats.

The polymer vehicle for Sustol at subcutaneous doses up to 0.295 g/day ([redacted] based on body surface

area) was found to have no adverse effect on fertility and reproductive performance of male and female rats.

2 Drug Information

2.1 Drug

Trade Name: Sustol®

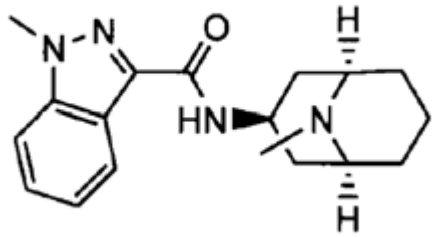
Generic Name: Granisetron (b) (4)

Code Name (Formulation): APF530

Chemical Name (Granisetron): 1-methyl-N-[(1R,SS)-9-methy1-9- azabicyclo[3.3.1]non-7-yl]indazole-3- carboxamide

Molecular Formula/Molecular Weight (Granisetron): $C_{18}H_{24}N_4O$/312.4

Structure (Granisetron):



Pharmacologic Class: Granisteron is a serotonin-3 (5-Hydroxytryptamine 3/5-HT3) receptor antagonist

2.3 Drug Formulation

Sustol, the drug product, (APF530) is a sterile viscous subcutaneous injectable formulation supplied in 1.0 mL (b) (4) pre-filled long barrel glass syringes. A single administration of Sustol (APF530) delivers 500.0 mg of polymeric formulation, containing 10.0 mg granisetron base. The proposed drug product contains 2.0% (w/w) granisetron base in 78.4% (w/w) tri(ethylene glycol) poly(ortho ester) (TEG-POE/APF135) and 19.6% (w/w) polyethylene glycol monomethyl ether (MPEG-(b) (4)). The components and quantitative composition of APF530 is shown below (from page 1 of Section 3.2.P.1, Description and Composition of the Drug Product).

Table 1: Composition of Granisetron Injection, extended release

Component	Code	Concentration (% w/w)	To deliver Concentration (mg/unit)	To fill Concentration (mg/unit)	Function	Reference to Quality Standard
Granisetron, base	RM36	2.0	10.0	(b) (4)	Drug Substance	Manufacturer[1]
TEG-POE	AP135	78.4	392.0		Excipient	Manufacturer[2]
MPEG-	(b) (4)	19.6	98.0		Excipient	NF
Totals		**100.0**	**500.0**			

[1] Acceptance Criteria based on manufacturer, A.P. Pharma specifications, and USP/EP monographs for Granisetron HCl

[2] Acceptance Criteria based on manufacturer, A.P. Pharma specifications

[3] See Section 3.2.P.2.2.2 for details on overfill

2.5 Comments on Impurities/Degradants of Concern

The revised specification for the final drug product (FDP) is shown below (from page 2-3 of Section 3.2.P.5.1).

Table 3: Final Granisetron Injection, extended release (APF530 FDP)

Test Identity	Acceptance Criteria	Reference
Description	(b) (4)	AP-P-347
Identity (IR)		AP-QC-020
Identity (HPLC)		AP-P-391
Identity (^{1}H-NMR)		AP-QC-024
Identity (^{13}C-NMR)		AP-QC-024
Granisetron Assay (HPLC)		AP-P-391
Granisetron Related Substances (HPLC)		AP-P-392
Delivered Dose Uniformity		AP-QC-022
Dynamic Viscosity @ 37°C		AP-QC-013
Molecular Mass Distribution (GPC)		AP-QC-015
In Vitro Release @ 37°C		AP-QC-012

Test Identity	Acceptance Criteria		Reference
(b) (4)	(b) (4)		(b) (4)
Particulate Matter (Light Obscuration)		Complies	AP-QC-035
		Complies	
Endotoxin (LAL)			P026-AP-001
Sterility			P006-AP-006
Container Closure Integrity		Pass	AP-QC-017
		Pass	

Granisetron-related substances (Impurity (b) (4) in the drug product were qualified from the nonclinical standpoint (pharmacology review dated October 30, 2012). In the previous specification, the level of Impurity (b) (4) was set at NMT (b) (4)%, which was qualified (above-mentioned pharmacology review). In this submission, the applicant revised the specification for Impurity (b) (4) at NMT (b) (4)%. This revised specification for Impurit (b) (4) was set based on the USP monograph for granisetron hydrochloride injection. Based on this, the revised specification for Impurity (b) (4) at NMT (b) (4)% is acceptable from the nonclinical perspective. In addition, in the above revised specifications of the drug product, the applicant included three (b) (4) (TEG-POE polymer)-related impurities, (b) (4)

(b) (4)

(b) (4)

2.6 Proposed Clinical Population and Dosing Regimen

APF530 is recommended for the following indications in cancer patients:

- Moderately emetogenic cancer chemotherapy-prevention of acute and delayed nausea and vomiting associated with initial and repeat courses
- Highly emetogenic cancer chemotherapy-prevention of acute nausea and vomiting associated with initial and repeat courses

The recommended dose of Sustol is a single subcutaneous (SC) injection administered approximately 30 minutes before the initiation of chemotherapy. Sustol has been administered as frequently as once every 7 days and in up to 4 consecutive cycles of chemotherapy.

2.7 Regulatory Background

The original NDA was submitted on May 14, 2009 and the applicant was issued a Complete Response (CR) letter dated March 16, 2010. The current resubmission is a Complete Response addressing the deficiencies listed in the above CR letter dated March 16, 2010.

3 Studies Submitted

3.1 Studies Reviewed

N/A

3.2 Studies Not Reviewed

The applicant did not submit any new nonclinical study report in this submission. The applicant submitted a protocol for Syrian hamster embryo (SHE) cell assay entitled "Clonal Transformation Assay at pH 6.7 Using Syrian Golden Hamster Embryo (SHE) Cells (24-Hour and 7-Day Exposure to Test Article" in the Section 1.8 (Special Protocol Assessment Request) of this submission. The above study is not a standard carcinogenicity bioassay and the Executive Carcinogenic Assessment Committee (ECAC) dose not review this type of protocol for an *in vitro* assay and the protocol of this study is not considered as a Special Protocol Assessment (SPA) and will not be reviewed by the ECAC.

3.3 Previous Reviews Referenced

1. Pharmacology review of NDA 22445 dated February 4, 2010
2. Pharmacology review of NDA 22445 dated October 30, 2012
3. Pharmacology review of NDA 203595 dated October 4, 2012

11 Integrated Summary and Safety Evaluation

Overall, the sponsor's proposed specification of the final drug product (FDP) appears to be acceptable from the nonclinical standpoint. Specification for [redacted] at NMT [redacted]% in the FDP appears to be acceptable based on the available toxicology information. In addition, combined specification for [redacted] of NMT [redacted]% in the FDP also appears to be acceptable based on the PDE. From the nonclinical standpoint, this NDA is recommended for approval with recommended labeling changes.

12 Appendix/Attachments

None

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/s/

TAMAL K CHAKRABORTI
02/20/2013

SUSHANTA K CHAKDER
02/20/2013

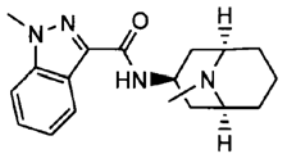
DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	22445
Supporting document/s:	021
Applicant's letter date:	March 1, 2010
CDER stamp date:	March 1, 2010
Product:	Sustol®/APF530
Indication:	Chemotherapy induced nausea and vomiting (CINV)
Applicant:	A.P. Pharma
Review Division:	DGIEP
Reviewer:	Tamal K. Chakraborti, Ph.D.
Supervisor:	Sushanta K. Chakder, Ph.D.
Division Director:	Donna Griebel, M.D.
Project Manager:	Jagjit Grewal, M.P.H.

Drug: Sustol®/APF530

Structure:



Pharmacological Category: Serotonin-3 (5-Hydroxytryptamine 3/5-HT3) receptor antagonist

Indication: APF530 is recommended for the following indications in cancer patients:

- Moderately emetogenic cancer chemotherapy-prevention of acute and delayed nausea and vomiting associated with initial and repeat courses
- Highly emetogenic cancer chemotherapy-prevention of acute nausea and vomiting associated with initial and repeat courses.

Submission Contents: The applicant submitted responses to the Division letter dated December 15, 2009 the regarding qualification of proposed total impurity specification level and the genotoxic potential of the impurities in the drug product. The Division asked the applicant to address the following:

1. The proposed total impurity specification level of less than or equal to (b) (4) % was not qualified in the nonclinical studies. Please qualify the proposed total impurity specification level of less than or equal to (b) (4) % or reduce the specification level to less than or equal to (b) (4) %.
2. In addition, please provide information regarding the genotoxic potential of the impurities.

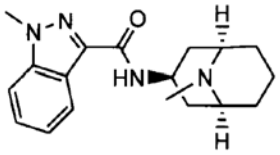
The applicant reduced the total impurity specification from (b) (4) % to (b) (4) % as recommended by the Division. The new specifications are shown in the following table (from page 3 of the applicant's submission).

Table 3 : APF530 Final Drug Product (FDP)

Test Identity	Acceptance Criteria
Description	(b) (4)
Identity by IR	
Identity Test by Proton NMR	
Identity Test by Carbon NMR	
Granisetron by HPLC	
Granisetron Related Substances by HPLC	
MMD by GPC	
MMD by GPC	
Dynamic Viscosity @ 37°C	
(b) (4)	
In Vitro Release of Granisetron @ 37°C	
Particulate Matter by Light Obscuration	
Sterility	
Filled Mass Determination	
Endotoxin by LAL	

Impurities in the drug product (AP530) were related to the active pharmaceutical ingredient (API), granisetron. The impurities represented related substances that were carried into the drug product as an impurity of the API and either increased or resulted from the manufacturing of the drug product. As per the above table, the current proposed specifications for the drug product included limits for six impurities at ≤ (b) (4) %. The related impurities were (b) (4) The proposed limit for each of the specified impurities (b) (4) was consistent with the qualification threshold for the drug product that is recommended at a maximum daily dose of 10 mg. Another impurity which might be present in the drug product, (b) (4), was controlled at a limit of (b) (4) %. The structures of granisetron and these impurities are shown below from the applicant's submission.

Granisetron:



(b) (4)

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(b) (4)

In response to the Division letter regarding the genotoxic potential of these impurities, the applicant has performed an *in silico* quantitative structure activity relationship (QSAR) analysis on four of the identified impurities in the drug product, specifically (b) (4). In the above analysis, the following selection criteria were used.

(b) (4)

(b) (4)

Comments: The applicant reduced the total impurity specification from (b) (4)% to ≤ (b) (4)% as recommended by the Division. Six specified impurities (b) (4) in the drug product (AP530) are substances related to the API, granisetron. The Division asked the applicant to provide information regarding the genotoxic potential of these impurities. The applicant conducted QSAR analysis on four of these impurities, i.e., (b) (4) including granisetron using Leadscope program. In the above analysis, (b) (4) were found to be negative for all 20 genotoxicity models. Like granisetron, positive prediction calls in the Ames test, UDS human lymphocyte model, and *in vivo* micronucleus test were obtained for (b) (4). Positive prediction probability values for (b) (4) were similar to those for granisetron. As discussed above, per the above FDA Guidance "In some cases, the structure of an impurity leading to the structural alert is shared with the API. The genotoxic potential of

such an impurity can be evaluated through the standard testing of the API if the chemical environment for the alerting structure of the compounds is deemed comparable for the reactivity potential." Structures of (b) (4) are related to granisetron, and no additional testing is necessary because the genotoxic potential of the API, granisetron, has already been adequately characterized. Based on the structural similarities between the other three impurities specified in the drug product (b) (4) and granisetron, the standard genotoxicity testing for the API, granisetron, was also considered appropriate for addressing the genotoxic potential of these impurities in the drug product and therefore no additional testing are required. Overall, it appears that these impurities do not appear to be associated with an increase in the genotoxic potential of the drug product, APF530, and that the thresholds identified in the ICH Q3B(R2) would be appropriate for the control of these impurities in the drug product. The applicant's proposed specification for the total and individual impurities in the drug product at (b) (4)%, respectively, is consistent with the qualification threshold for these impurities in the drug product when administered at a maximum daily dose of 10 mg [ICHQ3B(R2)]. From a nonclinical perspective, the proposed specification for the total and individual impurities in the drug product appears to be acceptable.

RECOMMENDATION: Applicant's proposed specification for the total impurities at ≤ (b) (4)% and individual granisetron-related impurities at ≤ (b) (4)% in the drug product appears to be acceptable.

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/s/

TAMAL K CHAKRABORTI
10/30/2012

SUSHANTA K CHAKDER
10/30/2012

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-445**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**5/16/2009**
PRODUCT:	**Sustol®/APF530 (2% Granisetron Injection, Extended Release, for Subcutaneous Use)**
INTENDED CLINICAL POPULATION:	**Cancer Patients with Chemotherapy-Induced Nausea and Vomiting (CINV)**
SPONSOR:	**A.P. Pharma**
DOCUMENTS REVIEWED:	**Nonclinical, 505(b)(2)**
REVIEW DIVISION:	**Division of Gastroenterology Products (DGP)**
PHARM/TOX REVIEWER:	**Tamal K. Chakraborti, Ph.D.**
PHARM/TOX SUPERVISOR:	**Sushanta K. Chakder, Ph.D.**
DIVISION DIRECTOR:	**Donna Griebel, M.D.**
PROJECT MANAGER:	**Todd Phillips, Pharm.D.**

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations:

A. Recommendation on Approvability: From a nonclinical standpoint, this NDA is recommended for approval.

B. Recommendation for Nonclinical Studies: None

C. Recommendations on Labeling: The draft labeling of Sustol® generally conforms to the format specified under 21CFR 201.57(c)(14) Requirements for PLR (Physician's Labeling Rule) Prescription Drug Labeling. However, the following changes are recommended.

8.1 Pregnancy

Sponsor's Version:

8.1 Pregnancy

(b) (4)

Evaluation: The text is in accordance with 21CFR 201.57(c)(14). However, the text should be modified as recommended below.

Recommended Version:

(b) (4)

Reproduction studies with granisetron hydrochloride have been performed in pregnant rats at intravenous doses up to 9 mg/kg/day ((b) (4) times the recommended (b) (4) human dose based on body surface area) and oral doses up to 125 mg/kg/day ((b) (4) times the recommended (b) (4) human dose based

on body surface area). Reproduction studies have been performed in pregnant rabbits at intravenous doses up to 3 mg/kg/day ((b) (4) times the (b) (4) human dose based on body surface area) and at oral doses up to 32 mg/kg/day ((b) (4) about 52 times the (b) (4) human dose based on body surface area). These studies did not reveal any evidence of impaired fertility or harm to the fetus due to granisetron. (b) (4)

8.3. Nursing Mothers

(b) (4)

Evaluation: The text is in accordance with 21CFR 201.57(c)(14).

Recommended Version: None

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Sponsor's Version

(b) (4)

Evaluation: The label is in accordance with 21CFR 201.57(c)(14)(i). However, the text should be modified as recommended below

Recommended Version:

"13.1 Carcinogenesis, Mutagenesis and Impairment of Fertility

In a 24-month carcinogenicity study, rats were treated orally with granisetron 1, 5 or 50 mg/kg/day . The 50 mg/kg/day dose was reduced to 25 mg/kg/day during week 59 due to toxicity. doses represent about times the recommended (10 mg/week mg/m^2/week, There was a statistically significant increase in the incidence of hepatocellular carcinomas and adenomas in males treated with 5 mg/kg/day (times the recommended human dose) and above, and in females treated with 25 mg/kg/day (times the recommended human dose with

(b) (4) No increase in liver tumors was observed at a dose of 1 mg/kg/day ((b) (4) time the recommended (b) (4) human dose (b) (4)) in males and 5 mg/kg/day (b) (4) (b) (4) times the recommended (b) (4) human dose (b) (4) in females.

In a 12-month oral toxicity study, treatment with granisetron 100 mg/kg/day ((b) (4) times the recommended (b) (4) human dose (b) (4) produced hepatocellular adenomas in male and female rats while no such tumors were found in the control rats. A 24-month mouse carcinogenicity study of granisetron did not show a statistically significant increase in tumor incidence, but the study was not conclusive.

Because of the tumor findings in rat studies, SUSTOL should be prescribed only at the dose and for the indication recommended *[see Indications and Usage, and Dosage and Administration]*.

Granisetron was not mutagenic in an *in vitro* Ames test and mouse lymphoma cell forward mutation assay, and *in vivo* mouse micronucleus test and *in vitro* and *ex vivo* rat hepatocyte UDS assays. It, however, was positive in a mouse lymphoma assay with metabolic activation and produced a significant increase in UDS in HeLa cells *in vitro* and a significant increased incidence of cells with polyploidy in an *in vitro* human lymphocyte chromosomal aberration test.

Granisetron at subcutaneous doses up to 6 mg/kg/day (b) (4) (b) (4) and oral doses up to 100 mg/kg/day (b) (4) (b) (4) was found to have no effect on fertility and reproductive performance of male and female rats."

13.2 Animal Toxicology

Sponsor's Version

The sponsor did not include this section in their proposed label.

Evaluation: The sponsor conducted toxicology studies with the novel excipient (b) (4) The findings should be included in this section.

Recommended Version:

"In rats, the major target organ appeared to be the injection site (injection site reactions characterized by edema, hemorrhage, fibrosis, epidermal hyperplasia, erosion/ulceration, and suppurative inflammation; skeletal muscle degeneration/necrosis; and inflammation).

In 90-day toxicology study in rats, lymphoid hyperplasia in the draining lymph nodes, splenic hematopoiesis and bone marrow myeloid hyperplasia were also observed when SUSTOL was administered once every 24 hours at 2.8 and 5.9 mg granisetron/dose."

II. Summary of Nonclinical Findings

This is a 505(b)(2) application. The sponsor depended on the previous findings of the Agency for the nonclinical safety or effectiveness of granisetron.

A. Brief Overview of Nonclinical Findings: APF530 is a new formulation containing 2% granisetron. Granisetron is an approved drug. Since, APF530 is a new formulation containing a novel excipient vehicle, (b) (4) the sponsor was asked to conduct nonclinical studies with (b) (4) as per the CDER Guidance entitled "Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients". APF530 is intended to be used for the prevention of acute and delayed nausea and vomiting associated with initial and repeat courses of moderately and highly emetogenic cancer chemotherapy. The recommended dosage of APF530 is a single 10 mg subcutaneous (SC) dose administered approximately 30 minutes before the start of single-day chemotherapy or it may be given once every 7 days, and only on the day chemotherapy is given. Based on its intermittent dosage, 3-month toxicology studies were recommended in a rodent and a non-rodent species by SC route of administration as per the above Guidance (Section C: Potential Excipients Intended for Intermediate Use). The sponsor conducted absorption, distribution, metabolism and excretion (ADME), acute, subacute/subchronic (28- and 90-day) toxicology, genotoxicity, and reproductive toxicology studies with (b) (4) as per the Division recommendations. In addition, AP Pharma will be conducting a 2 year carcinogenicity study in the rat and an *in vitro* Syrian Hamster Embryo (SHE) cell transformation assay with (b) (4) Toxicology studies were conducted in both rats and dogs using SC route, the intended route of administration in humans using the maximum feasible dose (MFD).

In ADME studies in rats, the highest mean concentrations of radioactivity (^{14}C-APF530) were observed at the injection site and in the liver, bone marrow, urinary bladder, plasma, small intestine and kidneys at 24 hours post-dose. The major route of excretion of radioactivity was *via* the urine. The biodisposition of (b) (4) is considered dependent on the hydrolysis of the polymer. Based on the size and hydrophobic nature of the polymer, intact polymer itself is not expected to be absorbed systemically. Under *in vivo* conditions (i.e. large amounts of water), the polymer vehicle is expected to undergo hydrolysis yielding a spectrum of breakdown product (b) (4) The findings of the radiolabel studies in rats indicated that the excreted radioactivity was associated primarily with TEG, and was

consistent with *in vivo* hydrolysis of (b) (4). Hydrolysis of the (b) (4) polymer vehicle is species independent and, therefore, no novel metabolite is expected to be formed in humans. The data in humans suggested that the majority of the polymer vehicle will be eliminated over the seven-day period with low levels of hydrolysis occurring during the initial one to two days following dosing. Peak elimination is expected to occur approximately one to two days following the Tmax for granisetron (i.e. at 48 to 72 hours), and low to negligible levels of hydrolyzed polymer is expected to be excreted over at least the next three to four days.

In toxicology studies, the major target organ appeared to be restricted to the injection site (inflammatory reactions). Sequelae secondary to the inflammatory reactions at the site of injection appeared to be more pronounced in the rat compared to the dog. In the 90-day toxicology study (once every 24 hours administration) in rats, lymphoid hyperplasia in the draining lymph nodes, splenic hematopoiesis and bone marrow myeloid hyperplasia were observed. However, similar doses did not produce such histopathological changes when administered subcutaneously once every 72 hours in the 28-day toxicology study in rats.

(b) (4) (vehicle) was negative in the Ames test, mouse lymphoma assay, and the *in vivo* rat bone marrow micronucleus test. APF530 was also negative in the standard battery of genotoxicity studies. Neither (b) (4) nor APF530 exhibited any mutagenic or clastogenic activity in any of the assays and, therefore, were considered nongenotoxic.

In reproductive toxicology studies, (b) (4) did not appear to have adverse effects on fertility and early embryonic development in male and female rats and were not teratogenic in rats and rabbits. In addition, in pre- and postnatal development study in rats, (b) (4) did not cause any significant adverse effect on pre- and postnatal developmental parameters.

APF530 has been characterized for its impurity profile. The specification acceptance criteria for individual and total impurities were based on the material qualified in the non-clinical and clinical studies. The known impurities qualified during the studies were (b) (4) The following is the highest level of total impurities tested in nonclinical studies: (b) (4)%. The following amounts of total impurities were qualified in toxicology studies: Rats: (b) (4) Dogs: (b) (4). Overall, individual impurities in the drug product are qualified at the specified levels in nonclinical studies. The proposed specification level of (b) (4)% for total impurities in the drug product was not qualified in the nonclinical studies. The sponsor should be asked to qualify the proposed total impurity specification level of (b) (4)% or reduce the specification level to (b) (4)%. In

addition, the sponsor should be asked to provide information regarding the genotoxic potential of the impurities.

Overall, the constituents of APF530, granisetron and the (b) (4) (novel vehicle excipient), have been adequately characterized in general toxicology, genetic toxicology and reproductive toxicology studies and support approval of APF530. In addition, APF530 has been fully evaluated in genetic toxicology studies as well as single and repeat dose toxicology studies. In conclusion, this 505(b)(2) application contains adequate nonclinical studies for the marketing approval of APF530 for the intended patient population at the recommended doses. Nonclinical studies conducted with Sustol® provided adequate assurance of safety for its proposed use. Therefore, from a nonclinical standpoint, this NDA is recommended for approval.

B. Pharmacologic Activity: The primary pharmacology of granisetron HCl has been characterized in a series of in vitro and in vivo studies under NDA 20-239. No additional primary pharmacology studies have been conducted with APF530. Granisetron exhibited efficacy in cisplatin, doxorubicin, cyclophosphamide and X-ray irradiation models of emesis in the ferret. The *in vitro* and *in vivo* data demonstrated that granisetron is relatively specific for the 5-hydroxytryptamine type 3 (5HT3) receptor with negligible to no activity at dopaminergic or opioid receptors. The anti-5HT3 activity translates into efficacy in chemotherapy and radiation induced emesis.

C. Nonclinical Safety Issues Relevant to Clinical Use: The proposed specification level of (b) (4)% for total impurities in the drug product was not qualified in the nonclinical studies. The sponsor should be asked to qualify the proposed total impurity specification level of (b) (4)% or reduce the specification level to (b) (4)%. In addition, the sponsor should be asked to provide information regarding the genotoxic potential of the impurities.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

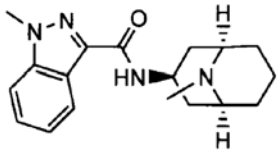
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-445
Review number: 001
Sequence number/date/type of submission: 000/May 14, 2009/Original Application
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: A.P. Pharma, Redwood City, CA
Manufacturer for drug substance: Ningbo Team Pharmaceutical Factory, Ningbo, Zhejiang, China

Reviewer name: Tamal K. Chakraborti, Ph.D.
Division name: Division of Gastroenterology Products (DGP)
Review completion date: February 4, 2010

Drug:

Trade name: Sustol®/APF530
Generic name: Granisetron
Code name: RM36
Chemical name: 1-methyl-N-[(1R,SS)-9-methy1-9- azabicyclo[3.3.1]non-7-yl]indazole-3- carboxamide
CAS registry number: 109899-09-0
Molecular formula/molecular weight: $C_{18}H_{24}N_4O$/312.4
Structure:



Relevant IND/NDA:

1. IND 68,213 (APF530, A.P. Pharma)
2. IND (b) (4)
3. NDA 21-238 [Kytril (Granisetron HCl) Oral Solution, Roche]
4. NDA 20-305 [Kytril® (Granisetron HCl) Tablets, Roche]
5. NDA 20-239 [Kytril® (Granisetron HCl) Injection, Roche]

Drug class: 5-Hydroxytryptamine 3 (5-HT3) receptor antagonist/Antiemetic

Intended clinical population: APF530 is indicated for the following in cancer patients:

- Moderately emetogenic cancer chemotherapy-prevention of acute and delayed nausea and vomiting associated with initial and repeat courses
- Highly emetogenic cancer chemotherapy-prevention of acute nausea and vomiting associated with initial and repeat courses.

Clinical formulation: APF530 is a new polymeric formulation of granisetron, which has been developed to provide sustained release of granisetron for the prevention of acute and delayed chemotherapy-induced nausea and vomiting (CINV). The polymer, AP135, which was used in APF530 is a triethylene glycol based poly (ortho ester) or TEG-POE, a class of polymer which undergoes controlled hydrolysis in the presence of water. The TEG-POE polymer (APF135) and methoxy polyethylene glycol (b) (4) provides the vehicle of this formulation, (b) (4). In this formulation, granisetron has been incorporated at a level of 2% w/w. The drug product is a viscous and clear injectable for SC administration. The following table shows the composition of the drug product.

Component	Quantity (% w/w)	Function
AP135	78.4	Excipient
MPEG (b) (4)	19.6	Excipient
Granisetron	2	Active Ingredient

Route of administration: Subcutaneous (SC)

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Data reliance: Any information or data necessary for approval of NDA 22-445 that A.P. Pharma 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 described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that A.P. Pharma does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 22-445.

Studies reviewed within this submission: The nonclinical package that is provided to support marketing of APF530 consists of studies designed to characterize the pharmacology and toxicology of granisetron HCl and granisetron free base (RM36), the drug product (e.g. APF530), and the novel excipient vehicle (b) (4). Nonclinical studies with granisetron have already been reviewed under the above mentioned NDAs. Nonclinical studies conducted with APF530 and (b) (4) to support this 505(b)(2) application (as per the Agency's recommendations, Meeting Minutes dated August 23, 2005 and January 11, 2006) are reviewed here. In addition, nonclinical studies with APF530 and (b) (4) that were reviewed under IND 68,213 are incorporated in the

appropriate sections of this review from the pharmacology review of IND 68,213 dated December 15, 2006. The following table lists the studies reviewed in this submission.

STUDY	REPORT NO.			REV. PAGE #
PHARMACOLOGY				13
ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION (ADME)				14
ABSORPTION				14
Comparison of pharmacokinetics of various granisetron formulations following subcutaneous administration to rats	AP23-17/7436-104			15
Determination of the pharmacokinetics of granisetron and tolerance of APF530 following subcutaneous administration to rats	AP23-19/7436-105			16
Determination of the pharmacokinetics of granisetron and tolerability of APF530 following subcutaneous administration to dogs	AP23-20/7436-106			17
DISTRIBUTION				18
Distribution and excretion study in rats administered a single subcutaneous dose of ^{14}C-(b) (4)	AP24-21/7436-123			18
Distribution and excretion study in rats administered a single subcutaneous dose of ^{14}C-(b) (4)	AP27-01/7436-140			19
METABOLISM				20
Metabolic profiling of radioactivity in selected urine samples following administration of a single subcutaneous dose of ^{14}C-(b) (4) to rats	AP25-01/7436-127			20
EXCRETION				21
Mass balance study in rats administered a single subcutaneous dose of ^{14}C-APF530	AP27-06/7436-145			21
TOXICOLOGY				23
Acute				28
Rat				28
Single, SC	AP24-01/7436-108			28
Dog				28
Single, SC	AP24-02/7436-109			28
Subacute/Subchronic				29
Rat				29
28-Day, SC	AP24-12/7436-114			29
90-Day, SC	AP25-07/7436-129			34
Dog				41
28-Day, SC	AP24-13/7436-115			41
90-Day, SC	AP-25-08/7436-130			47
GENOTOXICITY				53
Ames test, APF530	AP24-14/7436-116			53
Ames test, (b) (4)	AP24-17/7436-121			55
Ames test, Granisetron	AP24-18/7436-122			57
L5178Y TK$^{+/-}$ Mouse lymphoma assay, APF530	AP24-15/7436-117			59
L5178Y TK$^{+/-}$ Mouse lymphoma assay, (b) (4)	AP24-19/7436-119			62
L5178Y TK$^{+/-}$ Mouse lymphoma assay, Granisetron	AP24-20/7436-120			65
In vivo rat micronucleus assay, APF530 and (b) (4)	AP24-16/7436-118			68

Studies not reviewed within this submission: The sponsor provided literature references regarding pharmacology/toxicology of granisetron, which were not reviewed here. The above mentioned NDAs are referred for the nonclinical studies for granisetron. Method validation studies were not reviewed.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

The sponsor did not conduct any primary or safety pharmacology studies with APF530.

2.6.2.2 Primary pharmacodynamics

None

2.6.2.3 Secondary pharmacodynamics

None

2.6.2.4 Safety pharmacology

None

2.6.2.5 Pharmacodynamic drug interactions

None

2.6.3 PHARMACOLOGY TABULATED SUMMARY

None

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The *in vivo* studies conducted with APF530 in the rat and dog provided a comparison of the pharmacokinetics (PK) of granisetron in the approved formulation (Kytril®) to the PK of the new formulation, APF530. In a PK study with APF530 in rats, maximum plasma concentration (Cmax) values for granisetron in the plasma were observed at 0.5 to 1 hour postdose. In rats, Cmax and area under the curve (AUC_{0-t}) values for granisetron were about 35% higher for females than that of males following administration of APF530. The half-life ($T_{1/2}$) of granisetron following treatment with APF530 at the high dose was approximately 10 hours in rats, which was longer than the $T_{1/2}$ (approximately 1 hour) for an oral, intravenous (IV) or SC dose of an aqueous formulation (Kytril) of granisetron in rats. Cmax of granisetron in an aqueous formulation (e.g., Kytril) administered by either the IV or SC routes were greater (791 ng/mL for Kytril at 8 mg/kg of granisetron, SC vs. 202 ng/mL at 4.72 mg granisetron/rat for APF530) than for the APF530 formulations. In another PK and tolerability study with APF530 in dogs, Cmax was generally comparable in males and females. In dogs, mean $T_{1/2}$ values ranged from approximately 20-30 hours following SC administration of APF530 compared to whereas $T_{1/2}$ for an i.v. dose of the saline formulation of granisetron has been reported to be about 5.33 hours.

2.6.4.2 Methods of Analysis

Methods were described under individual study reviews.

2.6.4.3 Absorption

Comparison of the Pharmacokinetics of Various Granisetron Formulations Following Subcutaneous Administration to Rats ((b) (4) Study No. 7436-104 and AP Pharma Study No. AP 23-17)

Methods: This study compared the pharmacokinetics (PK) of granisetron in three different TEG-POE formulations following a single subcutaneous (s.c.) dose to male Sprague-Dawley (SD) rats. The PK of the various TEG-POE formulations was compared to aqueous granisetron (Kytril®) following s.c. administration.

In this study, male SD rats (n = 5 males/group) were administered a single s.c. injection of 8 mg Kytril (0.1% granisetron HCl in saline)/kg (Group 1); 2 mg (b) (4) (b) (4) (Group 2); 2 mg (b) (4) (Group 3); and 4 mg APF530 (2% granisetron in (b) (4) polymer vehicle)/animal (Group 4). Blood was collected from each animal at 1, 6, 12, 24, 48, 72, 96, 120, 144, and 168 hours after dosing. Plasma was analyzed for granisetron concentration by liquid chromatography and tandem mass spectrometry (LC/MS/MS).

Results: Plasma concentrations of granisetron in the three test formulations were generally measurable through 72 hours postdose. For Group 1, concentrations of granisetron were measurable through 6 hours postdose. Cmax values for granisetron in plasma were generally observed at approximately 1 hour postdose for the control Kytril, (b) (4) test formulations. The Tmax values for the APF530 ranged from 1 to 48 hours postdose but generally occurred at 6 to 12 hours. The results indicated that APF530 increased the duration of detectable plasma levels of granisetron when compared to Kytril (saline formulation). The following table (from page 19 of Vol. 3.14 of sponsor's submission) outlines the PK parameters for the various formulations.

Table 1: Selected Mean Pharmacokinetic Parameters for Granisetron in Plasma Following a Single Subcutaneous Dose to Male Rats

Group	Formulation	Target Dose Level	C_{max} (ng/mL)	T_{max} (hours)	AUC_{0-t} (hr·ng/mL)	AUC_{0-t}/D (hr·ng/mL)/(mg/kg)
1	Kytril®	8 mg/kg	791	1.00	2380	302
						(b) (4)
4	APF530	4 mg/animal	361[1] (202)[2]	14.6[1] (6.2)[2]	10480[1] (3598)[2]	750[1] (254)[2]

D Actual calculated dose administered.
NC Not calculated.
[1] Data represent N=5; data from all animals included
[2] Data represent N = 4; data from the one animal that exhibited a different pattern of plasma concentrations from the other animals were not included in the calculations; the results with the one rat removed more closely approximates the findings in the definitive PK study (AP 23-19).

Determination of the Pharmacokinetics of Granisetron and Tolerability of APF530 Following Subcutaneous Administration of APF530 to Rats ((b) (4) Study No. 7436-105 and AP Pharma Study No. AP 23-19)

Methods: The study assessed the pharmacokinetics of granisetron and the tolerability of APF530 following subcutaneous administration of APF530 to male and female rats. The objective of first phase of the study was to determine the systemic and local tolerability of the apparent maximum feasible dose (MFD) of APF530. Male and female Sprague-Dawley rats (N = 3/sex/group) in Group 1 were administered a total volume of 1 ml of APF530/animal by injecting 0.25 ml/site at four sites (approximately 20 mg of granisetron /animal or 60-80 mg/kg of granisetron). Previous studies with the TEG-POE polymer vehicle indicated that 0.25 mL was the maximum feasible dose (MFD) per site in rats, based on leakage of test article. There were no apparent test article related effects in Phase 1 following a 72 hour observation period. Based on this Phase 1 observation, a total of 1ml of APF530/animal was selected as the high dose for the second phase.

The objective of the second phase of the study was to assess the pharmacokinetics of granisetron and the tolerability of APF530 in male and female Sprague-Dawley rats (N = 5/sex/group) at a total s.c. dose volume of 0.25, 0.5, and 1 ml of APF530/animal (0.25 ml at one, two, and four sites, respectively) in Groups 2, 3, and 4. The total dose of granisetron administered was approximately 5, 10, and 20 mg/animal or approximately 15-20, 30-40, and 60-80 mg/kg of granisetron, respectively. Blood samples were collected from animals (5/group/timepoint) at 0.5, 1, 6, 12, 24, 48, 72, 96, 120, 144, and 168 hours postdose. Plasma concentrations of granisetron were determined by LC/MS/MS. Clinical observations were performed daily and body weights were determined at sacrifice (Day 8). Gross necropsy and microscopic evaluation were conducted on the injection site and selected organs. Organ weights were also obtained for the selected organs.

Results: There was no mortality. There were no apparent treatment-related effects on clinical observations, injection site, body weight, gross necropsy, organ weights, and histopathology of the kidney, heart, liver, brain, and lungs.

Cmax values for granisetron in the plasma were generally observed 0.5 to 1 hour postdose for all animals in Groups 2 through 4. Generally, plasma concentrations were measurable through 72, 96, and 120 hours postdose at 0.25, 0.5, and 1.0 ml of APF530, respectively. There appeared to be a biphasic peak in the mean plasma concentrations of granisetron with the first and second peaks occurring at approximately 1 and 72 hours. In general, Cmax and AUC values were about 35% higher for females than males. The Cmax values increased approximately 1:2.6:5.7-fold and AUC_{0-t} values increased approximately 1:2.4:5.9-fold with the 1:2:4-fold increase in dose level, respectively. There was no apparent gender difference in pharmacokinetic parameters. The $t_{1/2}$ (approximately 10 hours) was considerably longer than the $t_{1/2}$ that has been reported for an oral, i.v., or s.c. dose of an aqueous formulation (e.g. approximately 1 hour) of granisetron in rats. The pharmacokinetic parameters are outlined in the following table (from page 22 of Vol. 3.14 of sponsor's submission).

Table 2: Selected Mean Pharmacokinetic Parameters for Granisetron in Plasma Following a Single Subcutaneous Dose of APF530 to Male and Female Rats

Group	Dose Level (mg/animal)	Gender	C_{max} (ng/mL)	T_{max} (hours)	$t_{1/2}$ (hours)	AUC_{0-t} (hr·ng/mL)	AUC_{0-t}/D (hr·ng/mL)/(mg/kg)
2	5	M	100	15.2	NC	2936	181
		F	152	15.1	13.8	3289	166
		Overall	126	15.2[1]	13.8	3112	174
3	10	M	301	1.80	15.8	5563	171
		F	365	0.900	21.3	9178	238
		Overall	333	1.35	18.6	7370	204
4	20	M	692	0.700	12.0	14802	225
		F	733	1.70	7.38	21770	288
		Overall	713	1.20	9.94	18286	257

D Actual calculated dose administered.
NC Not calculated.
[1]The t_{max} in 10/12 animals was 0.5 to 1 hour and in 2 rats was 72 hours

Determination of the Pharmacokinetics of Granisetron and Tolerability of APF530 Following Subcutaneous Administration of APF530 to Dogs ((b) (4) Study No. 7436-106 and AP Pharma Study No. AP 23-20)

Methods: The objective of this study was to examine the pharmacokinetics of granisetron and the tolerability of APF530 following subcutaneous administration of APF530 to male and female beagle dogs. In the first phase, one dog/sex was administered a total dose of 4 ml of APF530 (maximum of 1 ml/site). The dose appeared to be well tolerated over a 72 hour observation period and a total dose volume of 4 ml of APF530 was selected as the high dose for the second phase. Male and female beagle dogs (N=3/sex/group) were administered 0.25, 1, and 4 mL of APF530/animal

(maximum of 1 ml/site at up to four sites). The total administered dose of granisetron was approximately 5, 20, and 80 mg/animal or approximately 0.5, 2, and 8 mg/kg of granisetron, respectively. Blood samples were collected form animals (3/group/timepoint) at 0.5, 1, 6, 12, 24, 48, 72, 96, 120, 144, and 168 hours postdose for determination of granisetron concentrations in the plasma and blood by LC/MS/MS. Clinical observations were performed daily and body weights were determined at necropsy. Dogs were sacrificed on Day 10. Gross necropsy and microscopic evaluations were conducted on the injection site and selected organs. Organ weights were also obtained for the selected organs.

Results: There was no mortality and no apparent treatment-related effects on clinical observations, body weight, and histopathology of the kidney, heart, liver, brain, and lungs.

Concentrations of granisetron in the plasma and whole blood were generally observed through 24, 96, and 120 hours postdose for the 5, 20, and 80 mg/animal doses, respectively. In this study, unlike the rat, the mean plasma and blood concentrations of granisetron did not exhibit a clear biphasic peak. The Cmax increased roughly dose-proportionately, but exposure was greater than dose-proportional. The Cmax was similar for males and females within each dose group, but slightly higher exposure was observed for females. The mean $t_{1/2}$ for a s.c. dose of APF530 ranged from approximately 20-30 hours. The $t_{1/2}$ in dogs for an i.v. dose of the saline formulation (Kytril) has been reported to be a mean of 5.33 hours. The PK parameters for plasma concentrations of granisetron are outlined in the following table (from page 25 of Vol. 3.14 of sponsor's submission).

Table 3: Selected Mean Pharmacokinetic Parameters for Granisetron in Plasma Following a Single Subcutaneous Dose of APF530 to Male and Female Dogs

Group	Dose Level (mg/animal)	Gender	C_{max} (ng/mL)	T_{max} (hours)	$t_{1/2}$ (hours)	AUC_{0-t} (hr·ng/mL)	AUC_{0-t}/D (hr·ng/mL)/(mg/kg)
2	5	M	4.94	1.00	34.0	96.0	305
		F	4.86	0.833	17.4	115	265
		Overall	4.90	0.917	28.5	105	285
3	20	M	16.0	4.33	29.6	657	426
		F	22.9	6.00	30.4	693	334
		Overall	19.5	5.17	30.0	675	380
4	80	M	73.3	2.67	22.1	2596	398
		F	59.9	6.33	20.8	3200	389
		Overall	66.6	4.50	21.4	2898	394

D Actual calculated dose administered.

2.6.4.4 Distribution

Distribution and Excretion Study in Rats Administered a Single Subcutaneous Dose of ^{14}C (b) (4) (AP24-21/7436-123)

Methods: The tissue distribution and excretion of radioactivity were examined following a single SC administration of ^{14}C (b) (4) to 18 male Sprague Dawley (SD) rats. In this

study, rats were administered a single 0.25-mL/animal (270-mg/animal) SC dose of ^{14}C-(b) (4) (specific activity = 0.11 µCi/mg, position of the radiolabel was not mentioned). Blood was collected from three animals/time point at 24, 72, 168, 336, 504, and 672 hours postdose, and animals were sacrificed and tissues were collected. Urine, feces, and expired air were collected at designated intervals throughout the study from the animals scheduled for sacrifice at 672 hours postdose. Blood, plasma, tissues, urine, feces, and expired air were analyzed for total radioactivity.

Results: The distribution of ^{14}C-(b) (4) derived radioactivity following a single SC administration was measurable in all samples at all collection time points, except for thymus at 504 hours postdose and bone marrow, plasma, prostate, and thymus at 672 hours postdose. Cmax of radioactivity in the blood and plasma were 36.4 and 54.5 µg equivalents ^{14}C-(b) (4)/g, respectively, at 24 hours postdose. The Cmax values were highest in the injection site, liver, bone marrow, urinary bladder, and plasma. The lowest Cmax values were observed in the fat and brain. ^{14}C-(b) (4) was detected at low levels in the brain, suggesting the drug-derived radioactivity crossed the blood brain barrier. The mean tissue:plasma concentration ratios were less than one in all tissues collected at 24 hours postdose except injection site, bone marrow, liver, and urinary bladder. The number of tissues with ratios exceeding one increased with time and by 336 hours postdose, all tissue:plasma concentration ratios exceeded one. Urine was the primary route of elimination with urine, feces, daily cage rinse, and expired air containing 85.3, 2.05, 3.39, and 3.38% of administered dose, respectively. The overall mass balance of administered radioactivity including excreta, tissues, and carcass was 95.3%.

Distribution and Excretion Study in Rats Administered a Single Subcutaneous Dose of ^{14}C-APF530 (APF27-01/7436-140)

Methods: The tissue distribution and excretion of radioactivity were examined following a single SC administration of ^{14}C-APF530 to 18 male and 18 female SD rats. Animals were administered a single 0.25-mL/animal (approximately 270-mg/animal) SC dose of ^{14}C-APF530 (specific activity = 0.0833-0.105 µCi/mg, position of the radiolabel was not mentioned). Blood samples were collected from 3 animals/sex/time point at 24, 72, 168, 336, 504, and 672 hours postdose. Following blood collections, animals were sacrificed and selected tissues were collected. Urine and feces were collected at designated intervals at 672 hours postdose. Blood, plasma, selected tissues, urine, and feces were analyzed for total radioactivity.

Results: The distribution of ^{14}C-APF530-derived radioactivity following a single SC administration was measurable in all samples at all collection time points, except male prostate and female thymus at 504 hours postdose and male prostate, large intestine, cecum and thymus, and female large intestine and cecum, salivary glands, small intestine and thymus at 672 hours postdose. At 72 hours postdose, Cmax of radioactivity in the blood and plasma were 48.7 and 69.6 µg equivalents ^{14}C-APF530/g, respectively, in males and 64.6 and 86.9 µg equivalents ^{14}C-APF530/g, respectively, in females. Cmax values were highest in the injection site, urinary bladder, liver, kidneys, plasma, and

prostate in both sexes. Lowest Cmax values were observed in the fat and brain in males and fat and bones in females. ^{14}C-APF530 was detected at low levels in the brain, suggesting that drug-derived radioactivity crossed the blood brain barrier. Mean tissue:plasma concentration ratios were less than one in over 60% of tissues at 24 hours and in all tissues collected at 72 hours postdose except the injection site, urinary bladder, kidneys, and liver. The number of tissues with ratios exceeding one increased with time and by 336 hours postdose, all tissue:plasma concentration ratios exceeded one. Urine was the primary route of elimination with mean recoveries in the urine and feces of 71.6 and 3.42% of administered dose, respectively, in males and 69.6 and 2.17% of administered dose, respectively, in females. The overall mean mass balance of administered radioactivity including excreta, tissues, and carcass was 77.4 % in males and 73.1 % in females.

2.6.4.5 Metabolism

Metabolic Profiling of Radioactivity in Selected Urine Samples Following Administration of a Single Subcutaneous Dose ^{14}C-(b) (4) to Rats (AP25-01/7436-127)

Methods: The purpose of this non-GLP (Good Laboratory Practice) study was to assess the metabolite profiles of radioactivity in selected urine samples following administration of a single SC dose of ^{14}C-(b) (4) to rats obtained from the Study 24-21. Nine urine samples from male Animal Nos. C20204, C20205, and C20206 were collected from 0-6, 6-12, and 12-24 hours postdose for analysis.

Results: Profiling of the ^{14}C-(b) (4)-derived radioactivity in the urine samples showed two peaks. A major radioactive peak was observed at 22.7 to 24.2 minutes and a minor peak at 24.2 to 26.1 minutes. The same peaks were observed in a sample fortified with ^{14}C-(b) (4). The major peak showed chromatographic characteristics consistent with triethylene glycol (TEG).

2.6.4.6 Excretion

Mass Balance Study in Rats Administered a Single Subcutaneous Dose of ^{14}C-APF530 (AP27-067/7436-145)

Methods: The excretion of radioactivity was examined following a single SC administration of ^{14}C-APF530 to four male Sprague Dawley (SD) rats (n = 4). Animals were administered a single 0.25 mL/animal (approximately 235 mg/animal, about 21 μCi/animal) SC dose of ^{14}C-APF530 (specific activity = 0.0883-0.0939 μCi/mg). Urine, feces, and expired air were collected through 144 hours postdose and analyzed for total radioactivity. This is a non-GLP study.

Results: The major route of elimination of radioactivity was *via* the urine. At 144 hours postdose, the urine accounted for a mean of 77.7% of the administered radioactivity and the feces accounted for 3.03 % of the administered dose. The daily cage rinse, expired air, and expired volatiles accounted for 9.02, 4.54, and 0.01% of the administered radioactivity, respectively. The cage wash, cage wipe, and residual carcass represented 0.15, 0.18, and 3.15% of administered dose, respectively. The overall mean mass balance of administered radioactivity was 97.7%.

2.6.4.7 Pharmacokinetic drug interactions

None

2.6.4.8 Other Pharmacokinetic Studies

None

2.6.4.9 Discussion and Conclusions

The PK studies conducted with APF530 in the rat and dog provided a comparison of the PK of granisetron as the approved formulation (Kytril) to the PK of the new formulation, APF530. Overall, the results from the PK studies in rats and dogs suggested a sustained blood level of granisetron following APF530 administration when compared to either IV or SC administration of the approved (Kytril) formulation of granisetron.

In general, plasma concentrations were measurable through at least 72 to 120 hours post dose at 0.25 to 1.0 mL of APF530 in rats and 24 to 120 hours post dose at 0.25 to 4.0 mL of APF530 in dogs. Plasma levels following SC administration of Kytril in the rat were not detected after 6 hours. Typically, a marked delay in Tmax and a prolongation in apparent $T_{½}$ were observed for APF530 compared to aqueous granisetron HCl. In rats, $T_{½}$ values, over a dose range of 0.25 to 1.0 mL of APF530 (5.9 to 23.6 mg granisetron/rat), ranged from approximately 10 to 19 hours. In dogs, $T_{1/2}$ values, over a dose range of 0.25 to 4.0 mL of APF530 (5.9 to 94.4 mg granisetron/dog), ranged from approximately 21 to 30 hours. However, Cmax of granisetron in an aqueous formulation administered by either the IV or SC routes was greater than that for the APF530 formulation. Generally in the rat and dog, an increase in dose of APF530 resulted in an increase in exposure to granisetron that was often dose proportional or greater than dose proportional. There was no apparent difference in exposure between genders for granisetron from APF530.

Biodisposition studies were conducted in the rat using (b) (4) vehicle or APF530 formulation with radiolabel randomly distributed throughout the polymer molecule (AP135) on the triethylene glycol (TEG) molecule. For both (b) (4) and APF530, the radioactivity was extensively distributed throughout the body with measurable quantities in multiple tissues through 672 hours. By 672 hours post dose, however, only approximately 1 to 2% of a radiolabeled dose of APF530 or (b) (4) was detected. Tissues with the highest radioactivity concentrations for both (b) (4) and APF530 included the injection site, liver, urinary bladder and kidney. While radioactivity

persisted for a relatively long duration, the majority of the radioactive dose (>80%) was eliminated within the first 24 hours for (b) (4). The major route of excretion of the radioactivity was in the urine with approximately 76% eliminated within 24 hours after administration of (b) (4). The majority of the radioactivity was eliminated by 72 to 96 hours after dosing. Approximately 45% of the radioactive dose was excreted at 0-72 hours after dosing with APF530 and 65% was excreted at 0-96 hours post dose.

2.6.4.10 Tables and figures to include comparative TK summary

The following table (from the sponsor's submission) compares selected PK parameters in rats and dogs following administration of APF530.

Table 17: Comparison of the Weekly APF530 and Granisetron Exposures in Rats and Dogs to Humans

Extrapolation of APF530 Exposure					
Species	APF530 Dose: Maximum total mg/dose	APF530 Dose: mg/kg dose: Daily	APF530 Dose: mg/kg dose: Weekly	APF530 Dose: mg/m² dose: Weekly	Multiples of Human Exposure (Based on Surface Area)[a]
Humans	500[b]	NA	10[c]	348	NA
Rats	295[d]	639[e]	4473[e]	38366	110X
Dogs	1180[d]	114[e]	798[e]	15527	45X

Extrapolation of Granisetron Exposure						
	Maximum total mg dose of granisetron	AUC (ng·hr/mL): Daily	AUC (ng·hr/mL): Weekly	Multiples of Human Exposure	C_{max} (ng/mL)	Multiples of Human Exposure
Humans	10[b]	NA	1385.1[f]	NA	17.8	NA
Rats	5.9[d]	1799[g]	12593	9X	252.8[h]	14.2X
Dogs	23.6[d]	563.5[g]	3944.5	3X	36.4[g]	2X

[a] Multiples of the human dose, which is administered weekly, based on the weekly dose in the rat and the dog;
[b] Anticipated maximum human dose;
[c] Based on a 50 kg individual;
[d] Maximum dose in the 90 day study in the rat (Table 2.6.7.7-2/ (b) (4) Study 7436-129 [AP Pharma Study AP 25-07]) (File located 4.2.3.2) and dog (Table 2.6.7.7-4/ (b) (4) Study 7436-130 [AP Pharma Study AP 25-08] (File located 4.2.3.2); AUC_{0-24}
[e] Mean for males and females combined. Dose calculated based on Week 14 mean body weights for rats and dogs, respectively, and total mass dose of APF530 administered on that day; the data represent a conservative estimate since the animals will be heaviest at the later time point;
[f] Data from C2005-01 study, AUC_{0-168}
[g] Values represent the mean value for males and females combined on Day 88 or Day 90 for rats and dogs, respectively;
AUC_{0-24hr}
[h] Values represent the mean value for males and females combined on Days 28 and 88 for rats;
AUC_{0-24} – Area under the concentration time curve from time 0 to 24 hours; AUC_{0-t} – Area under the concentration time curve from time 0 to time of last sample;
kg – Kilogram, mg – Milligram; ng – Nanogram; mL – Milliliter; hr - Hour
NA – not applicable

Extrapolation of APF530 Exposure				
Species	APF530 Dose			Multiples of Human Exposure (Based on Surface Area)a
	Maximum total mg/dose	mg/kg dose		
		Daily	Weekly	
Humans	500[b]	NA	App. 8 mg/kg[c]	NA
Rats	295[d]	639[e]	4473[e]	93X
Dogs	1180[d]	114[e]	819[e]	51X
Extrapolation of Granisetron Exposure				
	Maximum total mg dose of granisetron	AUC (ng·hr/mL)		Multiples of Human Exposure
		Daily	Weekly	
Humans	10	NA	1385.1±1348.4[f]	NA
Rats	5.9	1799[f]	12593	9X
Dogs	23.6	563.5[f]	3944.5[f]	3X

a Multiples of the human dose, which is administered weekly, based on the weekly dose in the rat and the dog;
b Anticipated maximum human dose;
c Based on a 60 kg individual;
d Maximum dose in the 90 day study in the rat (Table 2.6.7.7-2/ (b) (4) Study 7436-129 [AP Pharma Study AP 25-07] (File located 4.2.3.2))and dog (Table 2.6.7.7-4/ (b) (4) Study 7436-130 [AP Pharma Study AP 25-08] (File located 4.2.3.2));
e Mean for males and females combined. Dose calculated based on Week 14 mean body weights for rats and dogs, respectively, and total mass dose of APF530 administered on that day; the data represent a conservative estimate since the animals will be heaviest at the later time point;
f AUC0-t;
g Values represent the mean value for males and females combined on Day 88 or Day 90 for rats and dogs, respectively;

AUC0-24hr
AUC0-24 – Area under the concentration time curve from time 0 to 24 hours
kg – Kilogram, mg – Milligram; ng – Nanogram; mL – Milliliter; hr - Hour
NA – not applicable

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

None

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General Toxicology: As recommended by the Division, the sponsor conducted acute, subacute/subchronic (28- and 90-day), genotoxicity, and reproductive toxicology studies with (b) (4). In addition, AP Pharma will be conducting a 2 year carcinogenicity study in the rat and SHE cell assay with (b) (4). Toxicology studies were conducted in both rats and dogs using subcutaneous route, the intended route of administration in humans.

Acute S.C. toxicity studies were conducted with APF530 in rats and dogs (20 and 80 mg/kg of granisetron, 1.0 and 4.0 ml of APF530). These doses were non-lethal to both the species. In rats, test article-related effects were confined to the injection site i.e.,

erythema and edema associated with inflammatory infiltrate, degeneration and necrosis of cutaneous muscle (not present in humans). In dogs, test article-related effects were also confined to the injection site i.e., hemorrhage, subacute inflammation, degeneration and necrosis of cutaneous muscle (not present in humans) and edema.

In a 28-Day S.C. toxicity study with APF530 in rats, animals were treated with 0.125 and 0.25 ml/rat of APF530 (2.5 and 5 mg of granisetron per dose or 7.5-10 mg/kg and 15-20 mg/kg of granisetron, respectively) once every 72 hours. One group of animals received (b) (4) only and another group received the saline. All rats survived to terminal necropsy. There were no APF530 or (b) (4)-related changes in body weight, food consumption, ophthalmologic, organ weight, or macroscopic parameters. There were no histopathological changes in any organs with the exception of findings at the injection sites (edema, hemorrhage, fibrosis, epidermal hyperplasia, erosion/ulceration, and suppurative inflammation; skeletal muscle (panniculus carnosus) degeneration/necrosis; and inflammation). In addition, inflammatory infiltrates in the dermis, subcutis, and associated skeletal muscle (*Panniculus carnosus*) was also observed. These findings tended to be more pronounced and occurred at a greater frequency in APF530 and (b) (4) treated rats compared to saline. Lymphoid hyperplasia was noted in some of the lymph nodes (left and right axillary, inguinal, and popliteal) in all dose groups. In some cases, the incidences of lymphoid hyperplasia in the (b) (4) and/or APF530 groups appeared to be slightly increased compared to incidences in the saline control. The target organ of toxicity appeared to be the injection site. The NOAEL could not be identified as treatment-related increased incidences of lymphoid hyperplasia were noted at all tested doses.

In a 90-day subcutaneous toxicity study in rats, animals were treated once every 24 hours with APF530 at 2.8 (0.12 ml APF530/animal or 8.4-11.2 mg/kg of granisetron) and 5.9 (0.25 ml APF530/animal or 17.7-23.6 mg/kg of granisetron) mg granisetron/dose. The target organs of toxicity appeared to be the injection site, lymph node (lymphoid hyperplasia), bone marrow (myeloid hyperplasia) and spleen (hematopoiesis). It is to be mentioned here that similar doses did not produce histopathological changes in the lymph nodes, bone marrow and spleen when administered subcutaneously once every 72 hours for 28 days.

In a 28-day subcutaneous toxicity study with APF530 in dogs, animals were treated at 0.5 and 1.0 mL (10 and 20 mg of granisetron/dose or 30-40 mg/kg and 60-80 mg/kg of granisetron, respectively) per animal once every 72 hours. One group of animals received (b) (4) only and another group received saline. All dogs survived to scheduled necropsy. There was no evidence of systemic toxicity with either (b) (4) or APF530. No APF530 or (b) (4)-related changes in body weight, food consumption, ophthalmologic, electrocardiographic, clinical pathology or organ weight parameters were observed. There were no clinical observations and gross or histopathological effects on any organs or tissues with the exception of findings at the injection sites (edema, thickening, and inflammatory changes which included hemorrhage,

degeneration/necrosis of skeletal muscle, fibrosis etc.). The NOAEL may be considered as 60-80 mg/kg of granisetron.

In a 90-day subcutaneous toxicity study in beagle dogs, animals were treated with APF530 at 11.8 (35.4-47.2 mg/kg of granisetron) and 23.6 (70.8-94.4 mg/kg of granisetron) mg granisetron/animal or 0.5 and 1.0 ml/dog/day. The target organ of toxicity could not be determined in the absence of any significant histopathological findings in any organ or tissues. The dose selection does not appear to be appropriate. In a 28-day study, these doses did not produce any toxicity when administered once every 72 hours. The sponsor could have administered higher doses.

Genetic Toxicology: (b) (4) was negative in the Ames test, the mouse lymphoma assay, and the *in vivo* rat bone marrow micronucleus test. APF530 was also negative in the standard battery of genotoxicity studies. Overall, neither (b) (4) nor APF530 exhibited any mutagenic or clastogenic activity in any of the assays and, therefore, are nongenotoxic.

Reproductive Toxicology: In a Segment I fertility and early embryonic development study in male and female rats, (b) (4) was tested at 0.142 and 0.295 g/animal?/day. All rats survived to scheduled euthanasia. Clinical signs at the injection included erythema and edema and scab formation. There were no treatment related effects on body weights, food consumption, mating and fertility parameters or necropsy observations in either sex. Caesarean-sectioning parameters were comparable among the three dosage groups. It is to be mentioned here that only two doses have been tested. At least three doses should have been administered.

In a Segment II teratology study in rats, pregnant animals were treated (Groups I, II and III) with (b) (4) daily on gestation day 7 (GD7) through gestation day 17 (GD17) at dosages of 0.250 (negative control, saline), 0.140, and 0.295 g/day, respectively. Rats in Group IV were administered the test article subcutaneously once every 72 hours on GDs 7, 10, 13 and 16 at a dosage of 0.295 g/day. There were no significant treatment-related effects on maternal C-section parameters. There were no significant treatment-related fetal external, visceral and skeletal alterations. (b) (4) did not appear to be teratogenic in this study. It is to be mentioned here that the sponsor did not include any APF530-treated group as recommended in the Division letter dated November 4, 2005.

In a Segment II teratology study in rabbits, animals were administered (b) (4) subcutaneously once daily from GD 7 through GD 19 at 1.0 (negative control, saline, Group I), 0.59 (Group II) and 1.18 g/day (Group III). Group IV animals were administered the test article subcutaneously once every 72 hours on GD 7 at 1.18 g/day. There were no significant treatment-related effects on maternal C-section parameters. There were no significant treatment-related fetal external, visceral and skeletal alterations. (b) (4) did not appear to be teratogenic in this study.

In a Segment III peri- and postnatal development study in rats, pregnant female rats were administered (b) (4) subcutaneously once daily from DG 7 through DL 20 at dosages of 0.250 (negative control, saline), 0.142, and 0.295 g/day, respectively. No significant treatment-related effects were observed in the F1 generation male and female rats with regard to clinical signs, body weights or body weight gains, feed consumption, sexual maturation, learning and memory or fertility and reproductive capacity. There were no significant treatment-related gross necropsy observations in the F1 generation rats. There were no (b) (4)-related effects in the F2 generation fetuses. Overall, (b) (4) did not have any significant adverse effect on peri- and postnatal development in rats.

Local Tolerance: In a pharmacokinetic and tolerability study of APF530 in rats, animals (n =3/sex/dose) were treated with APF530 by SC route at 5, 10 and 20 mg granisetron/animal or 250, 500 and 1000 mg APF530/animal. APF530 appeared to be well tolerated. No apparent test article-related effects were observed on body weight, organ weights, and histopathology of the brain, kidney, liver, lung, and heart. Test article-related effects were observed at subcutaneous injection sites during histopathology. The primary histopathology finding included minimal to moderate inflammatory response, which appeared to be more severe in females than in males.

In a pharmacokinetic and tolerability study of APF530 in dogs, animals (n =3/sex/dose) were treated with APF530 by SC route at 5, 20 and 80 mg granisetron/animal or 250, 1000 and 4000 mg APF530/animal. Overall, APF530 appeared to be well tolerated. No apparent test article-related effects were observed on body weight, organ weights, and histopathology of the brain, kidney, liver, lung, and heart. Test article-related effects were observed for subcutaneous injection sites during histopathology. The primary histopathology finding was minimal to moderately severe inflammation response characterized by a cellular infiltrate of predominantly neutrophils as well as varying numbers of macrophages, multinucleated giant cells, lymphocytes, plasma cells and proliferating fibroblasts. The severity of the finding appeared to be dependent on dose volume/injection as well as total dose volume.

Special Toxicology: In a systemic and local toxicity study of (b) (4) in rats, male SD rats received a single intraperitoneal injection containing the control (saline), placebo (b) (4), test (b) (4) solution. All animals survived to their scheduled sacrifices. There were no clinical observations noted throughout the study with two exceptions. One placebo-treated and one (b) (4) -treated rat exhibited red nasal discharge on Days 22 and 29, respectively. Body weight and food consumption values were generally comparable between control and treated groups. There were no significant treatment-related gross or histopathology findings.

In a toxicity Study with (b) (4) in rabbits following incisional administration, male and female New Zealand white rabbits were assigned to 4 groups (15/sex/group). Rabbits were administered saline (control article), (b) (4) (placebo

article), (b) (4) (test article; (b) (4)), or (b) (4) solution in Groups 1, 2, 3, and 4, respectively. (b) (4) related effects were observed at the site of injection, which included erythema and edema. (b) (4) related hematology changes included increase in fibrinogen concentration and increase in serum ALT activity. Lymphoid hyperplasia of the inguinal lymph node was observed in APF 18A treated males.

In a toxicity study with (b) (4) in rats following incisional and subcutaneous administration, male and female SD rats were assigned to 4 groups (25/sex/group). Rats were administered saline (control article), (b) (4) (placebo article), (b) (4) (placebo article containing (b) (4)), or a (b) (4) solution (comparison article) in Groups 1, 2, 3, and 4, respectively. There was no evidence of significant systemic toxicity following incisional and subcutaneous administration of (b) (4). Most treatment-related effects were seen at the site of the injection. The healing process at incisional sites was essentially complete by Day 30.

2.6.6.2 Single-dose toxicity

The sponsor submitted draft reports of the acute toxicology studies in rats and dogs under the IND 68,213 Original submission. These reports were previously reviewed under the above IND. The reviews are incorporated below from the pharmacology review of IND 68,213 dated December 15, 2006. The results, interpretations and conclusions of the final reports of these studies do not seem to differ from the draft reports.

Acute toxicity studies in rats and dogs

Report No.	Testing Laboratory	Species/ Route	Date Started	Date Completed	Batch No.
24-01	(b) (4)	SD Rats	1/8/04	2/24/04 (Inlife Phase)	81741
24-02		Beagle dogs	1/22/04	2/24/04 (Inlife Phase)	81743

GLP Compliance: The sponsor submitted audited draft reports of these studies. Compliance statements were not signed.

Methods: Rats (n = 20/sex/group) were injected subcutaneously (single injection) with APF530 at 1 (0.25 ml/site, 4 sites) and 4 (1 ml/site, 4 sites) ml per animal (20 and 80 mg/kg of granisetron). Similarly, Beagle dogs (n = 20/sex/group) were also injected subcutaneously (single injection) with APF530 at 1 (0.25 ml/site, 4 sites) and 4 (1 ml/site, 4 sites) ml per animal (20 and 80 mg/kg of granisetron). Rats were sacrificed on Day 4, 8, and 15 postdose. The following observations were made on rats: mortality, clinical signs, dermal irritation, body weight, food consumption, clinical pathology, and histopathology. Dogs were sacrificed on Day 15 postdose and the following observations were made: clinical signs, dermal irritation, physical, ophthalmoscopic and electrocardiographic examinations, body weight, food consumption, clinical pathology, and histopathology.

Results: Both the tested doses (1.0 and 4.0 ml of APF530) were non-lethal to rats and dogs. In rats, test article-related effects were confined to the injection site i.e., erythema and edema associated with inflammatory infiltrate, degeneration and necrosis of cutaneous muscle. In dogs, test article-related effects were confined to the injection site i.e., hemorrhage, subacute inflammation, degeneration and necrosis of cutaneous muscle and edema.

Acute Toxicity Studies With APF-530 in Rats and Dogs

Species	Route	Dose (ml/animal)	Maximum Nonlethal Dose (ml/animal)		Minimum Lethal Dose (ml/animal)		Time to death
			Male	Female	Male	Female	
SD Rats, 20/sex/dose	s.c.	1, 4	4	4	NA	NA	None
Beagle dogs, 6/sex/dose	s.c.	1,4	4	4	NA	NA	None

Summary: Acute s.c. toxicity studies were conducted with APF530 in rats and dogs (20 and 80 mg/kg of granisetron, 1.0 and 4.0 ml of APF530). These doses were non-lethal to both the species. In rats, test article-related effects were confined to the injection site i.e., erythema and edema associated with inflammatory infiltrate, degeneration and necrosis of cutaneous muscle (not present in humans). In dogs, test article-related effects were confined to the injection site i.e., hemorrhage, subacute inflammation, degeneration and necrosis of cutaneous muscle (not present in humans) and edema.

2.6.6.3 Repeat-dose toxicity

The sponsor submitted draft reports of the following toxicology study under the IND 68,213 Original submission. The reports were reviewed under the above IND 68,213. The reviews are incorporated below from the pharmacology review of IND 68,213 dated December 15, 2006. The results, interpretations and conclusions of the final reports of these studies do not seem to differ from the draft reports.

Study Title: 28-Day Subcutaneous Toxicity Study with APF530 in Rats

Key Study Findings: In a 28-Day s.c. toxicity study with APF530 in rats, animals were treated with APF530 at 0.125 and 0.25 ml/rat (2.5 and 5 mg of granisetron per dose or 7.5-10 mg/kg and 15-20 mg/kg of granisetron, respectively) once every 72 hours (total of 10 doses). A group of rats also received the polymer vehicle, (b) (4) and another group received saline. All rats survived to terminal necropsy. There were no APF530 or (b) (4) -related changes in body weight, food consumption, ophthalmologic, organ weight, or macroscopic parameters. There were no histopathological effects on any organs with the exception of findings at the injection sites (edema, hemorrhage, fibrosis, epidermal hyperplasia, erosion/ulceration, and suppurative inflammation; skeletal muscle (panniculus carnosus) degeneration/necrosis; and inflammation). In addition, inflammatory infiltrates in the dermis, subcutis, and associated skeletal muscle (*Panniculus carnosus*) was also observed. These findings tended to be more pronounced and occurred at a greater frequency in APF530 and (b) (4) treated rats compared to saline. Lymphoid hyperplasia was noted in some of the lymph nodes (left and right axillary, inguinal, and popliteal) in all dose groups. In some cases, the incidences of lymphoid hyperplasia in the (b) (4) and/or APF530 groups appeared to be slightly increased compared to incidences in the saline control. The target organ of toxicity appeared to be the injection site.

Study No.: (b) (4) Study No. 7436- 114 and AP Pharma Study No. AP 24-12

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Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: August 31, 2004

GLP Compliance: The sponsor submitted a draft report and GLP and QA statements were not signed.

QA Report: yes ( ) no (x)

Drug, Lot #, and % Purity: APF530 (containing 2% granisetron), Lot. 81762; (b) (4) (vehicle), Lot. 81763 and 81740.

Methods: This study examined the toxicity of APF530 in rats when administered at dose levels of 0.125 and 0.25 ml/rat (2.5 and 5 mg of granisetron/dose or 7.5-10 mg/kg and 15-20 mg/kg of granisetron, respectively) once every 72 hours (total of 10 doses for toxicity groups and 9 doses for toxicokinetic groups) via s.c. injection for 28 days. Treated groups were compared to a negative control group given saline and a vehicle control group given (b) (4) The dose volume was 0.12 ml/rat for the 2.5 mg/dose and 0.25 ml/rat for all other groups.

Doses: 0.125 and 0.25 ml of APF530/rat (2.5 and 5.0 mg granisetron per dose)

Basis of Dose Selection: Not mentioned

Species/Strain: Sprague-Dawley rats

Number/Sex/Group: 10/sex/dose

Route, Formulation, Volume: Subcutaneous, viscous solution, 0.125 and 0.25 ml/dose

Satellite Groups Used for Toxicokinetics: 12/sex/dose

Age: 12 weeks old

Weight: Males: 350-403 g; Females: 237-275 g

Sampling Times: Blood samples were drawn from 3 rats/sex/group on Day 1 (one at predose and at approximately 5 minutes and 0.5, 1, 6, 12, 24, 48 and 72 hours postdose) and on Day 25 (one at predose and at approximately 5 minutes and 0.5, 1, 6, 12, 24, 48, 72, 96, 120 and 144 hours postdose).

Study Design: The study design (from Vol. 3.27, page 11 of sponsor's submission) and dose administration (from Vol. 3.27, page 12 of sponsor's submission) is shown below.

Group	No. of Animals		Dose Volume[a]	Granisetron	
	Male	Female	(mL/dose)	Dose Level (mg/dose)	Dose Concentration (mg/mL)
Toxicity Animals					
1 (Negative Control) Saline	10	10	0.25	0	0
2 (Vehicle Control) (b) (4)	10	10	0.25	0	0
3 (APF530)	10	10	0.12	2.5	20
4 (APF530)	10	10	0.25	5	20
Toxicokinetic Animals					
5 (APF530)	12	12	0.12	2.5	20
6 (APF530)	12	12	0.25	5	20

a Animals were dosed once every 72 hours. Toxicity animals received a total of 10 injections, and toxicokinetic animals received a total of 9 injections.

Study Procedures

Procedure	Frequency/Comment
Dose Preparation	
Negative Control	Dosed as provided by the manufacturer.
Test and Vehicle Control Articles[a]	Dosed as provided by the sponsor after being transferred to precalibrated syringes. Dose preparations were stored refrigerated (2 to 8°C) and removed from the refrigerator the afternoon before and/or the morning of dosing. Samples from the test, vehicle control, and negative control groups were retained refrigerated (2 to 8°C) and shipped to the sponsor after completion of the inlife phase for postdose analysis.
Dose Administration[a]	Administered by subcutaneous injection using a 16 gauge needle every 72 hours (total of 10 doses for toxicity groups and 9 doses for toxicokinetic groups) by rotating through 6 dermal dose sites as follows: Injection Site 1 – Left Cranial Back – Day 1 Injection Site 2 – Right Cranial Back – Days 4, 28 Injection Site 3 – Left Mid Back – Days 7, 19 Injection Site 4 – Right Mid Back – Days 10, 22 Injection Site 5 – Left Caudal Back – Days 13, 25 Injection Site 6 – Right Caudal Back – Day 16 The dose volume was 0.12 mL/rat for Group 3/5 and 0.25 mL/rat for Groups 1, 2, and 4/6. Syringes were weighed before and after dosing. Dosing was completed within 24 hours of the preparation's removal from the refrigerator. Dose sites were clipped free of hair the day before the first dose and weekly thereafter, and each site was marked and numbered throughout the study.

Observation and Times:

Mortality: Mortality was observed twice daily.

Clinical Signs: Clinical signs were observed twice daily.

Body Weights: Body weights were recorded on a weekly basis.

Food Consumption: Food consumption was recorded on a weekly basis.

Ophthalmoscopy: Ophthalmoscopy was conducted at pre-study and during Week 4.

Hematology: Hematology was conducted at necropsy.

Clinical Chemistry: Clinical chemistry was conducted at necropsy.

Urinalysis: Urinalysis was conducted at necropsy.

Gross Pathology: Gross pathology was conducted at necropsy.

Organ Weights: The following organs were weighed from all animals at necropsy: adrenal glands, brain, heart, kidneys, liver, lung, ovaries, spleen and testes.

Histopathology: Adequate Battery: yes (x), no ( )

Peer review: yes ( ), no (x)

Histopathological examinations were conducted on the following organ/tissues from all animals at the scheduled necropsies: adrenals, brain, cecum, colon, duodenum, esophagus, eyes, femur with bone marrow, heart, ileum, injection site, jejunum, kidneys, lesions, liver, lungs, lymph nodes (mesenteric), mammary area, ovary, pancreas, pituitary, prostate, rectum, regional lymph nodes to injection sites, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, thoracic and lumbar), spleen, sternum, stomach, testes, thymus, thyroid, tongue, trachea, urinary bladder, uterus, and vagina.

Toxicokinetics: Blood samples were drawn from 3 rats/sex/group on Day 1 (one at predose and at approximately 5 minutes and 0.5, 1, 6, 12, 24, 48 and 72 hours postdose) and on Day 25 (one at predose and at approximately 5 minutes and 0.5, 1, 6, 12, 24, 48, 72, 96, 120 and 144 hours postdose).

Results:

Mortality: None

Clinical Signs: Treatment-related swelling or masses were observed at 2.5 (females) and 5 mg granisetron/dose. Additional clinical signs included erythema, edema, exudates at the site of injection.

Body Weights: The mean initial and final weights of control (saline) males were 379 and 453 g, respectively. The mean initial and final body weights of control (saline) females were 259 and 297 g, respectively. There were no significant treatment-related changes.

Food Consumption: The mean initial and final food consumption in control males were 30.57 and 31.57 g/animal/day, respectively. The mean initial and final food consumption in control females were 24.57 and 25.28 g/animal/day, respectively. There were no significant treatment-related changes.

Ophthalmoscopy: No treatment-related changes were observed.

Hematology: There were no meaningful treatment-related changes.

Clinical Chemistry: No treatment-related changes were observed.

Urinalysis: There were no treatment-related changes.

Gross Pathology: Macroscopic examinations included thickening and scab formations at the site of injection.

Organ Weights: No significant treatment-related changes were observed.

Histopathology: There were no histopathological findings in any organs with the exception of findings at the injection sites (edema, hemorrhage, fibrosis, epidermal hyperplasia, erosion/ulceration, and suppurative inflammation; skeletal muscle (panniculus carnosus) degeneration/necrosis; and inflammation). In addition, inflammatory infiltrates in the dermis, subcutis, and associated skeletal muscle (*Panniculus carnosus*) was also observed. These findings tended to be more pronounced and occurred at a greater frequency in APF530 and (b) (4) treated rats compared to saline-treated animals. Lymphoid hyperplasia was noted in some of the lymph nodes (left and right axillary, inguinal, and popliteal) in all dose groups. In some cases, the incidences of lymphoid hyperplasia in the (b) (4) and/or APF530 groups appeared to be slightly increased compared to incidences in the saline control.

Toxicokinetics: Exposure to granisetron increased as the APF530 dose level increased. Mean values for Cmax and AUC were generally lower on Day 25 than on Day 1. The mean Tmax value was 0.5 hours. Plasma granisetron levels declined slowly over time and measurable levels were present in all animals at 72 hours postdose on both days (Day 1 and 25). No marked (>2-fold) gender differences in the mean Cmax and $AUC_{0\text{-}72h}$ values were observed on the two collection days, with the exception of the 2.5 mg/dose males on Day 1, which had a markedly lower AUC value than females. These results indicated no apparent accumulation of granisetron following multiple dosing of APF530. The toxicokinetic parameters are shown in the following table (from page 14 of Vol. 3.27 of sponsor's submission).

Toxicokinetic Parameters of Granisetron in Rat Plasma

Dose Group	APF530 Dose Level (mg/dose)	Gender	C_{max} (ng/mL)	T_{max} (hr)	$AUC_{0\text{-}t}$ (ng·hr/mL)	$AUC_{0\text{-}72hr}$ (ng·hr/mL)
			Day 1			
5	2.5	M	36.6	0.500	875	875
		F	58.0	0.500	1771	1771
6	5	M	69.2	72.0	2327	2327
		F	81.5	1.00	2190	2190
			Day 25			
5	2.5	M	28.7	0.500	410	410
		F	27.7	0.500	460	394
6	5	M	74.0	0.500	1458	1315
		F	60.3	0.500	1453	1275

Summary: In a 28-Day s.c. toxicity study with APF530 in rats, animals were treated with APF530 at 0.125 and 0.25 ml/rat (2.5 and 5 mg of granisetron per dose or 7.5-10 mg/kg and 15-20 mg/kg of granisetron, respectively) once every 72 hours. All rats survived to terminal necropsy. There were no APF530 or (b) (4)-related changes in body weight, food consumption, ophthalmologic, organ weight, or macroscopic parameters. There were no histopathological effects on any organs with the exception of findings at the injection sites (edema, hemorrhage, fibrosis, epidermal hyperplasia, erosion/ulceration, and suppurative inflammation; skeletal muscle (panniculus carnosus) degeneration/necrosis; and inflammation). In addition, inflammatory infiltrates in the dermis, subcutis, and associated skeletal muscle (Panniculus carnosus) was also observed. These findings tended to be more pronounced and occurred at a greater frequency in APF530 and (b) (4) treated rats compared to saline. Lymphoid hyperplasia was noted in some of the lymph nodes (left and right axillary, inguinal, and popliteal) in all dose groups. In some cases, the incidences of lymphoid hyperplasia in the (b) (4) and/or APF530 groups appeared to be slightly increased compared to incidences in the saline control. The target organ of toxicity appeared to be the injection site. The NOAEL could not be identified as treatment-related increased incidences of lymphoid hyperplasia were observed at all tested doses.

The sponsor submitted the reports of the 90-day toxicology studies in rats and dogs under the IND 68,213 Amendment 021 dated November 9, 2006. These reports were previously reviewed under the above IND. The reviews are incorporated below from the pharmacology review of IND 68,213 dated December 18, 2006. The results, interpretations and conclusions of the final reports of these studies do not seem to differ from the draft reports.

Study Title: 90-Day Subcutaneous Injection Toxicity and Toxicokinetic Study with APF530 in Rats with a 6-Week Recovery Period

Key Study Findings: In a 90-day subcutaneous toxicity study in rats, animals were treated once every 24 hours with APF530 at 2.8 (0.12 ml APF530/animal or 8.4-11.2 mg/kg of granisetron) and 5.9 (0.25 ml APF530/animal or 17.7-23.6 mg/kg of granisetron) mg granisetron/dose. The target organ of toxicity appeared to be the injection site, lymph node (lymphoid hyperplasia), bone marrow (myeloid hyperplasia) and spleen (hematopoiesis). It is to be mentioned here that similar doses did not produce histopathological changes in the lymph nodes, bone marrow and spleen when administered subcutaneously (once every 72 hours) for 28 days.

Study No.: (b) (4) 7436-129; AP Pharma 25-07

Volume # Page #: Vol. 1, page 1

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: October 17, 2005

GLP Compliance: A statement of compliance was included.

QA Report: yes (X) no ( )

Drug, Lot #, and % Purity: APF530 and (b) (4). The lot numbers are shown below (from page 11 and 12 of the study report).

Test Article	Lot No.	Storage	Purity	Expiration Date	Reserve (Archive) Sample
APF530 (active ingredient – 2% granisetron)	980040 980041 980042 974080 034-005-009A 034-005-009B 974094 034-005-010	Refrigerated at 2 to 8°C with desiccant	NA	See dose analysis results, Appendix 4	1 syringe per lot

NA Not applicable for polymers.

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Control Article	Lot/Batch No.	Storage	Purity	Expiration Date	Reserve (Archive) Sample
(b) (4)	980032 980033 980034 980035 980036	Refrigerated at 2 to 8°C with desiccant	NA	See dose analysis results, Appendix 4	1 syringe per lot

NA Not applicable for polymers.

Methods:

Doses: 2.8 and 5.9 mg granisetron/dose. The basis of dose selection was not mentioned.

Species/Strain: Rats/Sprague Dawley

Number/Sex/Group or Time Point (Main Study): 10/sex/group

Route, Formulation, and Volume: Subcutaneous, suspension, 0.12-0.25 ml/rat
Satellite Groups Used for Toxicokinetics: 4-10/sex/group

Age: 13 weeks

Weight: Males: 352-440 g; Females: 232-274 g

Study Design or Methodology: The animals were dosed once every 24 hours for a total of 92 or 93 injections. The following table (from page 13 of the study report) shows the study design.

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Group Designation and Dose Levels

Group	Number of Animals				Dose Volume[a,b] mL/animal
	Male		Female		
	Main[c, e]	Recovery[d]	Main[c, e]	Recovery[d]	
Toxicity Animals					
1 (Negative Control) Saline	10	5	10	5	0.25
2 (Vehicle Control) (b) (4)	10	5	10	5	0.25
3 (APF530)	10		10		0.12
4 (APF530)	10	5	10	5	0.25
Toxicokinetic Animals					
5 (Negative Control) Saline	4		4		0.25
6 (Vehicle Control) (b) (4)	4		4		0.25
7 (APF530)	10		10		0.12
8 (APF530)	10		10		0.25

a Animals were dosed once every 24 hours for a total of 92 or 93 injections.
b Based on a density for APF530 of 1.18 g/mL, a total dose volume of 0.12 and 0.25 mL translates into a total mass dose of approximately 142 and 295 mg/dose of APF530, respectively. Granisetron is present in APF530 at 2% on a weight/weight basis. A total dose volume of 0.12 and 0.25 mL of APF530 translates into a total mass dose of approximately 2.8 and 5.9 mg/dose of granisetron, respectively.
c The first 10 toxicity animals/sex/group (by ascending animal number) were designated as main study animals and were sacrificed following at least 90 days of treatment.
d The last 5 toxicity animals/sex/group (Groups 1, 2, and 4, by ascending animal number) were designated as recovery animals and were sacrificed following at least 90 days of treatment and a 6-week recovery phase.
e Toxicokinetic Groups included 1 animal/sex/group as a possible replacement.

Observation and Times:

Clinical Signs: Clinical signs were observed twice daily.

Body Weights: Body weights were recorded once prior to treatment and then weekly.

Food Consumption: Food Consumption was recorded on a weekly basis.

Hematology: Hematology was conducted at necropsy.

Clinical Chemistry: Clinical chemistry was conducted at necropsy.

Urinalysis: Urinalysis was conducted at necropsy.

Ophthalmoscopy: Opthalmoscopic examinations were conducted once prior to treatment and at week 13.

Gross Pathology: Gross pathology was conducted at necropsy.

Organ Weights: The following organs were weighed at necropsy: adrenal, brain, heart, kidney, liver, lung, ovary, spleen and testes.

Histopathology: The following organs (from page 233 and 234of the study report) were examined for histopathology from all animals at necropsy.

adrenal (2)
brain
cecum
colon
duodenum
epididymis (2)
esophagus
eye (2)
femur with bone marrow (articular surface of the distal end)
heart
ileum
injection site(s) (all)
jejunum
kidney (2)
lesions
liver
lung with mainstem bronchi
lymph node (mesenteric and axillary)
mammary gland (females)
ovary (2)
pancreas
pituitary gland
prostate
rectum
salivary gland [mandibular (2)]
sciatic nerve
seminal vesicle (2)
skeletal muscle (thigh)
skin
spinal cord (cervical, thoracic and lumbar)
spleen
sternum with bone marrow
stomach
testis (2)
thymus
thyroid (2) with parathyroid
tongue
trachea
urinary bladder
uterus
vagina

Toxicokinetics: Blood samples were collected from three rats/sex/group in Groups 5 and 6, on Day 1, once predose (Group 6 only) and at approximately 1 hour postdose. Blood samples were collected from thee rats/sex/group in Groups 7 and 8, on Days 1 and 28 (once predose and at approximately 5 minutes and 0.5, 1, 6, 12, and 24 hours postdose) and Day 88 (once predose and

at approximately 5 minutes and 0.5, 1, 6, 12, 24, 48, 72, 96, 120, and 144 hours postdose). Samples were assayed for granisetron using a high-performance liquid chromatography method with tandem mass spectrometric (LC/MS/MS) detection.

Irritation Observation: The designated subcutaneous injection sites from all animals were evaluated for effects prior to and after dosing on Day 1 and at necropsy. The injection sites were scored/graded using a modified Draize technique.

Results:

Mortality: There were no APF530-related deaths. One (b) (4) vehicle-dosed female was sacrificed on dosing phase Day 42 due to extensive injection site erosion/ulceration. One (b) (4) male was found dead of undetermined causes on recovery phase on Day 40.

Clinical Signs: There were no significant treatment-related clinical signs.

Body Weights: The mean initial and final weights of control males were 401 and 621 g, respectively. The mean initial and final weights of control females were 254 and 310 g, respectively. There were no significant treatment-related effects.

Food Consumption: The mean initial and final food consumption in control males were 34.9 and 37.7 g/animal/day, respectively. The mean initial and final food consumption in control females were 25.4 and 26.6 g/animal/day, respectively. There were no significant treatment-related effects.

Hematology: No significant treatment-related effects were observed.

Clinical Chemistry: There were no significant treatment-related effects.

Urinalysis: There were no significant treatment-related effects.

Gross Pathology: Treatment-related macroscopic observations were confined to changes at the injections sites. At the injection sites, administration of either APF530 or (b) (4) was associated with abrasions and thickening. Abrasions occurred exclusively in the (b) (4) rats of both sexes. Thickening of various injection sites was noted in all male and female groups, although the incidence was lower in the saline control animals.

Organ Weights: There were no significant treatment-related changes in the organ weights.

Histopathology: Histopathological changes were observed at the site of injection which included epidermal hyperplasia, epidermal erosion/ulceration, and suppurative inflammation. Additional nonepidermal microscopic lesions at various injection sites included edema and hemorrhage, fibrosis and skeletal muscle (panniculus carnosus) degeneration/ necrosis. Inflammatory infiltrates in the dermis, subcutis, and associated skeletal muscle (panniculus carnosus) ranged from chronic active to chronic. Chronic active infiltrates consisted of small mononuclear cells,

macrophages, and plasma cells admixed with abundant neutrophils, and eosinophils. Chronic infiltrates were comprised of small mononuclear cells and macrophages, and in some cases, assumed a more granulomatous appearance with numerous epithelioid cells and multinucleated giant cells, a few eosinophils, hemosiderin pigment-laden macrophages, and mineralized foci.

Lymphoid hyperplasia was observed in many lymph nodes (left and right axillary, inguinal, and popliteal). In both sexes, hyperplasia incidences were generally similar in the saline control, (b) (4) vehicle and APF530 groups. However, the severity was greater in the (b) (4) vehicle and APF530 groups than the saline controls.

Myeloid hyperplasia of the sternal and femoral bone marrow was noted in 1 of 10 males from the saline control group, 3 of 10 males and 3 of 11 females from the (b) (4) group, and 3 of 10 males and 1 of 10 females from the 0.25 ml APF350 group.

Treatment-related increased incidences of splenic hematopoiesis were observed in (b) (4) vehicle, 0.12 ml APF530, and 0.25 ml APF530 females compared to the saline controls.

The following table shows the histopathological changes.

Tissue Finding	Males				Females			
	1*	2	3	4	1	2	3	4
Lymph node, left, axillary, lymphoid hyperplasia	10	10	7	10	8	9	8	7
Lymph node, right, axillary, lymphoid hyperplasia	6	9	7	9	6	10	9	10
Lymph node, left, inguinal, lymphoid hyperplasia	5	5	3	6	6	7	8	5
Lymph node, right, inguinal, lymphoid hyperplasia	5	4	3	6	6	5	9	5
Lymph node, left, popliteal, lymphoid hyperplasia	2	6	8	8	5	9	9	6
Lymph node, right, politeal, lymphoid hyperplasia	4	9	9	5	4	7	10	6
Bone marrow, femur, myeloid hyperplasia	1	3	0	3	0	3	0	1
Bone marrow, sternum, myeloid hyperplasia	1	3	0	3	0	3	0	1
Spleen, hematopoiesis	9	10	10	9	3	7	9	7

*:
1: Saline Control (negative control)
2: (b) (4) (vehicle control, 0.25 ml/rat)
3: APF530 (0.12 ml/rat)
4: APF530 (0.25 ml/rat (0.25 ml/rat)

Toxicokinetics: Exposure to granisetron generally increased with the increase in APF530 dose level. After subcutaneous injection of APF530, granisetron readily appeared in plasma with Tmax values ranging from 0.0833 to 12.0 hours on Days 1, 28, and 88. No marked sex differences were observed in Cmax or $AUC_{0\text{-}24h}$ values, except for Group 8 males on Day 88, which showed a markedly lower Cmax value than females. Generally, values for both Cmax and $AUC_{0\text{-}24h}$ were markedly higher on Days 28 and 88 than on Day 1, indicating accumulation of granisetron after multiple dosing of APF530 in both sexes. The increases in Cmax and AUC were generally less than dose proportional. The following table shows the TK data (from page 17 of the study report).

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Toxicokinetic Parameters for Granisetron in Male and Female Rat Plasma

Dose Group	Dose Level[a] (mg/kg)	Sex	C_{max} (ng/mL)	DN C_{max}	T_{max} (hr)	$AUC_{0\text{-}t}$ (ng·hr/mL)	$AUC_{0\text{-}24}$ (ng·hr/mL)	DN $AUC_{0\text{-}24}$	$AUC_{0\text{-}144}$ (ng·hr/mL)
					Day 1				
7	6.71	M	39.5	5.88	6.00	498	498	74.3	NA
	11.5	F	71.4	6.22	12.0	1260	1260	110	NA
8	14.5	M	72.7	5.02	1.00	798	798	55.1	NA
	22.7	F	72.7	3.20	0.0833	1009	1009	44.4	NA
					Day 28				
7	6.67	M	59.3	8.88	1.00	984	984	147	NA
	11.1	F	145	13.1	0.0833	1409	1409	127	NA
8	12.8	M	110	8.54	1.00	1795	1795	140	NA
	21.7	F	252	11.6	12.0	4234	4234	195	NA
					Day 88				
7	5.54	M	81.7	14.7	1.00	2769	997	180	2950
	10.3	F	173	16.9	0.500	3849	1446	141	3849
8	10.2	M	126	12.4	0.500	5017	1281	126	5017
	18.6	F	523	28.1	0.0833	12721	2317	125	12721

a Actual mg/kg dose based on mean of the individual dose formulation and body weights.

Summary: In a 90-day subcutaneous toxicity study in rats, animals were treated once every 24 hours with APF530 at 2.8 (0.12 ml APF530/animal or 8.4-11.2 mg/kg of granisetron) and 5.9 (0.25 ml APF530/animal or 17.7-23.6 mg/kg of granisetron) mg granisetron/dose. The target organ of toxicity appeared to be the injection site, lymph node (lymphoid hyperplasia), bone marrow (myeloid hyperplasia) and spleen (hematopoiesis). It is to be mentioned here that similar doses did not produce histopathological changes in the lymph nodes, bone marrow and spleen when administered subcutaneously (once every 72 hours) for 28 days.

The sponsor submitted draft reports of the following toxicology study in dogs under the IND 68,213 Original submission. The reports were previously reviewed under the above IND. The reviews are incorporated below from the pharmacology review of IND 68,213

dated December 15, 2006. The results, interpretations and conclusions of the final reports of these studies do not seem to differ from the draft reports.

Study Title: 28-Day Subcutaneous Toxicity Study with APF530 in Dogs

Key Study Findings: In a 28-day subcutaneous toxicity study with APF530 in dogs, animals were treated with APF530 at 0.5 and 1.0 ml (10 and 20 mg of granisetron/dose) per animal once every 72 hours. A negative control group was administered saline (1 ml/dose) by s.c. injection and a vehicle control group was dosed subcutaneously with (b) (4) (polymer vehicle, 1 ml/dose). All dogs survived to scheduled necropsy. There was no evidence of systemic toxicity observed with either (b) (4) or APF530. No APF530 or (b) (4) -related changes in body weight, food consumption, ophthalmologic, electrocardiographic, clinical pathology or organ weight parameters were observed. There were no clinical observations and gross or histopathological effects on any organs or tissues with the exception of findings at the injection sites (edema, thickening, and inflammatory changes which included hemorrhage, degeneration/necrosis of skeletal muscle, fibrosis etc.). The target organ of toxicity appeared to be the site of injection.

Study No.: (b) (4) Study No. 7436- 115 and AP Pharma Study No. AP 24-13

Volume # Page #: 3.30, page 1

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: August 19, 2004

GLP Compliance: The sponsor submitted a draft report and GLP and QA statements were not signed.

QA Report: yes ( ) no (x)

Drug, Lot #, and % Purity: APF530 (containing 2% granisetron), Lot. 81762; (b) (4) (vehicle), Lot. 81740.

Methods: This study examined the toxicity of APF530 in Beagle dogs when administered at dose levels of 10 and 20 mg of granisetron/dose once every 72 hours (total of 10 doses) via s.c. injection for 28 days. Treated groups were compared to a negative control (0.9% saline) group given saline and a vehicle control group given (b) (4) (polymer vehicle). The dose volume was 1.0 ml/dog for all groups except for the 10 mg/dose, which was given 0.5 ml/dog.

Doses: 10 and 20 mg granisetron per dose

Basis of Dose Selection: Not mentioned

Species/Strain: Beagle dogs

Number/Sex/Group: 4/sex/dose

Route, Formulation, Volume: Subcutaneous, solution. The dose volume was 1.0 ml/dog for all groups except for the 10 mg/dose, which was given 0.5 ml/dog.

Satellite Groups Used for Toxicokinetics or Recovery: None

Age: 5 months old

Weight: Males: 6.9-9.5 kg; Females: 5.0-7.2 kg

Sampling Times: Blood samples were drawn on Day 1 and Day 25: one at predose and at approximately 5 minutes and 0.5, 1, 6, 12, 24, 48 and 72 hours postdose.

Study Design: The study design is shown in the following table.

Treatment	Male	Female	Dose Volume (ml/dose)	Dose (mg of granisetron/dose)	Dose Concentration (mg/ml)
1 (Saline)	4	4	1.0	0	0
2 (b) (4)	4	4	1.0	0	0
3 (APF530)	4	4	0.5	10	20
4 (APF530)	4	4	1.0	20	20

Observation and Times:

Mortality: Mortality was observed twice daily.

Clinical Signs: Clinical signs were observed twice daily.

Body Weights: Body weights were recorded on a weekly basis.

Food Consumption: Food consumption was recorded on a weekly basis.

Ophthalmoscopy: Ophthalmoscopy was conducted at pre-study and during Week 4.

Electrocardiography (ECG): Electrocardiography was conducted at pre-study and at Week 4.

Hematology: Hematology was conducted at necropsy.

Clinical Chemistry: Clinical chemistry was conducted at necropsy.

Urinalysis: Urinalysis was conducted at necropsy.

Gross Pathology: Gross pathology was conducted at necropsy.

Organ Weights: The following organs were weighed from all animals at necropsy: adrenal glands, brain, heart, kidneys, liver, lung, ovaries, spleen and testes.

Histopathology: Adequate Battery: yes (x), no ( )
Peer review: yes ( ), no (x)

Histopathological examinations were conducted on the following tissues from all animals at the scheduled necropsies: adrenals, brain, cecum, colon, duodenum, epididymides, esophagus, eyes, femur with bone marrow, gall bladder, heart, ileum, injection site, jejunum, kidneys, lesions, liver, lungs, lymph nodes (mesenteric), mammary area, ovary, pancreas, pituitary, prostate, rectum, regional lymph nodes to injection sites, salivary gland, sciatic nerve, skeletal muscle, skin, spinal cord (cervical, thoracic and lumbar), spleen, sternum, stomach, testes, thymus, thyroid, tongue, trachea, urinary bladder, uterus, and vagina.

Toxicokinetics: Blood samples were drawn on Day 1 and Day 25: one at predose and at approximately 5 minutes and 0.5, 1, 6, 12, 24, 48 and 72 hours postdose.

Results:

Mortality: None

Clinical Signs: Treatment-related edema was observed at the injection site.

Body Weights: The mean initial and final weights of control (saline) males were 8.1 and 9.2 kg, respectively. The mean initial and final body weights of control (saline) females were 6.1 and 7.2 kg, respectively. There were no significant treatment-related changes.

Food Consumption: The mean initial and final food consumption in control males were 256.3 and 390.6 g/animal/day, respectively. The mean initial and final food consumption in control females were 269.4 and 292.4 kg/animal/day, respectively. There were no significant treatment-related changes.

Ophthalmoscopy: No treatment-related changes were observed.

Electrocardiography: There were no significant treatment-related changes.

Hematology: There were no significant treatment-related changes.

Clinical Chemistry: No significant treatment-related changes were observed.

Urinalysis: There were no significant treatment-related changes.

Gross Pathology: Macroscopic examinations included thickening at the site of injection.

Organ Weights: No significant treatment-related changes were observed.

Histopathology: The following histopathologic changes were observed at the site of injection: inflammatory reactions characterized by cellular reaction, granulomatous inflammation, hemorrhage and degeneration/necrosis of skeletal muscle at all dose groups (although the incidence was lower in saline control compared to [redacted] (b) (4) and APF530), fibrosis, edema, and the presence of subcutaneous injection capsules (these were generally noted in [redacted] (b) (4) and APF530 groups).

Toxicokinetics: Exposure to granisetron increased as the APF530 dose level increased from 10 to 20 mg of granisetron/dose. Mean concentration of granisetron were generally similar on Days 1 and 25. The mean Tmax value was 1.0 to 7.5 hours on Day 1 and 2.25 to 9.00 hours on Day 25. Plasma granisetron levels declined slowly over time and measurable levels were present in all animals at 72 hours postdose on both days (Day 1 and 25). The mean $t_{1/2}$ values on Day 1 ranged from 18.9 to 20.9 hours and from 14.2 to 29.9 hours on Day 25. No marked (>2-fold) gender differences in the mean Cmax and $AUC_{0\text{-}72h}$ values were observed on the two collection days. There was no apparent accumulation of granisetron following multiple dosing of APF530. The toxicokinetic parameters on Day 1 and Day 25 are shown in the following table (from page 15 of Vol. 3.30 of sponsor's submission).

Summary of Mean Toxicokinetic Parameters of Granisetron in Dog Plasma: Day 1

Dose Group	APF530 Dose Level (mg/dose)	Gender		C_{max} (ng/mL)	T_{max} (hours)	AUC_{0-72hr} (ng·hr/mL)	$AUC_{0-\infty}$ (ng·hr/mL)	$t_{1/2}$ (hours)
3	10	M	Mean	14.5	3.75	349	376	18.9
			SD	3.6	5.50	33	49	3.5
			N	4	4	4	4	4
		F	Mean	15.8	5.00	427	467	20.2
			SD	0.8	5.23	89	100	1.0
			N	4	4	4	4	4
4	20	M	Mean	27.3	7.50	849	928	20.2
			SD	13.6	3.00	323	347	2.1
			N	4	4	4	4	4
		F	Mean	24.0	1.00	866	953	20.9
			SD	6.3	0	190	191	5.1
			N	4	4	4	4	4

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Summary of Mean Toxicokinetic Parameters of Granisetron in Dog Plasma: Day 25

Dose Group	APF530 Dose Level (mg/dose)	Gender		C_{max} (ng/mL)	T_{max} (hours)	AUC_{0-72hr} (ng·hr/mL)	$t_{1/2}$ (hours)
3	10	M	Mean	12.2	9.00	328	14.2
			SD	2.8	3.46	30	NA
			N	4	4	4	2
		F	Mean	14.8	8.00	449	23.8
			SD	2.3	10.92	118	NA
			N	4	4	4	2
4	20	M	Mean	31.7	6.25	920	21.5
			SD	13.0	4.50	282	NA
			N	4	4	4	2
		F	Mean	37.1	2.25	990	29.9
			SD	10.0	2.50	370	NA
			N	4	4	4	1

Summary: In a 28-day subcutaneous toxicity study with APF530 in dogs, animals were treated with APF530 at 0.5 and 1.0 mL (10 and 20 mg of granisetron/dose or 30-40 mg/kg and 60-80 mg/kg of granisetron, respectively) per animal once every 72 hours. A negative control group was administered saline (1 ml/dose) by s.c. injection and a vehicle control group was dosed subcutaneously with (b) (4) (1 ml/dose). All dogs survived to scheduled necropsy. There was no evidence of systemic toxicity observed with either

(b) (4) or APF530. No APF530 or (b) (4) -related changes in body weight, food consumption, ophthalmologic, electrocardiographic, clinical pathology or organ weight parameters occurred. There were no clinical observations and gross or histopathological effects on any organs with the exception of findings at the injection sites (edema, thickening, and inflammatory changes which included hemorrhage, degeneration/necrosis of skeletal muscle, fibrosis etc.). The target organ toxicity may be considered as the site of injection. The NOAEL may be considered as 60-80 mg/kg of granisetron.

The sponsor submitted the report of the 90-day toxicology study in dogs under the IND 68,213 Amendment 021 dated November 9, 2006. These reports were previously reviewed under the above IND. The reviews are incorporated below from the pharmacology review of IND 68,213 dated December 18, 2006. The results, interpretations and conclusions of the final reports of these studies do not seem to differ from the draft reports.

Study Title: 90-Day Subcutaneous Injection Toxicity and Toxicokinetic Study with APF530 in Dogs with a 6-Week Recovery

Key Study Findings: In a 90-day subcutaneous toxicity study in beagle dogs, animals were treated with APF530 at 11.8 (35.4-47.2 mg/kg of granisetron) and 23.6 (70.8-94.4 mg/kg of granisetron) mg granisetron/animal or 0.5 and 1.0 ml/dog/day. The target organ of toxicity could not be determined in the absence of any significant histopathological findings in any organ or tissues. The dose selection does not appear to be appropriate. In a 28-day study, these doses did not produce any toxicity when administered once every 72 hours. The sponsor could have administered higher doses.

Study No.: (b) (4) 7436-130; AP Pharma 25-08

Volume # Page #: Vol. 5, 1

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: November 18, 2005

GLP Compliance: A statement of compliance was included.

QA Report: yes (X) no ( )

Drug, Lot #, and % Purity: APF530, Lot Nos. 974094, 974082, 974093, 974081, 974102 and 974103

Methods:

Doses: 11.8 (35.4-47.2 mg/kg of granisetron) and 23.6 (70.8-94.4 mg/kg of granisetron) mg granisetron/animal or 0.5 and 1.0 ml/dog/day. The sponsor did not mention the basis of dose selection.

Species/Strain: Dogs/Beagle

Number/Sex/Group or Time Point (Main Study): 4-6/sex/group

Route, Formulation, Volume, and Infusion Rate: Subcutaneous, suspension, 0.5 and 1.0 ml/animal

Satellite Groups Used for Toxicokinetics: None

Age: 16-18 months

Weight: Males: 6.8-10.0 kg; Females: 5.5-8.4 kg

Study Design or Methodology: This study examined the toxicity of APF530 when administered to dogs at dose volumes of 0.5 and 1.0 ml/dog/day via subcutaneous injection for up to 93 days. The reversibility of effects following 1.0 ml/dog/day of APF530 was assessed during a 6-week recovery phase. Groups administered the test article, APF530, were compared to a negative control group (saline) and a vehicle control group (b) (4). The following table shows the study design (from page 12 of the study report).

Group	No. of Males		No. of Females		Dose Volume[a] mL/animal (mL/dog/day)
	Main[b]	Recovery[c]	Main[b]	Recovery[c]	
Toxicity Animals					
1 (Negative Control Saline)	4	2	4	2	1.0
2 (Vehicle Control (b) (4))	4	2	4	2	1.0
3 (APF530)	4		4		0.5
4 (APF530)	4	2	4	2	1.0

a Animals were dosed once every 24 hours for a total of 92 or 93 injections.
b The first four animals/sex/group (by ascending animal number) were designated as main study animals and were sacrificed following 92 or 93 days of treatment.
c The last two animals/sex/group (Groups 1, 2, and 4, by ascending animal number) were designated as recovery animals and were sacrificed following at least 90 days of treatment and a 6-week recovery phase.

Observation and Times:

Clinical Signs: Clinical signs were observed twice daily.

Body Weights: Body weights were recorded once prior to treatment and weekly thereafter.

Food Consumption: Food Consumption was recorded on a weekly basis.

Hematology: Hematology was conducted at predose and necropsy.

Clinical Chemistry: Clinical chemistry was conducted at predose and necropsy.

Urinalysis: Urinalysis was conducted at predose and necropsy.

Ophthalmoscopy: Ophthalmoscopy was conducted prior to initiation of treatment and at week 13.
Electrocardiography (ECG): Electrocardiography was conducted at predose and at week 13.

Gross Pathology: Gross pathology was conducted at necropsy.

Organ Weights: The following organs were weighed from all animals at necropsy: adrenal, brain, heart, kidney, liver with gallbladder, lung, ovary, spleen and testis.
Histopathology: The following organs (from page 159 of the study report) were examined for histopathology from all animals/group at necropsy.

adrenal (2)
brain
cecum
colon
duodenum
epididymis (2)[a]
esophagus
eye (2)[a]
femur with bone marrow (articular surface of the distal end)
gallbladder
heart
ileum
injection site(s)
jejunum
kidney (2)
lesions
liver
lung with mainstem bronchi
lymph node (mesenteric)
mammary gland (females)
ovary (2)
pancreas
pituitary gland
prostate
rectum
regional lymph node(s) to injection sites (axillary, inguinal, popliteal)
salivary gland [mandibular (2)]
sciatic nerve
skeletal muscle (thigh)
skin (untreated)
spinal cord (cervical, thoracic and lumbar)
spleen
sternum with bone marrow
stomach
testis (2)[a]
thymus
thyroid (2 lobes) with parathyroid
tongue
trachea
urinary bladder
uterus
vagina

a Modified Davidson's Fixative to be used.

Toxicokinetics: Blood samples were collected on Days 1, 28, and 90. For Groups 1 and 2, only a predose sample was collected for each animal. Samples were collected from Groups 3 and 4 at approximately 5 minutes and 0.5, 1, 6, 12 and 24 hours postdose.

Irritation Observation: The designated subcutaneous injection sites from all animals were evaluated for effects using a modified Draize technique.

Results:

Mortality: All animals survived to scheduled necropsy.

Clinical Signs: There were no test material-related clinical observations following subcutaneous administration of APF530.

Body Weights: The mean initial and final weights of control males were 8.8 and 10.9 kg, respectively. The mean initial and final weights of control females were 7.0 and 9.0 kg, respectively. There were no significant treatment-related effects.

Food Consumption: The mean initial and final food consumption in control males were 287.8 and 364 g/animal/day, respectively. The mean initial and final food consumption in control

females were 273.6 and 350.1 g/animal/day, respectively. There were no significant treatment-related effects.

Hematology: No significant treatment-related effects were observed.

Clinical Chemistry: There were no significant treatment-related effects.

Urinalysis: There were no significant treatment-related effects.

Gross Pathology: There were no test article-related gross pathology findings except at the injection sites (thickening).

Organ Weights: There were no significant treatment-related changes in the organ weights.

Histopathology: Histopathological effects at the injection sites included focal acanthosis, focal fibroplasia, focal chronic active inflammation, folliculitis, and scab formation. The incidence and severity of these lesions at different injection sites were similar between APF530 and (b) (4)

Toxicokinetics: After subcutaneous injection of APF530, granisetron readily appeared in the plasma with mean Tmax values ranging from 4.33 to 14.2 hours on Day 1, from 0.625 to 7.33 hours on Day 28, and from 0.625 to 4.50 hours on Day 90. Exposure to granisetron increased with the increase in APF530 dose. The increases in Cmax and $AUC_{0\text{-}24h}$ were generally dose proportional. No marked sex differences were observed in Cmax and AUC values. Both Cmax and AUC values were markedly higher on Days 28 and 90 compared to Day 1, indicating accumulation of granisetron after multiple dosing of APF530 in both sexes. The following tables (from page 17-18 of the study report) show the TK data on Day 1, 28 and 90.

(b) (4)7436-130

Summary of Toxicokinetic Parameters for Granisetron in Male and Female Dog Plasma
Day 1

Dose Group	Dose Level[a] (mg/kg)	Sex		C_{max} (ng/mL)	DN C_{max}	T_{max} (hr)	AUC_{0-24} (ng·hr/mL)	DN AUC_{0-24}
3	1.1	M	Mean	4.90	4.7	4.88	83.5	80
			SD	1.60	2.3	5.36	26.4	38
			N	4	4	4	4	4
	1.7	F	Mean	6.87	4.0	9.25	128	74
			SD	2.00	0.7	10.11	46	19
			N	4	4	4	4	4
4	2.5	M	Mean	9.41	3.7	4.33	182	72
			SD	3.58	1.3	4.58	56	20
			N	6	6	6	6	6
	3.3	F	Mean	12.4	3.7	14.2	243	73
			SD	2.6	0.8	8.7	33	11
			N	6	6	6	6	6

a Mean dose level is presented however the actual individual doses were used in toxicokinetic analysis.

Summary of Toxicokinetic Parameters for Granisetron in Male and Female Dog Plasma
Day 28

Dose Group	Dose Level[a] (mg/kg)	Sex		C_{max} (ng/mL)	DN C_{max}	T_{max} (hr)	AUC_{0-24} (ng·hr/mL)	DN AUC_{0-24}
3	1.2	M	Mean	19.2	15	0.750	341	267
			SD	8.0	4	0.289	172	92
			N	4	4	4	4	4
	1.5	F	Mean	21.7	14	0.625	282	181
			SD	6.4	3	0.250	70	24
			N	4	4	4	4	4
4	2.3	M	Mean	29.6	13	7.33	539	231
			SD	9.1	3	5.43	132	50
			N	6	6	6	6	6
	2.9	F	Mean	34.4	12	6.50	558	192
			SD	1.8	2	9.69	91	41
			N	6	6	6	6	6

a Mean dose level is presented however the actual individual doses were used in toxicokinetic analysis.

(b) (4) 7436-130

Summary of Toxicokinetic Parameters for Granisetron in Male and Female Dog Plasma
Day 90

Dose Group	Dose Level[a] (mg/kg)	Sex		C_{max} (ng/mL)	DN C_{max}	T_{max} (hr)	AUC_{0-24} (ng·hr/mL)	DN AUC_{0-24}
3	1.1	M	Mean	21.4	19	0.625	327	288
			SD	4.2	4	0.250	58	25
			N	4	4	4	4	4
	1.4	F	Mean	19.2	13	3.75	290	202
			SD	3.6	1	5.50	68	29
			N	4	4	4	4	4
4	1.97	M	Mean	38.8	19.8	4.50	576	295
			SD	11.1	6.0	9.56	82	53
			N	6	6	6	6	6
	2.7	F	Mean	34.0	13	1.67	551	208
			SD	5.3	2	2.14	81	25
			N	6	6	6	6	6

a Mean dose level is presented however the actual individual doses were used in toxicokinetic analysis.

Summary: In a 90-day subcutaneous toxicity study in beagle dogs, animals were treated with APF530 at 11.8 (35.4-47.2 mg/kg of granisetron) and 23.6 (70.8-94.4 mg/kg of granisetron) mg granisetron/animal or 0.5 and 1.0 ml/dog/day. The target organ of toxicity could not be determined in the absence of any significant histopathological findings in any organ or tissues. The dose selection does not appear to be appropriate. In a 28-day study, these doses did not produce any toxicity when administered once every 72 hours. The sponsor could have administered higher doses.

2.6.6.4 Genetic toxicology

The following reviews are incorporated from the pharmacology review of IND 68,213 dated December 15, 2006.

Study Title: Ames Test with APF530

Key Findings: Negative

Study No.: (b) (4)

Volume #, and Page #: 3.44, 1
Conducting Laboratory and Location: (b) (4)
Date of Study Initiation: August 27, 2004
GLP Compliance: This is a draft report and GLP compliance statement was not signed.
QA Reports: yes ( ) no (X)
Drug, Lot #, and % Purity: APF530, Lot No. 81762. Purity data not provided.

Methods: Plate incorporation method

Strains/cell line: *Salmonella typhimurium*, TA98, TA100, TA1535, TA1537 and WP2uvrA (*Escherichia coli*)

Doses used in definitive study: 100, 333, 1000, 3330 and 5000 µg/plate

Basis of dose selection: Cytotoxicity

Negative controls: Dimethyl sulfooxide (DMSO)

Positive controls: The positive controls are shown in the following table (from page 11 of sponsor's submission).

Table II. Positive Controls			
Tester Strain	S9 Mix	Positive Control	Dose (μg/plate)
TA98	+	benzo[a]pyrene	2.5
TA98	–	2-nitrofluorene	1.0
TA100	+	2-aminoanthracene	2.5
TA100	–	sodium azide	2.0
TA1535	+	2-aminoanthracene	2.5
TA1535	–	sodium azide	2.0
TA1537	+	2-aminoanthracene	2.5
TA1537	–	ICR-191	2.0
WP2*uvr*A	+	2-aminoanthracene	25.0
WP2*uvr*A	–	4-nitroquinoline-N-oxide	1.0

Incubation and sampling times: Plates were incubated for 52 hours and were evaluated immediately following the incubation period otherwise the plates were stored at 0-10^{0}C till evaluation.

Results:

Study validity: All doses of the test article, the vehicle controls and the positive controls were plated in triplicate. Revertant colonies were counted by automated colony counter. All criteria for a valid assay were met. The criteria for a positive result were as follows:

1. TA98, TA100 and WP2uvrA: A 2-fold increase in the mean revertants per plate of at least one of these tester strains over vehicle control.

2. TA1535 and TA1537: A 3-fold increase in the mean revertants per plate of at least one of these tester strains over vehicle control

Study outcome: Negative. The results are shown in the following table (from page 23 of sponsor's submission).

Table 5 : Mutagenicity Assay Results – Summary

Test Article ID: APF530

Assay No.: 26472-0-409OECD Trial No.: C1

Date Plated: 07-Oct-04 Vehicle: DMSO

Date Counted: 11-Oct-04, 12-Oct-04, 20-Oct-04 Plating Aliquot: 50 µL

		Mean Revertants Per Plate with Standard Deviation										Back-ground Lawn[a]
	Dose/Plate	TA98		TA100		TA1535		TA1537		WP2*uvrA*		
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: Rat Liver												
Vehicle Control		24	9	112	20	9	3	7	2	20	2	N
Test Article	100 µg	29	3	127	14	12	2	8	1	14	3	N
	333 µg	21	3	113	20	12	2	5	2	15	7	N
	1000 µg	18	7	116	9	15	3	6	3	12	6	N
	3330 µg	23	6	103	8	16	1	6	1	12	2	N
	5000 µg	24	7	125	9	17	3	7	2	17	6	N
Positive Control[b]		454	70	1035	53	167	19	246	40	604	46	N
Microsomes: None												
Vehicle Control		8	--	93	10	14	6	3	1	10	1	N
Test Article	100 µg	13	1	84	6	17	6	5	3	14	4	N
	333 µg	12	2	97	11	15	2	7	2	12	3	N
	1000 µg	13	5	105	9	16	6	3	1	10	3	N
	3330 µg	13	3	103	11	15	3	5	3	10	3	N
	5000 µg	9	5	117	14	18	2	8	2	9	6	NP
Positive Control[c]		510	267	1221	44	979	63	648	47	209	175	N

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

[b]

TA98	benzo[a]pyrene	2.5 µg/plate
TA100	2-aminoanthracene	2.5 µg/plate
TA1535	2-aminoanthracene	2.5 µg/plate
TA1537	2-aminoanthracene	2.5 µg/plate
WP2*uvr*A	2-aminoanthracene	25.0 µg/plate

[c]

TA98	2-nitrofluorene	1.0 µg/plate
TA100	sodium azide	2.0 µg/plate
TA1535	sodium azide	2.0 µg/plate
TA1537	ICR-191	2.0 µg/plate
WP2*uvr*A	4-nitroquinoline-N-oxide	1.0 µg/plate

Study Title: Ames Test with [redacted](b) (4) (Polymer Vehicle)

Key Findings: Negative

Study No.: [redacted](b) (4) Study No. 7436-121, Sponsor's Study No. AP 24-17

Volume #, and Page #: 3.45, 1
Conducting Laboratory and Location: [redacted](b) (4)
Date of Study Initiation: August 27, 2004

GLP Compliance: This is a draft report and GLP compliance statement was not signed.
QA Reports: yes ( ) no (X)
Drug, Lot #, and % Purity: (b) (4), Lot No. 81763. Purity data not provided.

Methods: Plate incorporation method

Strains/cell line: *Salmonella typhimurium*, TA98, TA100, TA1535, TA1537 and WP2uvrA (*Escherichia coli*)

Doses used in definitive study: 100, 333, 1000, 3330 and 5000 µg/plate

Basis of dose selection: Cytotoxicity

Negative controls: Dimethyl sulfooxide (DMSO)

Positive controls: The positive controls are shown in the following table (from page 11 of sponsor's submission).

Table II. Positive Controls

Tester Strain	S9 Mix	Positive Control	Dose (µg/plate)
TA98	+	benzo[a]pyrene	2.5
TA98	–	2-nitrofluorene	1.0
TA100	+	2-aminoanthracene	2.5
TA100	–	sodium azide	2.0
TA1535	+	2-aminoanthracene	2.5
TA1535	–	sodium azide	2.0
TA1537	+	2-aminoanthracene	2.5
TA1537	–	ICR-191	2.0
WP2*uvr*A	+	2-aminoanthracene	25.0
WP2*uvr*A	–	4-nitroquinoline-N-oxide	1.0

Incubation and sampling times: Plates were incubated for 52 hours and were evaluated immediately following the incubation period otherwise the plates were stored at 0-10^{0}C till evaluation.

Results:

Study validity: All doses of the test article, the vehicle controls and the positive controls were plated in triplicate. Revertant colonies were counted by automated colony counter. All criteria for a valid assay were met. The criteria for positive results were as follows:

3. TA98, TA100 and WP2uvrA: A 2-fold increase in the mean revertants per plate of at least one of these tester strains over vehicle control.

4. TA1535 and TA1537: A 3-fold increase in the mean revertants per plate of at least one of these tester strains over vehicle control

Study outcome: Negative. The results are shown in the following table (from page 24 of sponsor's submission).

Table 5 : Mutagenicity Assay Results –Summary

Test Article ID: (b) (4)

Assay No.: 26473-0-409OECD Trial No.: C1

Date Plated: 07-Oct-04 Vehicle: DMSO

Date Counted: 12-Oct-04 Plating Aliquot: 50 µL

		Mean Revertants Per Plate with Standard Deviation										Back-ground Lawn[a]
	Dose/Plate	TA98		TA100		TA1535		TA1537		WP2*uvr*A		
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: Rat Liver												
Vehicle Control		22	5	107	4	14	2	12	1	17	4	N
Test Article	100 µg	23	2	97	6	10	5	5	3	16	4	N
	333 µg	22	3	112	20	9	2	6	5	23	5	N
	1000 µg	20	8	109	2	16	4	6	3	22	5	N
	3330 µg	22	7	102	5	13	6	8	2	19	4	N
	5000 µg	20	2	104	9	13	3	6	5	20	2	N
Positive Control[b]		290	14	1106	43	170	6	160	29	636	124	N
Microsomes: None												
Vehicle Control		10	4	95	6	14	5	5	1	18	2	N
Test Article	100 µg	16	4	83	19	15	3	4	1	15	6	N
	333 µg	19	2	93	1	13	2	5	2	13	3	N
	1000 µg	13	2	93	15	15	2	3	2	14	3	N
	3330 µg	14	4	95	14	18	3	8	2	16	4	N
	5000 µg	17	6	91	15	17	5	2	2	13	3	N
Positive Control[c]		434	40	1087	110	850	35	705	105	344	102	N

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

[b] TA98	benzo[a]pyrene	2.5 µg/plate	[c] TA98	2-nitrofluorene	1.0 µg/plate
TA100	2-aminoanthracene	2.5 µg/plate	TA100	sodium azide	2.0 µg/plate
TA1535	2-aminoanthracene	2.5 µg/plate	TA1535	sodium azide	2.0 µg/plate
TA1537	2-aminoanthracene	2.5 µg/plate	TA1537	ICR-191	2.0 µg/plate
WP2*uvr*A	2-aminoanthracene	25.0 µg/plate	WP2*uvr*A	4-nitroquinoline-N-oxide	1.0 µg/plate

Study Title: Ames Test with Granisetron

Key Findings: Negative

Study No.: (b) (4) Study No. 7436-122, Sponsor's Study No. AP 24-18

Volume #, and Page #: 3.46, 1
Conducting Laboratory and Location: (b) (4)
Date of Study Initiation: August 27, 2004
GLP Compliance: This is a draft report and GLP compliance statement was not signed.
QA Reports: yes ( ) no (X)
Drug, Lot #, and % Purity: Granisetron, Lot No. 43PAL45A, and >99%

Methods: Plate incorporation method

Strains/cell line: *Salmonella typhimurium*, TA98, TA100, TA1535, TA1537 and WP2uvrA (*Escherichia coli*)

Doses used in definitive study: 100, 333, 1000, 3330 and 5000 µg/plate

Basis of dose selection: Cytotoxicity

Negative controls: Dimethyl sulfooxide (DMSO)

Positive controls: The positive controls are shown in the following table (from page 11 of sponsor's submission).

Table II. Positive Controls

Tester Strain	S9 Mix	Positive Control	Dose (µg/plate)
TA98	+	benzo[a]pyrene	2.5
TA98	–	2-nitrofluorene	1.0
TA100	+	2-aminoanthracene	2.5
TA100	–	sodium azide	2.0
TA1535	+	2-aminoanthracene	2.5
TA1535	–	sodium azide	2.0
TA1537	+	2-aminoanthracene	2.5
TA1537	–	ICR-191	2.0
WP2*uvr*A	+	2-aminoanthracene	25.0
WP2*uvr*A	–	4-nitroquinoline-N-oxide	1.0

Incubation and sampling times: Plates were incubated for 52 hours and were evaluated immediately following the incubation period otherwise the plates were stored at 0-10^{0}C till evaluation.

Results:

Table 3 : Mutagenicity Assay Results – Summary

Test Article ID: Granisetron

Assay No.: 26474-0-409OECD Trial No.: B1

Date Plated: 22-Sep-04 Vehicle: DMSO

Date Counted: 29-Sep-04 Plating Aliquot: 100 µL

		Mean Revertants Per Plate with Standard Deviation										Back-ground Lawn[a]
	Dose/Plate	TA98		TA100		TA1535		TA1537		WP2*uvrA*		
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: Rat Liver												
Vehicle Control		18	5	100	5	11	5	7	1	13	4	N
Test Article	100 µg	19	4	103	7	9	4	4	1	13	5	N
	333 µg	23	6	98	5	12	6	7	3	15	5	N
	1000 µg	18	3	83	11	7	3	7	4	13	4	N
	3330 µg	19	7	105	9	10	4	7	2	7	2	N
	5000 µg	25	2	111	8	12	3	3	1	4	0	N
Positive Control[b]		304	27	939	37	138	16	170	45	492	23	N
Microsomes: None												
Vehicle Control		8	3	85	6	15	4	4	2	17	5	N
Test Article	100 µg	9	1	81	7	15	7	6	1	9	1	N
	333 µg	10	2	90	13	20	8	5	2	10	8	N
	1000 µg	10	5	92	4	21	6	4	2	9	1	N
	3330 µg	11	6	93	9	32	8	4	2	6	2	N
	5000 µg	9	5	90	6	24	5	4	1	4	2	N
Positive Control[c]		342	56	1074	62	789	33	573	54	355	35	N

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

[b] TA98	benzo[a]pyrene	2.5 µg/plate	[c] TA98	2-nitrofluorene	1.0 µg/plate
TA100	2-aminoanthracene	2.5 µg/plate	TA100	sodium azide	2.0 µg/plate
TA1535	2-aminoanthracene	2.5 µg/plate	TA1535	sodium azide	2.0 µg/plate
TA1537	2-aminoanthracene	2.5 µg/plate	TA1537	ICR-191	2.0 µg/plate
WP2*uvrA*	2-aminoanthracene	25.0 µg/plate	WP2*uvrA*	4-nitroquinoline-N-oxide	1.0 µg/plate

Study Title: L5178Y TK$^{+/-}$ Mouse Lymphoma Forward Mutation Assay with APF530

Key Findings: Negative

Study No.: (b) (4) Study No. 7436-117, Sponsor's Study No. AP 24-15

Volume #, and Page #: 3.47, 1
Conducting Laboratory and Location: (b) (4)
Date of Study Initiation: September 16, 2004

GLP Compliance: This is a draft report and GLP compliance statement was not signed.
QA Reports: yes ( ) no (X)
Drug, Lot #, and % Purity: APF530, Lot No. 81762. Purity data were not provided.

Methods:

Strains/cell line: Mouse lymphoma L5178Y cell line

Doses used in definitive study: 19.7, 39.3, 78.5, 157, 313, 625, 1250 and 2500 µg/ml

Basis of dose selection: Cytotoxicity

Negative controls: Dimethyl sulfooxide (DMSO)

Positive controls: -S9: Methyl methane sulfonate (MMS, 6.5 µg/ml for 24 h treatment period and 13 µg/ml for 4-hour treatment period); +S9: Methylcholanthrene (MCA, 2-4 µg/ml)

Incubation and sampling times: -S9: 24 hours; +S9: 4 hours. Expression period: 2 days

Results:

Study validity: Three cultures were analyzed for each concentration. Both small and large colonies were counted. The test article was evaluated as positive if concentration-dependent increases of 2-fold or greater in mutant frequency are obtained over the concurrent background mutant frequency.

Study outcome: Negative. The results are shown in the following table (from page 26 and 30 of sponsor's submission).

(b) (4)7436-117
Genetic Toxicology Assay No. 26472-0-431ICH
Sponsor Study Number: 24-15

TABLE 5: SIZING DATA FOR CONFIRMATORY MUTATION ASSAY WITHOUT ACTIVATION

A. TEST ARTICLE: APF530
B. ASSAY NO.: 26472-0-431ICH
C. VEHICLE: DMSO
D. SELECTIVE AGENT: TFT 3.0 µg/mL
E. TREATMENT DATE: 11/30/2004

Test Condition	Conc.	Cum. RSG (%)[a] Day 1	Day 2	Day 3	Cloning Efficiency[b] Abs %	Rel %	Relative Growth[c] (%)	Mutant Frequency (x 10^{-6})[d] Total	Small	Large
Vehicle Control[e]	1%	103.0	104.5	110.8	134.6	107.2	118.9	47.8	28.6	19.2
	1%	105.6	101.5	96.1	119.8	95.5	91.7	46.7	27.6	19.1
	1%	91.4	94.0	93.1	122.0	97.2	90.6	47.4	28.0	19.4
MMS[f] (µg/mL)	6.5	77.2	84.6	43.0	53.6	42.8	18.4	346.4	187.1	159.3
	6.5	64.8	52.6	25.4	51.6	41.2	10.4	372.5	190.8	181.7
Test Article (µg/mL)	19.7	96.7	110.0	104.1	139.8	111.4	116.0	40.1	20.0	20.0
	39.3	103.8	109.6	110.5	123.6	98.6	108.9	48.8	28.2	20.6
	78.5	93.2	117.3	120.3	112.4	89.6	107.7	45.3	26.2	19.1
	157	87.9	115.3	76.8	159.6	127.2	97.8	43.7	21.6	22.1
	313	84.3	93.6	87.7	126.9	101.2	88.8	41.0	25.5	15.5
	625	69.2	95.6	78.7	135.5	108.0	85.0	55.8	25.0	30.9
	1250	64.8	64.0	58.3	153.3	122.2	71.3	43.9	25.9	18.0
	2500	31.1	33.5	25.3	126.2	100.6	25.4	47.6	26.5	21.0

[a]Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100
[c]Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10^{-4})
Decimal is moved to express the frequency in units of 10^{-6}
Expressed as Total Mutant Frequency, Small Colony Mutant Frequency and Large Colony Mutant Frequency
[e]Vehicle Control = DMSO
[f]Positive Control: MMS = Methyl methanesulfonate
Colony Counts increased by 9.099% to compensate for area of dish not scanned

(b) (4) 7436-117
Genetic Toxicology Assay No. 26472-0-431ICH
Sponsor Study Number: 24-15

TABLE 9: SIZING DATA FOR CONFIRMATORY MUTATION ASSAY WITH ACTIVATION

A. TEST ARTICLE: APF530
B. ASSAY NO.: 26472-0-431ICH
C. VEHICLE: DMSO
D. SELECTIVE AGENT: TFT 3.0 µg/mL
E. TREATMENT DATE: 11/30/2004

Test Condition	Conc.	Cum. RSG (%)[a] Day 1	Cum. RSG (%)[a] Day 2	Cloning Efficiency[b] Abs %	Cloning Efficiency[b] Rel %	Relative Growth[c] (%)	Mutant Frequency (x 10^{-6})[d] Total	Small	Large
Vehicle Control[e]									
	1%	94.4	105.1	99.6	94.7	99.6	65.0	36.1	28.8
	1%	103.5	100.2	107.5	102.1	102.3	53.1	30.1	23.0
	1%	102.1	94.7	108.6	103.2	97.7	53.9	32.2	21.8
MCA[f] (µg/mL)									
	2	67.4	64.7	104.4	99.2	64.1	232.8	108.7	124.0
	4	54.9	51.3	113.1	107.5	55.2	255.0	101.0	154.0
Test Article (µg/mL)									
	19.7	101.4	85.9	129.8	123.4	106.0	46.2	27.5	18.8
	39.3	106.9	83.7	134.6	127.9	107.0	46.2	24.6	21.6
	78.5	86.8	79.1	116.7	110.9	87.8	49.2	28.3	20.9
	157	93.1	80.3	118.9	113.0	90.8	51.7	24.5	27.2
	313	81.3	79.3	120.0	114.1	90.5	48.2	25.2	23.0
	625	91.7	88.0	109.3	103.9	91.4	50.2	26.6	23.6
	1250	78.5	82.3	128.7	122.4	100.7	48.0	29.4	18.6
	2500	81.9	74.7	113.1	107.5	80.3	42.1	26.7	15.4

[a]Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100
[c]Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10^{-4})
Decimal is moved to express the frequency in units of 10^{-6}
Expressed as Total Mutant Frequency, Small Colony Mutant Frequency and Large Colony Mutant Frequency
[e]Vehicle Control = DMSO
[f]Positive Control: MCA = Methylcholanthrene
Colony Counts increased by 9.099% to compensate for area of dish not scanned

Study Title: L5178Y TK$^{+/-}$ Mouse Lymphoma Forward Mutation Assay with (b) (4) (Polymer Vehicle)

Key Findings: Negative

Study No.: (b) (4) Study No. 7436-119, Sponsor's Study No. AP 24-19

Volume #, and Page #: 3.48, 1
Conducting Laboratory and Location: (b) (4)
Date of Study Initiation: September 16, 2004
GLP Compliance: This is a draft report and GLP compliance statement was not signed.

QA Reports: yes ( ) no (X)
Drug, Lot #, and % Purity: (b) (4)(Polymer vehicle), Lot No. 81740

Methods:

Strains/cell line: Mouse lymphoma L5178Y cell line

Doses used in definitive study: 500, 750, 1000, 1500, 2000, 3000, 4000 and 5000 µg/ml

Basis of dose selection: Cytotoxicity

Negative controls: Dimethyl sulfooxide (DMSO)

Positive controls: -S9: Methyl methane sulfonate (MMS, 6.5 µg/ml for 24 h treatment period and 13 µg/ml for 4-hour treatment period); +S9: Methylcholanthrene (MCA, 2-4 µg/ml)

Incubation and sampling times: -S9: 24 hours; +S9: 4 hours. Expression period: 2 days

Results:

Study validity: Three cultures were analyzed for each concentration. Both small and large colonies were counted. The test article was evaluated as positive if concentration-dependent increases of 2-fold or greater in mutant frequency are obtained over the concurrent background mutant frequency.

Study outcome: Negative. The results are shown in the following table (from page 26 and 30 of sponsor's submission).

(b) (4)7436-119
Genetic Toxicology Assay No. 26473-0-431ICH
Sponsor Study Number: 24-19

TABLE 5: SIZING DATA FOR CONFIRMATORY MUTATION ASSAY WITHOUT ACTIVATION

A. TEST ARTICLE: (b) (4) (polymer vehicle)
B. ASSAY NO.: 26473-0-431ICH
C. VEHICLE: DMSO
D. SELECTIVE AGENT: TFT 3.0 µg/mL
E. TREATMENT DATE: 11/16/2004

Test Condition	Conc.	Cum. RSG (%)[a] Day 1	Day 2	Day 3	Cloning Efficiency[b] Abs %	Rel %	Relative Growth[c] (%)	Mutant Frequency (x 10^{-6})[d] Total	Small	Large
Vehicle Control[e]										
	1%	97.5	105.4	110.1	123.6	104.7	115.3	30.3	10.9	19.4
	1%	99.8	95.3	102.3	114.9	97.3	99.6	33.5	11.4	22.2
	1%	102.7	99.3	87.5	115.6	97.9	85.7	37.7	11.6	26.1
MMS[f] (µg/mL)										
	6.5	67.7	45.2	25.1	45.8	38.8	9.7	289.7	161.9	127.8
	6.5	72.4	49.1	28.7	37.6	31.9	9.2	273.4	118.8	154.6
Test Article (µg/mL)										
	500	82.3	97.5	98.2	131.6	111.5	109.4	32.0	11.0	21.0
	750	67.1	68.0	60.6	124.7	105.6	64.0	44.3	12.2	32.1
	1000	75.9	44.5	47.8	143.5	121.5	58.1	30.9	9.9	21.0
	1500	65.4	47.4	49.1	127.6	108.1	53.0	34.5	10.8	23.6
	2000	42.6	31.4	37.3	107.3	90.9	33.9	32.5	11.2	21.4
	3000	50.2	33.5	27.0	106.2	89.9	24.3	34.9	12.3	22.6
	4000	43.8	27.7	22.8	114.4	96.9	22.1	30.2	7.6	22.6
	5000	24.5	14.4	13.1	134.6	114.0	14.9	43.5	14.9	28.6

[a]Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100
[c]Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10^{-4})
Decimal is moved to express the frequency in units of 10^{-6}
Expressed as Total Mutant Frequency, Small Colony Mutant Frequency and Large Colony Mutant Frequency
[e]Vehicle Control = DMSO
[f]Positive Control: MMS = Methyl methanesulfonate
Colony Counts increased by 9.099% to compensate for area of dish not scanned

(b) (4) 7436-119
Genetic Toxicology Assay No. 26473-0-431ICH
Sponsor Study Number: 24-19

TABLE 9: SIZING DATA FOR CONFIRMATORY MUTATION ASSAY WITH ACTIVATION

A. TEST ARTICLE: (b) (4) (polymer vehicle)
B. ASSAY NO.: 26473-0-431ICH
C. VEHICLE: DMSO
D. SELECTIVE AGENT: TFT 3.0 µg/mL
E. TREATMENT DATE: 11/16/2004

		Cum. RSG (%)[a]		Cloning Efficiency[b]		Relative Growth[c]	Mutant Frequency (x 10^{-6})[d]		
Test Condition	Conc.	Day 1	Day 2	Abs %	Rel %	(%)	Total	Small	Large
Vehicle Control[e]									
	1%	84.7	92.4	111.1	96.4	89.1	34.4	12.8	21.6
	1%	117.1	109.8	111.3	96.6	106.0	46.1	20.6	25.5
	1%	98.2	97.8	123.3	107.0	104.6	45.4	19.5	26.0
MCA[f] (µg/mL)									
	2	53.5	43.5	103.1	89.5	39.0	208.5	75.5	133.0
	4	72.9	62.8	100.7	87.4	54.9	266.1	101.1	165.0
Test Article (µg/mL)									
	100	98.2	102.5	152.4	132.2	135.5	32.7	14.8	17.9
	250	96.5	107.1	120.9	104.9	112.4	60.8	25.6	35.2
	500	101.2	107.5	131.8	114.4	123.0	30.3	9.1	21.2
	1000	105.9	110.5	122.4	106.2	117.3	36.3	13.7	22.6
	2000	72.9	83.8	110.9	96.3	80.6	45.9	17.0	28.9
	3000	75.9	87.2	108.6	94.2	82.1	46.6	16.1	30.5
	4000	84.1	81.3	99.6	86.5	70.3	35.4	10.6	24.8
	5000	78.2	76.4	97.3	84.4	64.5	38.5	15.7	22.8

[a]Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100
[c]Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10^{-4})
Decimal is moved to express the frequency in units of 10^{-6}
Expressed as Total Mutant Frequency, Small Colony Mutant Frequency and Large Colony Mutant Frequency
[e]Vehicle Control = DMSO
[f]Positive Control: MCA = Methylcholanthrene
Colony Counts increased by 9.099% to compensate for area of dish not scanned

Study Title: L5178Y TK$^{+/-}$ Mouse Lymphoma Forward Mutation Assay with Granisetron

Key Findings: Negative without metabolic activation and positive with metabolic activation.

Study No.: (b) (4) Study No. 7436-120, Sponsor's Study No. AP 24-20

Volume #, and Page #: 3.49, 1
Conducting Laboratory and Location: (b) (4)
Date of Study Initiation: September 16, 2004
GLP Compliance: This is a draft report and GLP compliance statement was not signed.
QA Reports: yes ( ) no (X)
Drug, Lot #, and % Purity: Granisetron, Lot No. 040528

Methods:

Strains/cell line: Mouse lymphoma L5178Y cell line

Doses used in definitive study: -S9: 25, 50, 100 and 150 µg/ml; +S9: 75, 100, 150, 200, 250, 300, 350 and 400 µg/ml

Basis of dose selection: Cytotoxicity

Negative controls: Dimethyl sulfooxide (DMSO)

Positive controls: -S9: Methyl methane sulfonate (MMS, 13 µg/ml for 4-hour treatment period); +S9: Methylcholanthrene (MCA, 2-4 µg/ml)

Incubation and sampling times: -S9: 24 hours; +S9: 4 hours. Expression period: 2 days

Results:

Study validity: Three cultures were analyzed for each concentration. Both small and large colonies were counted. The test article was evaluated as positive if concentration-dependent increases of 2-fold or greater in mutant frequency are obtained over the concurrent background mutant frequency.

Study outcome: Negative without metabolic activation and positive with metabolic activation. About 2.02-, 2.4, and 2.8-fold increase over control in total mutant frequencies at 300, 325 and 350 µg/ml, respectively. There was a dose-related increase in the number of small colonies (2-, 2.25-, 2.25-, 3.0-, 3.25- and 4.4-fold increase over control at 150, 200, 250, 300, 325 and 350 µg/ml, respectively). The results are shown in the following table (from page 28 and 33 of sponsor's submission).

(b) (4) 7436-120
Genetic Toxicology Assay No. 26474-0-431ICH
Sponsor Study Number: 24-20

TABLE 6: SIZING DATA FOR CONFIRMATORY MUTATION ASSAY WITHOUT ACTIVATION

A. TEST ARTICLE: Granisetron
B. ASSAY NO.: 26474-0-431ICH
C. VEHICLE: DMSO
D. SELECTIVE AGENT: TFT 3.0 µg/mL
E. TREATMENT DATE: 11/09/2004

Test Condition	Conc.	Cum. RSG (%)[a] Day 1	Day 2	Day 3	Cloning Efficiency[b] Abs %	Rel %	Relative Growth[c] (%)	Mutant Frequency (x 10^{-6})[d] Total	Small	Large
Vehicle Control[e]										
	1%	108.3	97.5	99.1	125.6	108.5	107.5	48.0	31.5	16.5
	1%	84.6	96.3	96.0	116.6	100.7	96.7	44.9	25.6	19.3
	1%	107.1	106.2	104.9	105.1	90.8	95.2	38.4	21.5	17.0
MMS[f] (µg/mL)										
	6.5	66.9	40.0	20.9	60.0	51.8	10.8	342.4	217.0	125.5
	6.5	74.8	30.9	18.6	70.7	61.1	11.3	304.4	179.9	124.4
Test Article (µg/mL)										
	25.0	65.7	72.4	79.9	146.6	126.6	101.2	34.5	19.1	15.4
	50.0	76.7	66.2	63.4	122.7	106.0	67.2	40.3	24.0	16.3
	100	62.1	40.5	44.7	96.0	82.9	37.0	51.5	30.7	20.8
	150	26.8	20.2	18.5	115.3	99.6	18.5	44.5	28.1	16.4

[a]Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100
[c]Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10^{-4})
Decimal is moved to express the frequency in units of 10^{-6}
Expressed as Total Mutant Frequency, Small Colony Mutant Frequency and Large Colony Mutant Frequency
[e]Vehicle Control = DMSO
[f]Positive Control: MMS = Methyl methanesulfonate
Colony Counts increased by 9.099% to compensate for area of dish not scanned

(b) (4) 7436-120
Genetic Toxicology Assay No. 26474-0-4311CH
Sponsor Study Number: 24-20

TABLE 11: SIZING DATA FOR CONFIRMATORY MUTATION ASSAY WITH ACTIVATION

A. TEST ARTICLE: Granisetron
B. ASSAY NO.: 26474-0-4311CH
C. VEHICLE: DMSO
D. SELECTIVE AGENT: TFT 3.0 µg/mL
E. TREATMENT DATE: 11/09/2004

		Cum. RSG (%)[a]		Cloning Efficiency[b]		Relative Growth[c]	Mutant Frequency (x 10^{-6})[d]		
Test Condition	Conc.	Day 1	Day 2	Abs %	Rel %	(%)	Total	Small	Large
Vehicle Control[e]									
	1%	106.3	104.1	119.6	104.4	108.7	37.1	15.8	21.3
	1%	101.0	100.5	110.0	96.0	96.6	38.7	15.9	22.8
	1%	92.7	95.4	114.0	99.5	94.9	31.9	12.4	19.5
MCA[f] (µg/mL)									
	2	68.3	57.1	84.4	73.7	42.0	294.4	136.2	158.2
	4	67.7	45.7	97.3	84.9	38.8	286.7	125.6	161.1
Test Article (µg/mL)									
	50.0	95.6	88.0	160.9	140.5	123.6	36.4	13.6	22.8
	100	86.7	92.2	128.0	111.7	103.0	40.9	13.1	27.8
	150	73.1	79.5	124.9	109.0	86.7	63.5	32.0	31.4
	200	80.2	71.0	112.6	98.3	69.8	64.6	36.2	28.4
	250	45.1	40.0	120.9	105.6	42.2	68.6	36.1	32.5
	300	29.1	32.4	94.6	82.5	26.8	75.0	47.7	27.3
	325	19.6	17.9	103.5	90.3	16.2	88.2	52.4	35.9
	350	10.1	12.0	99.8	87.1	10.5	105.6	70.3	35.3

[a]Cum. RSG = Cumulative Suspension Growth Relative to the Average Vehicle Control Suspension Growth
[b]Cloning Efficiency = Total Viable Colony Count/Number of Cells Seeded x 100
[c]Relative Growth = (Relative Suspension Growth x Relative Cloning Efficiency) / 100
[d]Mutant Frequency = (Total Mutant Colonies/Total Viable Colonies) x (2 x 10^{-4})
Decimal is moved to express the frequency in units of 10^{-6}
Expressed as Total Mutant Frequency, Small Colony Mutant Frequency and Large Colony Mutant Frequency
[e]Vehicle Control = DMSO
[f]Positive Control: MCA = Methylcholanthrene
Colony Counts increased by 9.099% to compensate for area of dish not scanned

Study Title: *In Vivo* Rat Micronucleus Assay with APF530 and (b) (4) by Subcutaneous Route

Key Findings: Negative.

Study No.: (b) (4) Study No. 7436-118, Sponsor's Study No. AP 24-16

Volume #, and Page #: 3.50, 1
Conducting Laboratory and Location: (b) (4)
Date of Study Initiation: September 10, 2004
GLP Compliance: This is a draft report and GLP compliance statement was not signed.
QA Reports: yes ( ) no (X)
Drug, Lot #, and % Purity: APF530: Lot No. 81762; (b) (4): Lot No. 81740

Methods:

Strains: Sprague Dawley male rats, 9 weeks old and body weight: 267-329 g

Doses used in definitive study: (b) (4): 750, 1500 and 3000 mg/kg/day; APF530: 750, 1500 and 3000 mg/kg, s.c. The study design is shown in the following table (from page 8 of sponsor's submission).

Approximate Dose Level (mg/kg)	Stock Concentration	Dosing Volume	Route of Administration	Animals/Harvest Timepoint[a] 24 Hour Male	48 Hour Male
Positive Control, 60	6 mg/mL	10 mL/kg	Oral Gavage	6	-
Negative Control, 0	0 mg/mL	1.0 mL total in 4 x 0.25 doses	SC	6	6
(b) (4)					
750	as supplied	0.25 mL total in 1 x 0.25 doses	SC	6	-
1500	as supplied	0.5 mL total in 2 x 0.25 doses	SC	6	-
3000	as supplied	1.0 mL total in 4 x 0.25 doses	SC	6	6
APF530					
750	as supplied	0.25 mL total in 1 x 0.25 doses	SC	6	-
1500	as supplied	0.5 mL total in 2 x 0.25 doses	SC	6	-
3000	as supplied	1.0 mL total in 4 x 0.25 doses	SC	6	6

Negative Control = 0.9% saline, Positive Control = Cyclophosphamide, SC = Subcutaneous injection
[a] Six animals were dosed to ensure the availability of five animals/harvest timepoint for analysis.
[b] Animals were dosed as potential replacements for the original high-dose groups.

Basis of dose selection: None

Negative controls: 0.9% sodium chloride solution

Positive controls: Cyclophosphamide (60 mg/kg, po)

Sampling times: 24 and 48 hours after treatment

Results:

Study validity: The criterion for a positive response was the detection of a statistically significant increase in micronucleated polychromatic erythrocytes (PCEs) for at least one dose level and a statistically significant dose-related response. A test article that did not induce both of these responses was considered negative.

Study outcome: Negative. The results are shown in the following table (from page 22 of sponsor's submission).

(b) (4)7436-118
Genetic Toxicology Assay No. 26472-0-454OECD
Sponsor Study Number: AP-24-16

Table 5: Micronucleus Assay – Summary Table

Assay No.: 26472-0-454OECD
Initiation of Dosing: 16 September 2004

Treatment	Dose	Harvest Time	% Micronucleated PCEs Mean of 2000 per Animal ± S.E. Males	Ratio PCE:NCE Mean ± S.E. Males
Controls				
Negative	0.9% Saline [a]	24 hr	0.08 ± 0.03	0.80 ± 0.03
		48 hr	0.16 ± 0.02	0.68 ± 0.04
Positive	CP 60 mg/kg	24 hr	2.24 ± 0.28*	0.71 ± 0.03
Test Articles				
(b) (4)	750 mg/kg	24 hr	0.08 ± 0.02	0.70 ± 0.04
(polymer vehicle)	1500 mg/kg	24 hr	0.10 ± 0.03	0.85 ± 0.05
	3000 mg/kg	24 hr	0.10 ± 0.03	0.76 ± 0.03
		48 hr	0.05 ± 0.02	0.68 ± 0.02
APF530	750 mg/kg	24 hr	0.10 ± 0.03	0.80 ± 0.02
(polymer vehicle	1500 mg/kg	24 hr	0.05 ± 0.04	0.83 ± 0.02
+ 2% granisetron)	3000 mg/kg	24 hr	0.04 ± 0.02	0.77 ± 0.03
		48 hr	0.03 ± 0.01	0.61 ± 0.06

[a]Dose = 1 mL/animal
* Significantly greater than the corresponding negative control, $p \leq 0.01$.
CP = Cyclophosphamide
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte

2.6.6.5 Carcinogenicity

None submitted.

2.6.6.6 Reproductive and developmental toxicology

Fertility and Early Embryonic Development

Study title: **Subcutaneous Fertility and General Reproduction Toxicity Study of** (b) (4) **in Rats**

Key study findings:

- All rats survived to scheduled euthanasia.
- Clinical signs at the injection site included erythema and edema and scab formation.
- There were no treatment related effects on body weights, food consumption, mating and fertility parameters or necropsy observations in either sex. Caesarean-sectioning parameters were comparable among the three dosage groups.

Study no.: AP27-02
Volume #, and page #: N/A
Conducting laboratory and location: (b) (4)
Date of study initiation: February 6, 2007
GLP compliance: A statement of compliance was included.
QA reports: yes (X) no ( )
Drug, lot #, and % purity: (b) (4), SCR-149-63, SCR-149-65, N/A

Methods:

Doses: 0.142 and 0.295 g/day
Species/strain: SD rats
Number/sex/group: 25
Route, and volume: Subcutaneous, 0.25 mL/dose
Satellite groups used for toxicokinetics: None
Study design: Seventy-five male and 75 female SD rats (n = 25/sex/group) were randomly assigned to three dosage groups (Groups I through III). The test article (b) (4), or the negative control article (0.9% Sodium Chloride Injection, USP), were administered subcutaneously at dosages of 0.250 (NaCl), 0.142, and 0.295 g/day, respectively, to male rats beginning 28 days before cohabitation, through cohabitation and continuing through the day before sacrifice, and to female rats once daily beginning 15 days before cohabitation and continuing through day 7 of presumed gestation (GD7). Dosages were administered on a mL/animal basis and were not adjusted daily for body weight changes. Injections were made using 16 gauge needles and injection sites were rotated to minimize irritation due to the depot characteristics of the polymer vehicle; documentation of these procedures was recorded in the raw data. Injections were made into six approximately equal areas on the dorsum (areas A, B, C, D, E and F; areas A and D being closest to the shoulders, areas E and B in the middle and areas C and F closest to the tail). Daily

dosages were injected in these areas in sequence (i.e., area A on the first day of the dosage period, area B on the second day of the dosage period, area C on the third day of the dosage period, continuing in this pattern until an injection had been made in each area and then repeating the sequence beginning on the seventh day of the dosage period). The study design is shown in the following table (from page 21 of the study report).

Dosage Group	Descriptor	Nominal Dosage Volume (mL/dose)	Nominal Mass Dosage (g/day)[a]	Number of Rats Per Sex	Assigned Rat Numbers	
					Male Rats	Female Rats
I	Negative Control Article[b]	0.25	0.250	25	4001 - 4025	4101 - 4125
II	(b) (4)	0.12	0.142	25	4026 - 4050	4126 - 4150
III		0.25	0.295	25	4051 - 4075	4151 - 4175

a. Nominal mass dosage was based on the specific gravity of the test material. The specific gravity of (b) (4) is 1.18.

b. 0.9% Sodium Chloride Injection, USP.

Parameters and endpoints evaluated: Mortality, clinical signs, body weights and food consumption values were recorded for all rats. Estrous cycling and mating behavior were recorded for female rats. Reproductive organs were weighed and retained for histopathological evaluations and sperm evaluations were also conducted. All female rats were sacrificed on GD13 and Caesarean-sectioned. The number and distribution of corpora lutea were recorded. The uterus of each rat was excised and examined for pregnancy, number and distribution of implantation sites and viable and nonviable embryos.

Results:

Mortality: None

Clinical signs: Clinical signs included erythema and edema at the injection sites and was attributed to the treatment procedure and the volume of test material being administered. Scab formation was noted in all dosage groups, including the negative control group. Other clinical observations included localized alopecia (limbs), chromorhinorrhea, a mass on the right side of the back, slight excess salivation, sparse hair coat (limbs and/or underside) and missing/broken incisors. Male rat 4057 in Group III (nominal mass dosage 0.295 g/day) was first observed with a mass on the right side of the back. The mass persisted until Day 41, when the size decreased. On Day 45, the mass was resolved and did not return; therefore, no gross lesion was available for retention at gross necropsy.

Body weight: The mean initial (Day 1) and final (Day 50) body weights of control males were 355.0 and 506.8 g, respectively. The mean initial (Day 1) and final (Day 50) body

weights of control males were 244.4 and 254.5 g, respectively. There were no significant treatment-related effects.

Food consumption: The mean initial (Day 1-8) and final (Day 42-50) food consumption of control males were 28.5 and 30.2 g/animal/day, respectively. The mean initial (Day 1-8) and final (Day 42-50) food consumption of control females were 18.7 and 19.7 g/animal/day, respectively. There were no significant treatment-related effects.

Necropsy: There were no significant treatment-related effects in either sex.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): In males, mating and fertility parameters were not affected by the treatment. All sperm parameters evaluated were unaffected by (b) (4). Values for number and percent motile sperm, number of nonmotile sperm and total sperm count from the vas deferens and cauda epididymal sperm count and density were comparable among the three dosage groups. All mating and fertility parameters in females (fertility index, number of pregnancies, etc.) were unaffected by (b) (4). No Caesarean-sectioning or litter parameters were affected by (b) (4). The litter averages for corpora lutea, implantations, viable and nonviable embryos and the percentage of nonviable embryos per litter were comparable among the three dosage groups. The following table (from page 106 of the study report) shows the Caesarean-sectioning data.

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PROTOCOL PJQ00003: SUBCUTANEOUS FERTILITY AND GENERAL REPRODUCTION TOXICITY STUDY OF (b) (4) IN RATS
(SPONSOR'S REFERENCE NUMBER: AP 27-02)

TABLE B12 (PAGE 1): CAESAREAN-SECTIONING AND LITTER OBSERVATIONS - SUMMARY - FEMALE RATS

DOSAGE GROUP DESCRIPTOR NOMINAL MASS DOSAGE (G/DAY)a		I NEGATIVE CONTROL ARTICLE 0.250	II (b) (4) 0.142	III (b) (4) 0.295
RATS TESTED	N	25	25	25
PREGNANT	N(%)	23(92.0)	22(88.0)	22(88.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 13 OF GESTATION	N	23	22	22
CORPORA LUTEA	MEAN±S.D.	16.5 ± 2.2	16.3 ± 1.8	16.1 ± 1.6
IMPLANTATIONS	MEAN±S.D.	16.0 ± 2.1	15.5 ± 1.8	15.7 ± 1.8
VIABLE EMBRYOS	N	335	316	328
	MEAN±S.D.	14.6 ± 2.7	14.4 ± 1.7	14.9 ± 2.1
NONVIABLE EMBRYOS	N	32	25	18
	MEAN±S.D.	1.4 ± 1.7	1.1 ± 1.3	0.8 ± 1.2
DAMS WITH ANY NONVIABLE EMBRYOS	N(%)	17(73.9)	14(63.6)	10(45.4)
DAMS WITH ALL NONVIABLE EMBRYOS	N(%)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE EMBRYOS	N(%)	23(100.0)	22(100.0)	22(100.0)
PLACENTAE APPEARED NORMAL	N(%)	23(100.0)	22(100.0)	22(100.0)
% NONVIABLE EMBRYOS/LITTER	MEAN±S.D.	8.9 ± 11.7	7.0 ± 7.9	5.2 ± 7.6

a. Dosage occurred on day 1 of study through day 7 of gestation.

Summary: In a Segment I fertility and early embryonic development study in male and female rats, (b) (4) was tested at 0.142 and 0.295 g/day. All rats survived to scheduled euthanasia. Clinical signs included erythema and edema and scab formation at the injection sites?. There were no treatment related effects on body weights, food consumption, mating and fertility parameters or necropsy observations in either sex. Caesarean-sectioning parameters were comparable among the three dosage groups. It is to be mentioned here that only two doses of (b) (4) have been tested. At least three doses should have been administered.

Embryofetal Development

Study Title: **Subcutaneous Segment II Teratology Study of (b) (4) in Rats**

The sponsor submitted the draft report of this study under the Original submission of IND (b) (4) which was previously reviewed (pharmacology review dated December 15, 2006). The sponsor submitted the final report of this study in this NDA. The results and interpretations do not seem to differ from that of the draft report. The review is incorporated below from the pharmacology review dated December 15, 2006.

Key study findings: In a Segment II study in rats, pregnant females were treated (Groups I, II and III) daily on gestation day 7 (GD7) through gestation day 17 (GD17) at dosages of 0.250 (negative control, saline), 0.140, and 0.295 g/day, respectively. Rats in Group IV were administered the test article subcutaneously once every 72 hours on GDs 7, 10, 13 and 16 at a dosage of 0.295 g/day. There were no significant treatment-related effects on maternal C-section parameters. There were no significant treatment-related fetal external, visceral and skeletal alterations. (b) (4) did not appear to be teratogenic in this study.

Study no.: AP 25-09
Volume #, and page #: Amendment 015, and 1
Conducting laboratory and location: (b) (4)
Date of study initiation: October 4, 2005
GLP compliance: This is a draft report.
QA reports: yes ( ) no (X)
Drug, lot #, and % purity: (b) (4), Lot No. 980028. The sponsor provided certificate of analyses fro (b) (4) ; however, the purity data was not mentioned.

Methods:

Doses: The following table (from page 15 of the study report) shows the dosage administration.

2.7.1. Dosage Administration

Dosage Group[a]	Descriptor	Nominal Dosage Volume (mL/dose)	Nominal Mass Dosage (g/day)[b]	Number of Rats	Assigned Rat Numbers
I	Negative Control Article[c]	0.25[d]	0.250	25	3801 - 3825
II	(b) (4)	0.12	0.140	25	3826 - 3850
III		0.25	0.295	25	3851 - 3875
IV		0.25	0.295	25	3876 - 3900

a. Test article was administered daily to rats in Groups I, II and III, and was administered every 72 hours to rats in Group IV.
b. Nominal mass dosage was based on the specific gravity of the test material. The specific gravity of (b) (4) is 1.18.
c. 0.9% Sodium Chloride Injection, USP.

Species/strain: Rats/ Crl:CD(SD)

Number/group: 25/dose

Route, formulation, and volume: Subcutaneous (s.c.), gel, 0.12-0.25 ml/dose,

Satellite groups used for toxicokinetics: None

Study design: One hundred pregnant female rats were randomly assigned to four dosage groups (Groups I through IV), 25 rats per group. Solutions of the test article, (b) (4), or the negative control article, 0.9% sodium chloride for Injection, USP, were administered subcutaneously once daily to rats in Groups I, II and III on days 7 through 17 of presumed gestation (GDs 7 through 17) at dosages of 0.250 (Negative Control Article), 0.140, and 0.295 g/day, respectively. Rats in Group IV were administered the test article subcutaneously once every 72 hours during the period of organogenesis, specifically on GDs 7, 10, 13 and 16 at a dosage of 0.295 g/day. The dosage volume was 0.25, 0.12, 0.25 and 0.25 ml/kg in the four respective dosage groups.

The dosages were selected based on earlier studies (not mentioned). The high dosage level was considered to be the maximum feasible dosage (MFD) that would minimize leakage from the injection site and confluence of adjacent sites following repeat dosage administration. The low dosage level was selected in order to produce graded responses to the test article.

Parameters and endpoints evaluated: Mortality was observed twice daily, and clinical observations were recorded daily. Body weights were recorded daily. Food consumption was recorded on GDs 0, 7, 10, 12, 15, 18 and 21. On GD 21, all surviving female rats were sacrificed and Caesarean-sectioned. All rats were examined for the number and distribution of corpora lutea, implantation sites and uterine contents. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Two skin samples were collected from each rat (one from the injection area of the last site injected and one from an untreated area) and retained for possible further evaluation. Fetuses were weighed, sexed and examined for gross external, visceral and skeletal alterations.

Results:

Mortality (dams): One negative control group rat delivered its litter on the day of scheduled sacrifice (GD21). There were no treatment-related mortalities.

Clinical signs (dams): Clinical signs included scab formation at the site of injection, erythema and edema. The scab formation occurred in each rat in all dosage groups, including the negative control.

Body weight (dams): The mean initial and final body weight of control animals were 236.3 and 393.1 g, respectively. There were no treatment-related effects.

Food consumption (dams): The mean initial and final food consumption in control animals were 20.8 and 22.7 g/animal/day, respectively. There were no treatment-related effects.

Toxicokinetics: None

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): No Caesarean-sectioning parameters were affected by treatment with (b) (4). The C-section data is shown in the following table (from pages 39 and 40 of the sponsor's submission).

PROTOCOL PJQ00001: SUBCUTANEOUS DEVELOPMENTAL TOXICITY STUDY OF (b) (4) IN RATS (SPONSOR'S STUDY NUMBER: AP 25-09)

TABLE 6 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

DOSAGE GROUP DOSAGE (G/DAY)a		I 0.250	II 0.140	III 0.295	IV 0.295
RATS TESTED	N	25	25	25	25
PREGNANT	N(%)	24(96.0)	24(96.0)	24(96.0)	23(92.0)
DELIVERED AND SACRIFICED	N(%)	1(4.2)	0(0.0)	0(0.0)	0(0.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	23	24	24	23
CORPORA LUTEA	MEAN±S.D.	16.3 ± 1.9	16.2 ± 2.0	15.4 ± 2.6	16.6 ± 2.2*
IMPLANTATIONS	MEAN±S.D.	15.0 ± 1.8	15.4 ± 1.8	14.2 ± 2.6	14.0 ± 2.9
LITTER SIZES	MEAN±S.D.	14.2 ± 2.2	15.0 ± 1.8	13.6 ± 2.5	13.3 ± 2.8
LIVE FETUSES	N	326	360	326	307
	MEAN±S.D.	14.2 ± 2.2	15.0 ± 1.8	13.6 ± 2.5	13.3 ± 2.8
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.8 ± 1.5	0.4 ± 0.6	0.7 ± 0.8	0.7 ± 0.9
EARLY RESORPTIONS	N	19	8	16	16
	MEAN±S.D.	0.8 ± 1.5	0.3 ± 0.6	0.7 ± 0.8	0.7 ± 0.9
LATE RESORPTIONS	N	0	1	0	0
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
DAMS WITH ANY RESORPTIONS	N(%)	12(52.2)	8(33.3)	11(45.8)	11(47.8)
DAMS WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE FETUSES	N(%)	23(100.0)	24(100.0)	24(100.0)	23(100.0)
PLACENTAE APPEARED NORMAL	N(%)	23(100.0)	24(100.0)	24(100.0)	23(100.0)

a. Dosage occurred on days 7 through 17 of gestation for Groups I through III or once every 72 hours (days 7, 10, 13 and 16 of gestation) for Group IV.
* Significantly different from the control group value ($p \leq 0.05$).

PROTOCOL PJQ00001: SUBCUTANEOUS DEVELOPMENTAL TOXICITY STUDY OF (b) (4) IN RATS (SPONSOR'S STUDY NUMBER: AP 25-09)

TABLE 7 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY

DOSAGE GROUP DOSAGE (G/DAY)a		I 0.250	II 0.140	III 0.295	IV 0.295
LITTERS WITH ONE OR MORE LIVE FETUSES	N	23	24	24	23
IMPLANTATIONS	MEAN±S.D.	15.0 ± 1.8	15.4 ± 1.8	14.2 ± 2.6	14.0 ± 2.9
LIVE FETUSES	N	326	360	326	307
	MEAN±S.D.	14.2 ± 2.2	15.0 ± 1.8	13.6 ± 2.5	13.3 ± 2.8
LIVE MALE FETUSES	N	161	172	168	168
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	49.5 ± 12.8	47.9 ± 15.1	50.7 ± 13.4	53.7 ± 14.0
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	4.94 ± 0.34	5.11 ± 0.34	5.15 ± 0.35*	5.31 ± 0.28**
MALE FETUSES	MEAN±S.D.	5.10 ± 0.40	5.23 ± 0.38	5.30 ± 0.39	5.43 ± 0.29**
FEMALE FETUSES	MEAN±S.D.	4.80 ± 0.31	4.99 ± 0.32	5.01 ± 0.33*	5.19 ± 0.31** [22]b
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	5.5 ± 9.8	2.4 ± 3.7	4.5 ± 5.5	4.8 ± 6.0

[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 17 of gestation for Groups I through III or once every 72 hours (days 7, 10, 13 and 16 of gestation) for Group IV.
b. Litter 3881 had no female fetuses.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Offspring (malformations, variations, etc.): There were no significant treatment-related external, visceral or skeletal fetal alterations (malformations or variations). The following table shows some of the fetal external, visceral and skeletal observations.

Parameter	0 g/day	0.140 g/day	0.295 g/day	0.295 g/day*
Fetal External Alterations				
Fetuses Examined/litter	326/23	360/24	326/24	307/23
Head: Domed	0	0	0	1[b]
Eye: Bulged depressed	0	2	0	1[b]
Fore/hind limb: Digits absent	0	0	0	1[b]
Tail: Short	0	0	0	1[b]
Fetal Visceral Alterations				
Fetuses/Litter Examined	158/23	173/24	157/24	148/23
Vessels: Umbilical artery descends to the left of the urinary bladder	3	2	2	0
Vessels: Aorta passes dorsal to the trachea and esophagus	0	0	1	0
Kidneys: Pelvis, slight dilation	0	0	1	0
Kidneys: Pelvis, moderate dilatation	0	2	2	1
Fetal Skeletal Alterations				
Fetuses/Litter Examined	168/23	187/24	169/24	159/23
Skull: Zygomatic, incomplete ossification	0	0	3**	0
Skull: Squamosal, incomplete ossification	0	0	3**	0

Sternal Centrum: incomplete ossification	0	2	1	2
Ribs: incomplete ossification (hypoplastic)	1	0	3**	0
Ribs: wavy	0	0	1	0
Pelvis: Pubis, incomplete ossification	0	0	2	0

*: Administered every 72 hours. Other groups were administered once daily.
**: Three fetuses (3858-5, -7 and -12) from one litter in Group III had incompletely ossified zygomatic bones. These same fetuses also had incompletely ossified squamosal bones, an observation that also occurred at a significantly higher fetal incidence in comparison to the negative control group value. Each of these fetuses had additional alterations.

b: Fetus 3890-6 had other gross external alterations

Summary: In a Segment II study in rats, pregnant females were treated (Groups I, II and III) daily on GD7 through GD17 at dosages of 0.250 (negative control, saline), 0.140, and 0.295 g/day, respectively. Rats in Group IV were administered the test article subcutaneously once every 72 hours on GDs 7, 10, 13 and 16 at a dosage of 0.295 g/day. There were no significant treatment-related effects on maternal C-section parameters. There were no significant treatment-related fetal external, visceral and skeletal alterations. (b) (4) did not appear to be teratogenic in this study.

Study Title: **Subcutaneous Segment II Teratology Study of (b) (4) in Rabbits**

The sponsor submitted the draft report of this study under the Original submission of IND 68,213, which was previously reviewed (pharmacology review dated December 15, 2006). The sponsor submitted the final report of this study in this NDA. The results and interpretations do not seem to differ from that of the draft report. The review is incorporated below from the pharmacology review dated December 15, 2006.

Study title: Embryofetal Development (Segment II) Study with (b) (4) in Rabbits by Subcutaneous Route

Key study findings: In a Segment II study in rabbits, animals were administered test article, APF18A, or 0.9% Sodium Chloride Injection, USP, subcutaneously once daily from GD7 through GD19 at 1.0 (negative control, Group I), 0.59 (Group II) and 1.18 g/day (Group III). Group IV animals were administered the test article subcutaneously once every 72 hours on GD7 at 1.18 g/day. There were no significant treatment-related effects on maternal C-section parameters. There were no significant treatment-related fetal external, visceral and skeletal alterations. (b) (4) did not appear to be teratogenic in this study. It is to be mentioned here that the sponsor did not include any APF530-treated group as recommended in the Division letter dated November 4, 2005.

Study no.: AP 25-10
Volume #, and page #: Amendment 015, 1
Conducting laboratory and location: (b) (4)
Date of study initiation: October 17, 2005
GLP compliance: This is a draft report.
QA reports: yes ( ) no (X)
Drug, lot #, and % purity: (b) (4) Lot No. 980029. Purity data not provided.

Methods:

Doses: 1.0 (negative control), 0.59 and 1.18 g/day (Group I, II and III, respectively, daily dosing). Group IV animals: 1.18 g/day (once every 72 hours dosing). The following table (from page 15 of the study report) shows the dosage administration.

2.7.1. Dosage Administration

Dosage Group[a]	Descriptor	Nominal Dosage Volume (mL/dose)	Nominal Mass Dosage[b] (g/day)	Number of Rabbits	Assigned Rabbit Numbers
I	Negative Control Article[c] (b) (4)	1.0	1.00	20	4001 - 4020
II		0.5	0.59	20	4021 - 4036, 7249[d], 4038 - 4040
III		1.0	1.18	20	4041 - 4060
IV		1.0	1.18	20	4061 - 4080

a. Test article was administered daily in Groups I, II and III, and was administered every 72 hours in Group IV.
b. Nominal mass dosage was based on the specific gravity of the test material. The specific gravity of (b) (4) is 1.18.
c. 0.9% Sodium Chloride Injection, USP.
d. Doe 4037 was excluded from study on 19 October 2005 due to body weight loss and the presence of liquid feces. This does was replaced with doe 7249.

Species/strain: Rabbit/New Zealand White

Number/group: 20/dose

Route, formulation, and volume: Subcutaneous, gel, and 0.5-1.0 ml/kg

Satellite groups used for toxicokinetics: None

Study design: Eighty timed-mated New Zealand White rabbits were randomly assigned to four dosage groups (Groups I through IV), 20 rabbits per group. Solutions of the test article, APF18A, or the negative control article, 0.9% Sodium Chloride Injection, USP, were administered subcutaneously once daily to rabbits in Groups I, II and III on GD7 through GD19 at 1.0 (negative control), 0.59 and 1.18 g/day, respectively. Group IV animals were administered the test article subcutaneously once every 72 hours on GD7 at 1.18 g/day. The dosage volume was 1.0, 0.5, 1.0 and 1.0 ml/dose in the four respective dosage groups.

Parameters and endpoints evaluated: Viability was checked twice daily and clinical observation was made daily. Body weight and food consumption values were recorded daily. On GD29, al1 does were sacrificed and Caesarean-

sectioned. All rabbits were examined for the number and distribution of corpora lutea, implantation sites and uterine contents. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Two skin samples were collected from each rabbit (one from the injection area of the last site injected and one from an untreated area) and retained for possible further evaluation. Uteri of apparently non-pregnant rabbits were examined. Fetuses were weighed, sexed and examined for gross external, visceral and skeletal alterations.

Results:

Mortality (dams): None

Clinical signs (dams): Clinical signs attributed to subcutaneous administration of the vehicle or (b) (4) included scab formation at the sites of injection, erythema and edema. Scab formation (single or multiple) occurred in 19 or 20 rabbits in Groups I through IV. This observation was considered to be related to the size of the dosing needle and/or the route of administration.

Body weight (dams): The mean initial and final body weight of control animals were 3.48 and 4.13 kg, respectively. There were no significant treatment-related effects.

Food consumption (dams): The mean initial and final food consumption in control animals were 179.8 and 165.5 g/animal/day. There were no significant treatment-related effects.

Toxicokinetics: None.

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.): There were no treatment-related effects on C-section parameters except number of implantation sites. There was significant reduction in the average number of implantation sites in Group IV animals. The C-section data is shown in the following table (from page 43 and 44 of the study report).

PROTOCOL PJQ00002: SUBCUTANEOUS DEVELOPMENTAL TOXICITY STUDY OF (b) (4) IN RABBITS (SPONSOR'S STUDY NUMBER: AP 25-10)

TABLE 7 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

DOSAGE GROUP DOSAGE (G/DAY)a		I 1.00b	II 0.59	III 1.18	IV 1.18
RABBITS TESTED	N	20	20	20	20
PREGNANT	N(%)	19(95.0)	20(100.0)	17(85.0)	19(95.0)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	19	20	17	19
CORPORA LUTEA	MEAN±S.D.	10.3 ± 1.9	10.4 ± 2.4	9.9 ± 1.9	9.1 ± 1.6
IMPLANTATIONS	MEAN±S.D.	9.8 ± 1.4	9.2 ± 1.8	8.7 ± 2.4	8.4 ± 1.1**
LITTER SIZES	MEAN±S.D.	9.4 ± 1.4	8.9 ± 1.9	8.3 ± 2.4	8.2 ± 1.2*
LIVE FETUSES	N	178	178	141	156
	MEAN±S.D.	9.4 ± 1.4	8.9 ± 1.9	8.3 ± 2.4	8.2 ± 1.2*
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.5 ± 0.6	0.2 ± 0.4	0.4 ± 0.7	0.2 ± 0.4
EARLY RESORPTIONS	N	6	4	6	3
	MEAN±S.D.	0.3 ± 0.5	0.2 ± 0.4	0.4 ± 0.6	0.2 ± 0.4
LATE RESORPTIONS	N	3	1	1	1
	MEAN±S.D.	0.2 ± 0.4	0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.2
DOES WITH ANY RESORPTIONS	N(%)	8(42.1)	5(25.0)	5(29.4)	4(21.0)
DOES WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DOES WITH VIABLE FETUSES	N(%)	19(100.0)	20(100.0)	17(100.0)	19(100.0)
PLACENTAE APPEARED NORMAL	N(%)	19(100.0)	20(100.0)	17(100.0)	19(100.0)

a. Dosage occurred on days 7 through 19 of gestation for Groups I through III or once every 72 hours (days 7, 10, 13, 16 and 19 of gestation) for Group IV.
b. Negative control article.
* Significantly different from the Group 1 value ($p \leq 0.05$).
** Significantly different from the Group 1 value ($p \leq 0.01$).

PROTOCOL PJQ00002: SUBCUTANEOUS DEVELOPMENTAL TOXICITY STUDY OF (b) (4) IN RABBITS (SPONSOR'S STUDY NUMBER: AP 25-10)

TABLE 8 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY

DOSAGE GROUP DOSAGE (G/DAY)a		I 1.00b	II 0.59	III 1.18	IV 1.18
LITTERS WITH ONE OR MORE LIVE FETUSES	N	19	20	17	19
IMPLANTATIONS	MEAN±S.D.	9.8 ± 1.4	9.2 ± 1.8	8.7 ± 2.4	8.4 ± 1.1**
LIVE FETUSES	N	178	178	141	156
	MEAN±S.D.	9.4 ± 1.4	8.9 ± 1.9	8.3 ± 2.4	8.2 ± 1.2*
LIVE MALE FETUSES	N	84	101	75	79
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	47.2 ± 11.0	55.9 ± 21.4	52.6 ± 22.2	51.0 ± 13.7
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	44.17 ± 3.07	43.29 ± 4.01	45.75 ± 3.92	44.41 ± 4.56
MALE FETUSES	MEAN±S.D.	45.07 ± 2.77	44.20 ± 5.17	46.71 ± 4.12	44.70 ± 4.42
FEMALE FETUSES	MEAN±S.D.	43.46 ± 3.89	41.57 ± 5.40	44.90 ± 4.32 [16]c	44.11 ± 4.85
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	4.7 ± 6.1	3.1 ± 5.7	4.9 ± 8.8	2.6 ± 5.1

[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 19 of gestation for Groups I through III or once every 72 hours (days 7, 10, 13, 16 and 19 of gestation) for Group IV.
b. Negative control article.
c. Litter 4052 had no female fetuses.
* Significantly different from the Group 1 value ($p \leq 0.05$).
** Significantly different from the Group 1 value ($p \leq 0.01$).

Offspring (malformations, variations, etc.): There were no significant treatment-related effects on fetal external, visceral and skeletal parameters. The following table shows some of the fetal external, visceral and skeletal observations.

Parameter	0 g/day	0.59 g/day	1.18 g/day	1.18 g/day*
Fetal External Alterations				
Fetuses Examined/litter	178/19	178/20	141/17	156/19
Fore or Hind limb: digits absent	0	0	2	0
Palate: cleft	1	0	0	0
Fore or Hind limb: digits short	1c	0	0	0
Fore or Hind limb: toenail absent	1c	0	0	0
Fetal Visceral Alterations				
Fetuses/Litter Examined	178/19	178/20	141/17	156/19
Heart: interventricular septal defect	0	1	0	0
Lungs: intermediate lobe absent	0	0	1	0
Diaphragm: hernia	0	1	0	0
Kidneys: pelvis dilated	0	1	0	0
Fetal Skeletal Alterations				
Fetuses/Litter Examined	178/19	178/20	141/17	156/19
Skull: incomplete ossification	1	0	0	0
Skull: Frontal: irregular ossification	0	2	0	1
Hyoid: angulated	1	3	2	6
Manubrim: fused	0	1	1	0
Sternal Centra: fused	4	4	1	8
Sternal Centra: asymmetric	0	2	1	0
Sternal Centra: incomplete ossification	0	0	0	2
Forelimb: digit absent	0	0	2	0
Forelimb: metacarpal not ossified	0	0	2	0

c: Fetus 4013-3 had other gross external alterations
*: Administered every 72 hours. Other groups were administered once daily.

Summary: In a Segment II study in rabbits, animals were administered test article, APF18A, or 0.9% Sodium Chloride Injection, USP, subcutaneously once daily from GD7 through GD19 at 1.0 (negative control, Group I), 0.59 (Group II) and 1.18 g/day (Group III). Group IV animals were administered the test article subcutaneously once every 72 hours on GD7 at 1.18 g/day. There were no significant treatment-related effects on maternal C-section parameters. There were no significant treatment-related fetal external, visceral and skeletal alterations. (b) (4) did not appear to be teratogenic in this study. It is to be mentioned here that the sponsor did not include any APF530-treated group as recommended in the Division letter dated November 4, 2005.

Prenatal and Postnatal Development

Study title: **Subcutaneous Developmental and Perinatal/Postnatal Reproduction Toxicity Study of (b) (4) in Rats, Including a Postnatal Behavioral/Functional Evaluation**

Key study findings:

- All F0 rats survived to scheduled sacrifice. Clinical signs included scab formations and erythema.
- Maternal body weights, body weight gains, feed consumption (absolute and relative) and necropsy observations were unaffected by treatment.
- One male and one female rat at 0.250 g/day (saline control) dosage group were found dead, and two male rats at 0.295 g/day (b) (4) dosage group were euthanized early due to clinical signs.
- No significant treatment-related effects were observed in the F1 generation male and female rats with regard to clinical signs, body weights or body weight gains, feed consumption, sexual maturation, learning and memory or fertility and reproductive capacity.
- There were no significant treatment-related gross necropsy observations in the F1 generation rats.
- There were no significant (b) (4)-related effects in the F2 generation fetuses.

Study no.: AP27-03
Volume #, and page #: N/A
Conducting laboratory and location: (b) (4)
Date of study initiation: January 7, 2007
GLP compliance: A statement of compliance was included.
QA reports: yes (X) no ( )
Drug, lot #, and % purity: (b) (4), SCR-149-65, N/A

Methods:

Doses: 0.142 and 0.295 g/day
Species/strain: SD rats
Number/sex/group: 25
Route, formulation, volume: SC, viscous gel, 0.12-0.25 mL/dose
Satellite groups used for toxicokinetics: None
Study design: Seventy-five presumed pregnant female rats were randomly assigned to three dosage groups (Groups I through III, 25 rats per group). The test article, (b) (4), or the negative control article, 0.9% Sodium Chloride Injection, USP, were administered subcutaneously once daily to these rats from day 7 of presumed gestation (DG 7) through day 20 of lactation (DL 20) or DG 24 (rats that did not deliver a litter) at dosages of 0.250 (saline control), 0.142, and 0.295

g/day, respectively. The dosage volume was 0.25, 0.12 and 0.25 mL/dose in the three respective dosage groups. F1 generation pups were not directly administered the test article or negative control article, but may have been exposed to the test article or negative control article during maternal gestation (*in utero* exposure) or via maternal milk during the lactation period. After completion of the 21-day postpartum period, female rats were sacrificed and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed.

Parameters and endpoints evaluated: The following parameters were evaluated: viability, F0 and F1 clinical observations including injection site assessment, maternal body weights/body weight gains, maternal feed consumption, adverse clinical signs during parturition, duration of gestation, litter sizes, pup viability at birth, maternal behavior, F0 and F1 necropsy observations.

F1 Generation Pups

The following parameters were recorded: viability, clinical observations, body weights, feed consumption, age of vaginal patency (female rats) or age of preputial separation (male rats), passive avoidance test (for learning, short-term retention and long-term retention), water-filled M-maze test (for overt coordination, swimming ability, learning and memory) and mating performance. At necropsy, gross pathologic findings, and the number and distribution of implantation sites were recorded. Female rats were sacrificed on DG 21 and Caesarean-sectioned. Fetuses were weighed and examined for gross external alterations and sex. Male rats were sacrificed after completion of the cohabitation period, and testes and epididymides were excised and paired organ weights were recorded.

Results:

F_0 in-life: There was no mortality. The primary clinical sign was scab formation (single or multiple) at the injection site. Discoloration at the sites of injection and grade 1 erythema were also observed across all dosage groups, including control animals, but occurred more frequently in the (b) (4)-treated groups during the gestation period and/or postpartum period. Maternal body weights, body weight gains, feed consumption (absolute and relative) were unaffected by the treatment.

F_0 necropsy: Pregnancy occurred in 25, 24 and 23 of the 25 mated female rats in the 0.250 (saline), 0.142 and 0.295 g/kg/day dosage groups, respectively. All pregnant dams delivered litters. Natural delivery and litter observations were unaffected by (b) (4). Values for the numbers of dams delivering litters, the duration of gestation, averages for implantation sites per delivered litter, the gestation index (number of dams with one or more liveborn pups/number of pregnant rats), the numbers of dams with stillborn pups and with all pups dying, litter sizes, viability index, surviving pups per litter, the percentage of male pups per number of pups sexed per litter, live litter size at weighing

and pup weight per litter were comparable among the three dosage groups and did not significantly differ. There were statistically significant reductions in the lactation index that occurred in the (b) (4)-treated groups. The following tables (from page 62 and 63 of the study report) show the Cesarean data.

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TABLE A11 (PAGE 1): NATURAL DELIVERY OBSERVATIONS - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP DESCRIPTOR DOSAGE (G/DAY)a		I NEGATIVE CONTROL ARTICLE 0.250	II (b) (4) 0.142	III (b) (4) 0.295
RATS ASSIGNED TO NATURAL DELIVERY	N	25	25	25
PREGNANT	N	25	24	23
DELIVERED A LITTER	N	25	24	23
DURATION OF GESTATION b	MEAN±S.D.	22.6 ± 0.5	22.6 ± 0.5	22.8 ± 0.4
IMPLANTATION SITES PER DELIVERED LITTER	N MEAN±S.D.	360 14.4 ± 2.2	345 14.4 ± 1.4	333 14.5 ± 1.4
DAMS WITH STILLBORN PUPS	N(%)	0(0.0)	2(8.3)	0(0.0)
DAMS WITH NO LIVEBORN PUPS	N(%)	0(0.0)	0(0.0)	0(0.0)
GESTATION INDEX c	% N/N	100.0 25/ 25	100.0 24/ 24	100.0 23/ 23
DAMS WITH ALL PUPS DYING DAYS 1-4 POSTPARTUM	N(%)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH ALL PUPS DYING DAYS 5-21 POSTPARTUM	N(%)	0(0.0)	0(0.0)	0(0.0)

a. Dosage occurred on day 7 of presumed gestation through day 20 postpartum or day 24 of presumed gestation (rats that did not deliver a litter).
b. Calculated (in days) as the time elapsed between confirmed mating (arbitrarily defined as day 0 of gestation) and the time the first pup was delivered.
c. Number of rats with live offspring/number of pregnant rats.

PROTOCOL PJQ00004: SUBCUTANEOUS DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF (b) (4) IN RATS, INCLUDING A POSTNATAL BEHAVIORAL/FUNCTIONAL EVALUATION (SPONSOR'S REFERENCE NUMBER: AP 27-03)

TABLE A12 (PAGE 1): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS

DOSAGE GROUP DESCRIPTOR DOSAGE (G/DAY)a		I NEGATIVE CONTROL ARTICLE 0.250	II (b) (4) 0.142	III (b) (4) 0.295
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	25	24	23
PUPS DELIVERED (TOTAL)	N MEAN±S.D.	336 13.4 ± 2.2	329 13.7 ± 1.8	317 13.8 ± 1.6
LIVEBORN	MEAN±S.D. N(%)	13.4 ± 2.2 336(100.0)	13.6 ± 1.7 327(99.4)	13.8 ± 1.6 317(100.0)
STILLBORN	MEAN±S.D. N(%)	0.0 ± 0.0 0(0.0)	0.1 ± 0.3 2(0.6)	0.0 ± 0.0 0(0.0)
SACRIFICED	N	0	8	5
PUPS FOUND DEAD OR PRESUMED CANNIBALIZED				
DAY 1	N/N(%)	1/336(0.3)	2/327(0.6)	2/317(0.6)
DAYS 2- 4	N/N(%)	4/335(1.2)	8/325(2.5)	3/315(1.0)
DAYS 5- 7	N/N(%)	1/331(0.3)	1/317(0.3)	1/312(0.3)
DAYS 8-14	N/N(%)	0/330(0.0)	0/308(0.0)	0/306(0.0)
DAYS 15-21	N/N(%)	0/330(0.0)	0/308(0.0)	0/306(0.0)
VIABILITY INDEX b	% N/N	98.5 331/336	96.9 317/327	98.4 312/317
LACTATION INDEX c	% N/N	99.7 330/331	97.2 308/317**	98.1 306/312*

DAY(S) = DAY(S) POSTPARTUM
a. Dosage occurred on day 7 of presumed gestation through day 20 postpartum or day 24 of presumed gestation (rats that did not deliver a litter).
b. Number of live pups on day 4 postpartum/number of liveborn pups on day 1 postpartum.
c. Number of live pups on day 21 postpartum/number of live pups on day 4 postpartum.
* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).

F_1 physical development: One male and one female rat in the 0.250 g/day (saline) dosage group were found dead, and two male rats in the 0.295 g/day (b) (4) dosage group were euthanized early. Male rat 24661 in the 0.295 g/day maternal dosage group was euthanized on Day 50 post-weaning because of declining clinical condition. Adverse clinical signs noted for this rat included decreased motor activity, ptosis, cold to touch, ungroomed coat, red substance on fur, scant feces, chromodacryorrhea, chromorhinorrhea, hyperpnea and urine-stained abdominal fur, each of which occurred during week 8 post-weaning. Necropsy examination revealed that all lobes of the lungs were dark red. Male rat 24675 in the 0.295 g/day maternal dosage group was euthanized on Day 3 post-weaning because of declining clinical condition associated with limited accessibility to the water source. Adverse clinical signs noted for this rat included decreased motor activity, ataxia, ptosis, apparent dehydration, urine-stained abdominal fur and scant feces, each of which occurred during week 1 post-weaning. All tissues appeared normal at necropsy examination. All other F1 generation rats survived to scheduled euthanasia.

Clinical observations in the F1 generation male and female (prior to cohabitation and during gestation) rats included misaligned/missing/broken incisors, localized alopecia (head, limbs or underside), chromodacryorrhea, sparse hair coat (limbs, underside, head or back), abnormal fecal output (scant or soft or liquid), ptosis, corneal opacity, enlargement of the right eye, decreased motor activity, urine-stained abdominal fur, chromorhinorrhea, apparent dehydration, discoloration (black) of the right eye, ulceration in the right eye, periorbital swelling, lacrimation, ataxia, cold to touch, hypernea, ungroomed coat, red substance on the fur, mass along the left axilla, swollen ears and scab on the mouth. All clinical observations in the F1 generation male and female (prior to cohabitation and during gestation) rats were considered unrelated to treatment of the F0 generation dams with (b) (4) for the following reasons: 1) the incidences were not dosage-dependent; 2) the observation occurred infrequently; 3) the observation was considered common in this species and strain; and/or 4) the observation occurred in only the maternal control group. Body weights and food consumption were not significantly affected by the treatment. (b) (4) did not affect sexual maturation of the F1 generation rats. The average day on which preputial separation and vaginal patency occurred and the body weight at sexual maturation was comparable among the three maternal dosage groups.

Gross necropsy findings included slight to extreme dilation of the pelvis in one or both kidneys; adhesions between the stomach; spleen and pancreas; reduction of the left uterine horn into a ligament; dark red lung lobes; red foci on the thymus; a misshapen and small lobe on the seminal vesicle; small testes; small left epididymis and absence of the right epididymis. All necropsy observations in the F1 generation rats were considered unrelated to treatment of the F0 generation dams with (b) (4) because: 1) the incidences were not dosage-dependent; and/or 2) the observation occurred in only one or two rats.

F_1 behavioral evaluation: There were no significant treatment-related effects on learning, short-term retention, long-term retention, or response inhibition in the F1 generation male and female rats, as evaluated by performance in a passive avoidance paradigm. Significant increases in the average number of trials to criterion and the average number of errors per trial in F1 generation male rats was not considered treatment-related because the increases were small in magnitude and the differences occurred only in one sex.

F_1 reproduction: There were no significant treatment-related effects on the mating and fertility parameters evaluated in the F1 generation male and female rats. Values for the number of days in cohabitation, the number of rats that mated, the fertility index (number of pregnancies per number of rats that mated), the number of rats with confirmed mating dates during the second week of cohabitation, and the number of pregnancies per number of rats in cohabitation were comparable among the three maternal dosage groups and did not significantly differ. The following tables (from page 150 and 151 of the study report) show the mating and fertility parameters.

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PROTOCOL PJQ00004: SUBCUTANEOUS DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF (b) (4) IN RATS, INCLUDING A POSTNATAL BEHAVIORAL/FUNCTIONAL EVALUATION (SPONSOR'S REFERENCE NUMBER: AP 27-03)

TABLE B18 (PAGE 1): MATING AND FERTILITY - SUMMARY - F1 GENERATION MALE RATS

MATERNAL DOSAGE GROUP		I	II	III
DESCRIPTOR		NEGATIVE CONTROL ARTICLE	(b) (4)	(b) (4)
MATERNAL DOSAGE (G/DAY)		0.250	0.142	0.295
RATS IN COHABITATION	N	24a	25	23a
DAYS IN COHABITATION b	MEAN±S.D.	3.2 ± 2.6	5.0 ± 4.8	3.6 ± 2.8
RATS THAT MATED c	N(%)	24(100.0)	22(88.0)	22(95.6)
FERTILITY INDEX d,e	N/N	23/24	20/22	19/22
	(%)	(95.8)	(90.9)	(86.4)
RATS WITH CONFIRMED MATING DATES	N	24	22	22
MATED WITH FEMALE f				
DAYS 1-7	N(%)	23(95.8)	19(86.4)	22(100.0)
DAYS 8-14	N(%)	1(4.2)	3(13.6)	0(0.0)
RATS PREGNANT/RATS IN COHABITATION e	N/N	23/24	20/25	19/23
	(%)	(95.8)	(80.0)	(82.6)

a. Excludes values for rats that were found dead or sacrificed due to adverse clinical observations.
b. Restricted to rats with a confirmed mating date and rats that did not mate.
c. Includes only one mating for each male rat.
d. Number of pregnancies/number of rats that mated.
e. Includes only one pregnancy for each rat that impregnated more than one female rat.
f. Restricted to rats with a confirmed mating date.

PROTOCOL PJQ00004: SUBCUTANEOUS DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF (b) (4) IN RATS, INCLUDING A POSTNATAL BEHAVIORAL/FUNCTIONAL EVALUATION (SPONSOR'S REFERENCE NUMBER: AP 27-03)

TABLE B19 (PAGE 1): MATING AND FERTILITY - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP DESCRIPTOR MATERNAL DOSAGE (G/DAY)		I NEGATIVE CONTROL ARTICLE 0.250	II (b) (4) 0.142	III (b) (4) 0.295
RATS IN COHABITATION	N	24a	25	25
DAYS IN COHABITATION b	MEAN±S.D.	3.2 ± 2.6	5.4 ± 5.7	3.7 ± 3.6
RATS THAT MATED	N(%)	24(100.0)	25(100.0)	25(100.0)
FERTILITY INDEX c	N/N (%)	23/24 (95.8)	23/25 (92.0)	22/25 (88.0)
RATS WITH CONFIRMED MATING DATES	N	24	25	25
MATED BY FIRST MALE d DAYS 1-7 DAYS 8-14	 N(%) N(%)	 23(95.8) 1(4.2)	 19(76.0)** 3(12.0)	 24(96.0) 0(0.0)
MATED BY SECOND MALE d DAYS 15-21	 N(%)	 0(0.0)	 3(12.0)	 1(4.0)
RATS PREGNANT/RATS IN COHABITATION	N/N (%)	23/24 (95.8)	23/25 (92.0)	22/25 (88.0)

a. Excludes values for rat 24717, which was found dead on day 9 postweaning.
b. Restricted to rats with a confirmed mating date and rats that did not mate.
c. Number of pregnancies/number of rats that mated.
d. Restricted to rats with a confirmed mating date.
** Significantly different from the control group value (p≤0.01).

(b) (4) did not affect absolute weights of testes or epididymides or the ratios of these weights to the terminal body weight in F1 generation male rats. The absolute paired weights for the epididymides were significantly increased at 0.295 g/day compared to the respective control group value. The ratio (%) of this paired organ weight to the terminal body weight was also significantly increased at this dose when compared to the respective negative control group value. The following table (from page 153 of the study report) shows the organ weight data.

PROTOCOL PJQ00004: SUBCUTANEOUS DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF (b) (4) IN RATS, INCLUDING A POSTNATAL BEHAVIORAL/FUNCTIONAL EVALUATION (SPONSOR'S REFERENCE NUMBER: AP 27-03)

TABLE B21 (PAGE 1): TERMINAL BODY WEIGHTS, ORGAN WEIGHTS AND RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT - SUMMARY - F1 GENERATION MALE RATS

MATERNAL DOSAGE GROUP DESCRIPTOR MATERNAL DOSAGE (G/DAY)		I NEGATIVE CONTROL ARTICLE 0.250	II (b) (4) 0.142	III (b) (4) 0.295
RATS TESTED	N	23a	25	23a
INCLUDED IN ANALYSES	N	22b	25	23
TERMINAL BODY WEIGHT	MEAN±S.D.	606.8 ± 73.5	615.0 ± 62.9	597.2 ± 68.0
EPIDIDYMIDES PAIRED	MEAN±S.D.	1.56 ± 0.14	1.58 ± 0.16	1.68 ± 0.17*
EPIDIDYMIDES PAIRED (%)	MEAN±S.D.	0.260 ± 0.035	0.258 ± 0.032	0.284 ± 0.028*
TESTES PAIRED	MEAN±S.D.	3.68 ± 0.34	3.66 ± 0.33	3.85 ± 0.30
TESTES PAIRED (%)	MEAN±S.D.	0.615 ± 0.090	0.600 ± 0.060	0.649 ± 0.061

ALL WEIGHTS WERE RECORDED IN GRAMS (G). RATIOS (%) = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.
a. Excludes values for rats that were found dead or sacrificed due to adverse clinical observations.
b. Excludes values for rat 24622, which had had abnormal organs (weight affected).
* Significantly different from the control group value (p≤0.05).

No Caesarean-sectioning or litter parameters for F1 generation were affected by (b) (4). The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights and the percentage of resorbed conceptuses were comparable among the three dosage groups. No dam had a litter consisting of only resorbed conceptuses, and there were no dead fetuses. There was a statistically significant reduction in the percentage of live male fetuses at 0.142 g/day; however, this was not dose-related. The only fetal alteration (i.e., an umbilical hernia) occurred in one fetus at 0.142 g/day maternal dosage group. This alteration was not dose-related.
The following tables (from pages 155-157 of the study report) show the Caesarean-sectioning data for the F1 rats.

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PROTOCOL PJQ00004: SUBCUTANEOUS DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF (b) (4) IN RATS, INCLUDING A POSTNATAL BEHAVIORAL/FUNCTIONAL EVALUATION (SPONSOR'S REFERENCE NUMBER: AP 27-03)

TABLE B23 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP DESCRIPTOR MATERNAL DOSAGE (G/DAY)		I NEGATIVE CONTROL ARTICLE 0.250	II (b) (4) 0.142	III (b) (4) 0.295
RATS TESTED	N	24	25	25
PREGNANT	N(%)	23(95.8)	23(92.0)	22(88.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	23	23	22
CORPORA LUTEA	MEAN±S.D.	17.8 ± 2.9	17.1 ± 2.6	17.5 ± 2.6
IMPLANTATIONS	MEAN±S.D.	15.5 ± 3.6	16.3 ± 2.6	16.3 ± 2.6
LITTER SIZES	MEAN±S.D.	14.6 ± 3.5	15.4 ± 2.5	15.9 ± 2.5
LIVE FETUSES	N	337	355	350
	MEAN±S.D.	14.6 ± 3.5	15.4 ± 2.5	15.9 ± 2.5
DEAD FETUSES	N	0	0	0
RESORPTIONS	MEAN±S.D.	0.9 ± 1.2	0.9 ± 1.3	0.4 ± 0.6
EARLY RESORPTIONS	N	19	21	8
	MEAN±S.D.	0.8 ± 1.1	0.9 ± 1.3	0.4 ± 0.6
LATE RESORPTIONS	N	1	0	1
	MEAN±S.D.	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.2
DAMS WITH ANY RESORPTIONS	N(%)	11(47.8)	10(43.5)	8(36.4)
DAMS WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE FETUSES	N(%)	23(100.0)	23(100.0)	22(100.0)
PLACENTAE APPEARED NORMAL	N(%)	23(100.0)	23(100.0)	22(100.0)

PROTOCOL PJQ00004: SUBCUTANEOUS DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF (b) (4) IN RATS. INCLUDING A POSTNATAL BEHAVIORAL/FUNCTIONAL EVALUATION (SPONSOR'S REFERENCE NUMBER: AP 27-03)

TABLE B24 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP		I	II	III
DESCRIPTOR		NEGATIVE CONTROL ARTICLE	(b) (4)	(b) (4)
MATERNAL DOSAGE (G/DAY)		0.250	0.142	0.295
LITTERS WITH ONE OR MORE LIVE FETUSES	N	23	23	22
IMPLANTATIONS	MEAN±S.D.	15.5 ± 3.6	16.3 ± 2.6	16.3 ± 2.6
LIVE FETUSES	N	337	355	350
	MEAN±S.D.	14.6 ± 3.5	15.4 ± 2.5	15.9 ± 2.5
LIVE MALE FETUSES	N	188	160	192
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	55.4 ± 11.8	44.8 ± 12.1**	54.3 ± 14.0
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	5.18 ± 0.39	5.00 ± 0.32	5.07 ± 0.33
MALE FETUSES	MEAN±S.D.	5.34 ± 0.42	5.09 ± 0.40	5.18 ± 0.36
FEMALE FETUSES	MEAN±S.D.	4.98 ± 0.36	4.92 ± 0.31	4.96 ± 0.33
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	5.5 ± 7.0	5.3 ± 7.3	2.5 ± 3.6

** Significantly different from the control group value (p≤0.01).

PROTOCOL PJQ00004: SUBCUTANEOUS DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF (b) (4) IN RATS. INCLUDING A POSTNATAL BEHAVIORAL/FUNCTIONAL EVALUATION (SPONSOR'S REFERENCE NUMBER: AP 27-03)

TABLE B25 (PAGE 1): FETAL GROSS EXTERNAL ALTERATIONS - SUMMARY - F1 GENERATION FEMALE RATS

MATERNAL DOSAGE GROUP		I	II	III
DESCRIPTOR		NEGATIVE CONTROL ARTICLE	(b) (4)	(b) (4)
MATERNAL DOSAGE (G/DAY)		0.250	0.142	0.295
LITTERS EVALUATED	N	23	23	22
FETUSES EVALUATED	N	337	355	350
LIVE	N	337	355	350
UMBILICAL HERNIA				
LITTER INCIDENCE	N	0(0.0)	1(4.3)	0(0.0)
FETAL INCIDENCE	N	0(0.0)	1(0.3)	0(0.0)

F_2 findings: There were no significant (b) (4)-related effects in the F2 generation fetuses.

Summary: In a Segment III peri and postnatal development study in rats, pregnant female rats were administered (b) (4) subcutaneously once daily from DG7 through DL20 at 0.250 (saline control), 0.142, and 0.295 g/day. No significant treatment-related effects were observed in the F1 generation male and female rats with regard to clinical signs, body weights or body weight gains, feed consumption, sexual maturation, learning and memory or fertility and reproductive capacity. There were no significant treatment-related gross necropsy observations in the F1 generation rats. There were no (b) (4)-related effects in the F2 generation fetuses. Overall, (b) (4) did not have significant adverse effect on pre- and post-natal development in rats.

2.6.6.7 Local tolerance

Determination of the Pharmacokinetics of Granisetron and Tolerability of APF530 Following Subcutaneous Administration of APF530 to Rats (AP23-19)

Methods: The purpose of this study was to determine the pharmacokinetics of granisetron and the tolerability of the test article formulation (APF530) following subcutaneous administration of APF530 to male and female rats. In this study, animals (n =3/sex/dose) were treated with APF530 by SC route at 5, 10 and 20 mg granisetron/animal or 250, 500 and 1000 mg APF530/animal. Animals in Groups 1 and 4 each received the full dose volume divided equally over a total of four injection sites. Animals in Groups 2 and 3 each received the full dose volume via one and two injection sites, respectively. The study design is shown below (from 2 of the study report).

STUDY DESIGN

Group	Number of Animals: Males	Number of Animals: Females	Dose Route	Target Dose Level (mg/animal)[a]	Target Dose Volume (mL/animal)	Target Dose Concentration (mg/mL)
1	3	3	SC	20	1[b]	20
2	5	5	SC	5	0.25[c]	20
3	5	5	SC	10	0.5[d]	20
4	5	5	SC	20	1[b]	20

SC Subcutaneous injection.
Note: Group 1 served as a tolerability dose group. Animals in Group 1 were dosed approximately 4 days prior to dosing animals in Groups 2 through 4.
a Values reflect the total amount of granisetron given. The values correspond to the following APF530 formulation values:

5 mg granisetron/animal = 250 mg APF530/animal.
10 mg granisetron/animal = 500 mg APF530/animal.
20 mg granisetron/animal = 1000 mg APF530/animal.

b Total amount delivered to each animal via 4 injection sites (0.25 mL/site).
c Total amount delivered to each animal via 1 injection site.
d Total amount delivered to each animal via 2 injection sites (0.25 mL/site).

Animals were observed for mortality and clinical signs on a daily basis. Body weights were recorded on the days of dose administration. Animals in Group 1 were not weighed on the scheduled day of dosing. For Groups 2 through 4, body weights were also recorded just prior to necropsy. For Groups 2 through 4, blood was collected from each animal at 0.5, 1, 6, 12, 24, 48, 72, 96, 120, 144, and 168 hours post-dose. No blood samples were collected for Group 1 animals. Gross necropsy was conducted on all rats from Groups 2 through 4. The following tissues were weighed and examined for histopathology: kidney, heart, liver, brain, lungs, individual injection sites, and any gross lesions.

Results: Clinical signs included leakage at the injection sites, swelling, white creamy discharge, and erythema. Body weights were not affected by the treatment. There were no significant treatment-related effects on gross necropsy findings and organ weights. Test article-related microscopic findings were present in one or more subcutaneous

injection sites in all animals in all study groups. The most common test article-related finding at injection sites was subcutaneous minimal to moderately severe granulomatous inflammation characterized by a cellular infiltrate of predominantly neutrophils as well as varying numbers of macrophages, multinucleated giant cells, lymphocytes, plasma cells, and proliferating fibroblasts. In addition, the inflammation tended to be more severe in females than in males. The granulomatous inflammation was frequently accompanied by hemorrhage and a cavity within the subcutis where the test article had likely been deposited. A minimal amount of mineralization was also seen in the inflammatory areas in the injection sites of some animals, primarily for males in Groups 3 and 4.

Cmax (granisetron) in the plasma were generally observed at 0.5 to 1 hour post-dose for all animals in Groups 2 through 4 with the exception of four animals (Tmax = 6-72 hr). Cmax was generally higher for females than males within each dose group, and higher exposure was observed for females based on the dose normalized AUC values. Concentrations of granisetron in the plasma were generally observed through 72, 96, and 120 hours postdose for Groups 2 (5 mg granisetron/animal), 3 (10 mg granisetron/animal), and 4 (20 mg granisetron/animal), respectively. In general, an increase in plasma concentrations was seen at later time points. The following table (from page 11 of the study report) shows the mean PK parameters.

Selected Mean Pharmacokinetic Parameters for Granisetron in Plasma Following a Single Subcutaneous Dose of APF530 to Male and Female Rats

Group	Dose Level (mg/animal)	Gender	C_{max} (ng/mL)	T_{max} (hours)	$t_{1/2}$ (hours)	AUC_{0-t} (hr·ng/mL)	AUC_{0-t}/D (hr·ng/mL)/(mg/kg)
2	5	M	100	15.2	NC	2936	181
		F	152	15.1	13.8	3289	166
		Overall	126	15.2	13.8	3112	174
3	10	M	301	1.80	15.8	5563	171
		F	365	0.900	21.3	9178	238
		Overall	333	1.35	18.6	7370	204
4	20	M	692	0.700	12.0	14802	225
		F	733	1.70	7.38	21770	288
		Overall	713	1.20	9.94	18286	257

D Actual calculated dose administered.
NC Not calculated.

Overall, APF530 appeared to be well tolerated. No apparent test article-related effects were observed on body weight, organ weights, and histopathology of the brain, kidney, liver, lung, and heart. Test article-related effects were mainly observed at the subcutaneous injection sites. Primary histopathology finding included minimal to moderate inflammatory response, which appeared to be more severe in females than in males.

Determination of the Pharmacokinetics of Granisetron and Tolerability of APF530 Following Subcutaneous Administration of APF530 to Dogs (AP23-20)

Methods: The purpose of this study was to determine the pharmacokinetics of granisetron and the tolerability of the test article formulation (APF530) following subcutaneous administration of APF530 to male and female dogs. In this study, animals (n =3/sex/dose) were treated with APF530 by SC route at 5, 20 and 80 mg granisetron/animal or 250, 1000 and 4000 mg APF530/animal. For Groups 1 and 4, the quadrants were distributed across the dorsum of the dog (i.e., right and left dorsal thorax, right and left dorsal abdomen.) The shaved area was divided appropriately using indelible ink or marker. Animals in Groups 1 and 4 each received the full dose volume divided equally over a total of four injection sites. Animals in Groups 2 and 3 each received the full dose volume via one injection site. The study design is shown below (from 2 of the study report).

STUDY DESIGN

	Number of Animals		Dose	Target Dose Level	Target Dose Volume	Target Dose Concentration
Group	Males	Females	Route	(mg/animal)[a]	(mL/animal)	(mg/mL)
1	1	1	SC	80	4[b]	20
2	3	3	SC	5	0.25[c]	20
3	3	3	SC	20	1[c]	20
4	3	3	SC	80	4[b]	20

SC Subcutaneous injection.
Note: Group 1 served as a tolerability dose group. Animals in Group 1 were dosed approximately 5 days prior to dosing animals in Groups 2 through 4.
a Values reflect the total amount of granisetron given. The values correspond to the following APF530 formulation values:

5 mg granisetron/animal = 250 mg APF530/animal.
20 mg granisetron/animal = 1000 mg APF530/animal.
80 mg granisetron/animal = 4000 mg APF530/animal.

b Total amount delivered to each animal via 4 injection sites (1 mL/site).
c Total amount delivered to each animal via 1 injection site.

Animals were observed for mortality and clinical signs on a daily basis. Body weights were recorded on the days of dose administration. Animals in Group 1 were not weighed on the scheduled day of dosing. For Groups 2 through 4 (PK group), body weights were also recorded just prior to necropsy. No blood samples were collected from animals in Group 1. For Groups 2 through 4, blood was collected from each animal at 0.5, 1, 6, 12, 24, 48, 72, 96, 120, 144, and 168 hours post-dose. Gross macroscopy was conducted on all rats from Groups 2 through 4. The following tissues were weighed and examined for histopathology: kidney, heart, liver, brain, lungs, individual injection sites, and any gross lesions.

Results: There were no significant clinical signs. Body weights were not affected by the treatment. No macroscopic observations were present in any animals given 0.25 mL of APF530 (5 mg of granisetron). Test article-related macroscopic findings, such as thickening and/or diffuse redness, were seen at the injection site at 20 and 80 mg of

granisetron doses. There were no significant treatment-related effects on organ weights. Test article-related microscopic findings were present in one or more subcutaneous injection sites in all animals in all study groups, which included subcutaneous inflammatory response. The most common test article-related finding at injection sites was a subcutaneous inflammatory response characterized by a cellular infiltrate of predominantly neutrophils as well as varying numbers of macrophages, multinucleated giant cells, lymphocytes, plasma cells, and proliferating fibroblasts. The finding appeared to be dose related with respect to severity and incidence. The inflammation in the two highest dose groups was occasionally accompanied by hemorrhage, scattered mineralization, and a cavity within the subcutis that may have contained the test article.

Increases in Cmax were roughly dose-proportional, but AUC was greater than dose-proportional. Mean plasma Cmax values increased approximately 1:4.0:13.6-fold and AUC_{0-t} values increased approximately 1:6.4:27.6-fold with the 1:4:16-fold increase in dose level (mg/animal). Cmax was similar for males and females within each dose group, but slightly higher exposure was observed for females. Concentrations of granisetron in the plasma and whole blood were generally observed through 24, 96, and 120 hours postdose for the 5-, 20-, and 80-mg/animal doses, respectively. In general, an increase in plasma concentrations was seen at later time points. The following table (from page 13 of the study report) shows the mean PK parameters.

Selected Mean Pharmacokinetic Parameters for Granisetron in Plasma Following a Single Subcutaneous Dose of APF530 to Male and Female Dogs

Group	Dose Level (mg/animal)	Gender	C_{max} (ng/mL)	T_{max} (hours)	$t_{1/2}$ (hours)	AUC_{0-t} (hr·ng/mL)	AUC_{0-t}/D (hr·ng/mL)/(mg/kg)
2	5	M	4.94	1.00	34.0	96.0	305
		F	4.86	0.833	17.4	115	265
		Overall	4.90	0.917	28.5	105	285
3	20	M	16.0	4.33	29.6	657	426
		F	22.9	6.00	30.4	693	334
		Overall	19.5	5.17	30.0	675	380
4	80	M	73.3	2.67	22.1	2596	398
		F	59.9	6.33	20.8	3200	389
		Overall	66.6	4.50	21.4	2898	394

D Actual calculated dose administered.

Selected Mean Pharmacokinetic Parameters for Granisetron in Whole Blood Following a Single Subcutaneous Dose of APF530 to Male and Female Dogs

Group	Dose Level (mg/animal)	Gender	C_{max} (ng/mL)	T_{max} (hours)	$t_{1/2}$ (hours)	AUC_{0-t} (hr·ng/mL)	AUC_{0-t}/D (hr·ng/mL)/(mg/kg)
2	5	M	5.08	1.00	11.7	65.6	208
		F	5.34	2.67	29.3	126	291
		Overall	5.21	1.83	17.6	96.0	250
3	20	M	16.6	6.00	32.1	619	401
		F	23.3	4.33	28.8	679	327
		Overall	19.9	5.17	30.8	649	364
4	80	M	72.6	4.33	27.0	2629	403
		F	68.4	8.67	27.7	3634	441
		Overall	70.5	6.50	27.4	3132	422

D Actual calculated dose administered.

Overall, APF530 appeared to be well tolerated. No apparent test article-related effects were observed on body weight, organ weights, and histopathology of the brain, kidney, liver, lung, and heart. Test article-related effects were observed for subcutaneous injection sites during histopathology. The primary histopathology finding was minimal to moderately severe inflammation response characterized by a cellular infiltrate of predominantly neutrophils as well as varying numbers of macrophages, multinucleated giant cells, lymphocytes, plasma cells and proliferating fibroblasts. The severity of the finding appeared to be dependent on dose volume/injection as well as total dose volume.

2.6.6.8 Special toxicology studies

Study title: **Twenty-Nine Day Toxicity Study with (b) (4) and (b) (4) in Rats Following Intraperitoneal Injection**

Key study findings:

- All animals survived to their scheduled sacrifices.
- One placebo-treated and one (b) (4)-treated rat exhibited red nasal discharge on Days 22 and 29, respectively.
- Body weight and food consumption values were generally comparable between control and treated groups.
- There were no significant treatment-related gross or histopathology findings.

Study no.: AP23-10
Volume #, and page #: N/A
Conducting laboratory and location: (b) (4)
Date of study initiation: March 10, 2003
GLP compliance: A statement of compliance was included.
QA reports: yes (X) no ( )
Drug, lot #, and % purity: (b) (4) (Lot No. 76590, (b) (4)) and (b) (4) (Lot No. 76589), 100%
Formulation/vehicle: Clear liquid

Methods: The purpose of this study was to assess both systemic and local toxicity of (b) (4) to the peritoneum and peritoneal organs when administered by intraperitoneal (IP) injection to rats as a single dose. Male SD rats were assigned to 4 groups (20/group). On Day 1, each group received a single IP injection containing the control (saline), placebo (b) (4), test (b) (4) at a dose volume of 0.22 mL. The dose volume translates into a mass dose of approximately 0.26 g of polymer in the (b) (4) groups. Parameters evaluated to assess toxicity included survival, clinical observations, body weights, quantitative food consumption, clinical pathology, organ weights, and macroscopic and microscopic evaluations. The doses were selected based on a comparison of the surface area of the peritoneal cavity in rats and humans. The peritoneal surface area in rats and humans is approximately 0.029 and 1.81 square meters, respectively (Wiseman, D. M., "Animal Adhesion Models: Design, Variables, and Relevance," Peritoneal Surgery, Ch. 38, pp. 459-477, Springer-Verlag, New York, New York, (2000)). At an IP dose of 0.26 grams in rats or the proposed maximum human dose of 10 grams, this would approximate a local dose of 9.0 and 5.5 grams per square meter. The local dose in the rat represented approximately a two-fold safety margin in the unlikely event that the entire human dose gains access to the peritoneal cavity. The study design is shown below (from page 10 of the report).

Test System and Study Design
Rats were randomly assigned to the following groups based on weight.

Group	No. of Animals[a] Male	Dose Volume (mL)	Mass Dose Level (b) (4) (mg)	Polymer (g)	Dose Concentration (mg/mL)
1 (Saline)	20	0.22	0	0	0
2 (b) (4)	20	0.22	0	0.26	0
3	20	0.22	6.6	0.26	30
4	20	0.22	6.6	0	30

Five males/group were sacrificed on Days 5, 9, 16, and 30.

Results: All animals survived to their scheduled sacrifices. There were no significant clinical signs with two exceptions. One placebo-treated and one (b) (4)-treated rat exhibited red nasal discharge on Days 22 and 29, respectively. Body weight and food consumption values were generally comparable between control and treated groups. There were no significant treatment-related gross or histopathology findings.

Study title: **Toxicity Study with (b) (4) in Rabbits Following Incisional Administration**

Key study findings:

- All rabbits survived to their scheduled sacrifice except for one (b) (4) male (F60597) which was sacrificed on Day 15 due to a back injury and limb paralysis.
- Clinical signs, body weights, food consumption, and organ weights showed no effects attributable to the treatment.
- Fibrinogen concentrations were elevated for a few males in the (b) (4) groups at Day 5. The alanine aminotransferase (ALT) activity was higher in some rabbits treated with (b) (4)
- Gross signs of irritation at the incision site were seen in all groups. Principal findings included erythema and edema. The overall frequency of erythema tended to be higher in the (b) (4) treated rabbits.
- Necropsy findings at incision sites mostly included swelling and dark discoloration in some of the rabbits at Days 5, 9, and 16.
- The skin incisions were mostly healed at the Day 16 and later sacrifice intervals. Changes in the deeper tissues of the abdominal wall, involving the subcutaneous connective tissue and abdominal muscles, were resolved by the Day 59 terminal sacrifice, but fibroplasia and granulomatous reaction to the sutures remained in some males and females.
- Minimal to slight lymphoid hyperplasia was seen in the inguinal lymph nodes at Day 16 in males and later sacrifices intervals in (b) (4) treated group.

Study no.: AP23-11
Volume #, and page #: N/A
Conducting laboratory and location: (b) (4)
Date of study initiation: March 10, 2003
GLP compliance: A statement of compliance was included.
QA reports: yes (X) no ( )
Drug, lot #, and % purity: (b) (4) (Lot No. 76590, (b) (4)) and (b) (4) (Lot No. 76589), 100%
Formulation/vehicle: Clear liquid

Methods: This study examined the toxicity, local tolerability, and effects on wound healing of (b) (4) following incisional administration in rabbits. In this study, male and female New Zealand white rabbits were assigned to 4 groups (15/sex/group). Rabbits were administered saline (control article), (b) (4) (placebo article), (b) (4) (test article; (b) (4), or a (b) (4) solution in Groups 1, 2, 3, and 4, respectively. A single incision approximately 2 cm in length was made through the ventral skin, to the right of the midline, beginning at the level of the umbilicus and extending caudal and extending through the abdominal musculature into the peritoneal cavity. The muscle layers were sutured closed. A subcutaneous pocket was then created around the wound, into which a dose volume of 1.0 mL was delivered. The skin was sutured closed. Dose volume was based on the maximum feasible dose (MFD) for the route of administration. Dose levels of (b) (4) delivered were 0, 0, 30, and 30 mg in saline, (b) (4), and (b) (4) groups, respectively. The approximate mass dose of the placebo article (poly ortho ester polymer) and of the test article (b) (4) administered to Groups 2 and 3 were 1.19 and 1.18 g, respectively. Three rabbits/sex/group were sacrificed on Day 5, 9, 16, 30, or 59. The following parameters were evaluated: survival, clinical signs, irritation scores for the incisional sites, body weights, weight changes, food consumption, clinical pathology, organ weights, and macroscopic and microscopic pathology. The following table (from page 11 of the report) shows the study design.

Group Designation and Dose Levels

Group	No. of Animals[a] Male	Female	Dose Volume mL	Dose Level (b) (4)[b] mg	Mass Dose[c] g
1 (Saline)	15	15	1.0	0	0
2 (b) (4)	15	15	1.0	0	1.19
3	15	15	1.0	30	1.18
4	15	15	1.0	30	0

a Three animals/sex/group were scheduled for sacrifice on Day 5, 9, 16, 30, or 59.
b (b) (4) concentration = 30 mg/mL.
c Approximate density (1.19 g/mL for (b) (4) and 1.18 g/mL for (b) (4)) provided by sponsor.

Results: All rabbits survived to their scheduled sacrifice except as follows: (b) (4) Male F60597 designated for sacrifice on Day 30 was sacrificed on Day 15 due to a back injury and limb paralysis. There were no other unscheduled deaths. There were no

significant treatment-related effects on clinical signs, body weights, weight changes, and food consumption. The fibrinogen concentrations were elevated in the (b) (4) (143% of control, control = 334 mg/dL), (b) (4), and (b) (4) groups at Day 5. In addition, the globulin concentrations were elevated (>4.0 g/dL) in (b) (4) treated males at Day 5, but globulin concentrations were also elevated in several (b) (4) treated females without concurrent increases in fibrinogen values. Mean serum ALT activity was higher for a few rabbits throughout the study [(b) (4) at Day 16 (187% of control, control = 46 U/L), (b) (4) at Day 30]. A trace amount of fine granular casts was observed in the urine of (b) (4) Male F60606 on Day 5. Gross signs of irritation at the incision site were seen in all groups. Principal findings included erythema and edema. The overall frequency of erythema tended to be higher in the (b) (4) treated rabbits. Increased kidney weight (relative to body weight) was seen in (b) (4) males. However, no correlating macroscopic or microscopic changes were seen in the kidneys to relate to this relative increase in weight. Necropsy findings at incision sites mostly included swelling and dark discoloration in some of the rabbits at Days 5, 9, and 16. The skin incisions were mostly recovered at Day 16 and later sacrifice intervals. Changes in the deeper tissues of the abdominal wall, involving the subcutaneous connective tissue and abdominal muscles, were resolved by the Day 59 terminal sacrifice, but fibroplasia and granulomatous reaction to the sutures remained in some males and females. Minimal to slight lymphoid hyperplasia was seen in the inguinal lymph nodes at Day 16 (0 of 3, 2 of 3, 0 of 2 and 1 of 3 in males in Groups 1, 2 or (b) (4), 3 and 4, respectively) and later sacrifices intervals. Also, a female (F60661, (b) (4) group) had a focal area of myocardial degeneration/necrosis. No other change similar to this was seen in any rabbit.

Overall, (b) (4) related effects were observed at the site of injection, which included erythema and edema. (b) (4) related clinical pathology changes included increase in fibrinogen concentration and increase in serum ALT activity. Lymphoid hyperplasia of the inguinal lymph node was observed in (b) (4) treated males.

Study title: **Toxicity Study with (b) (4) in Rats Following Incisional and Subcutaneous Administration**

Key study findings:

- One (b) (4) female was found dead on Day 3. Histopathology identified a large abscess within the pelvic cavity along with mucosal/submucosal necrosis within the urinary bladder. No other mortality occurred.
- Clinical findings, body weights, food consumption, and organ weights showed no effects attributable to incisional and subcutaneous administration.
- Slightly high neutrophil counts and fibrinogen concentrations were seen in most of the (b) (4) females at Day 5.
- Gross signs of irritation after dose administration were more frequent at incision versus subcutaneous sites. Principal findings included edema and erythema.

- Necropsy findings included gelatinous incision sites at Day 5 and dark or red raised areas, sores, and/or scabs at incision and/or subcutaneous sites at Days 5, 9, and 16.
- Histopathology findings on Day 5, 9 and 16 included degeneration and necrosis of cutaneous muscle fibers, accompanied by regeneration, reactive fibroplasia, and chronic inflammation. These findings were more severe in animals given (b) (4), or (b) (4) as compared to what?.
- On Day 30, wound healing process appeared to be completed.

Study no.: AP23-12
Volume #, and page #: N/A
Conducting laboratory and location: (b) (4)
Date of study initiation: March 10, 2003
GLP compliance: A statement of compliance was included.
QA reports: yes (X) no ( )
Drug, lot #, and % purity: (b) (4) (Lot No. 76590, (b) (4)) and (b) (4) (Lot No. 76589), 100%
Formulation/vehicle: Clear liquid

Methods: This study examined the toxicity, local tolerability, and effects on wound healing of (b) (4) following incisional and subcutaneous administration in rats. In this study, male and female SD rats were assigned to 4 groups (25/sex/group). Rats were administered saline (control), (b) (4) (placebo article), (b) (4) (placebo article containing (b) (4)), or a (b) (4) solution (comparison article) in Groups 1, 2, 3, and 4, respectively. A single incision approximately 1 cm in length was made through the dorsal skin, parallel to the long axis of the body and caudal to the right scapula. A subcutaneous pocket was created into which a dose volume of 0.5 mL was delivered, after which the skin was sutured closed. A second dose of 0.25 mL was injected subcutaneously at a site parallel to, and off to the left side, of the dorsal midline. Dose volumes were based on the MFD for the route of administration. Dose levels of (b) (4) delivered were 0, 0, 15, and 15 mg at the incisional site and 0, 0, 7.5, and 7.5 mg at the subcutaneous site in saline, (b) (4) groups, respectively. The approximate mass doses of the placebo article (b) (4) and of the test article ((b) (4)) administered to Groups 2 and 3 were 0.60 and 0.59 g at incision sites and 0.30 and 0.29 g at subcutaneous injection sites, respectively. Five rats/sex/group were sacrificed on Day 5, 9, 16, 30, or 59. The following parameters were evaluated: survival, clinical signs, irritation scores for the incision and injection sites, body weights, weight changes, food consumption, clinical pathology results, organ weights, and macroscopic and microscopic pathology. The study design is shown below (from page 12 of the study report).

Group Designation and Dose Levels

Group	No. of Animals[a] Male	Female	Dose Volumes SC mL	Incisional mL	Dose Level (b) (4)[b] SC mg	Incisional mg	Mass Dose[c] SC g	Incisional g
1 (Saline)	25	25	0.25	0.5	0	0	0	0
2 (b) (4)	25	25	0.25	0.5	0	0	0.30	0.60
3	25	25	0.25	0.5	7.5	15	0.29	0.59
4	25	25	0.25	0.5	7.5	15	0	0

a Five rats/sex/group were sacrificed on Day 5, 9, 16, 30, or 59.
b (b) (4) concentration = 30 mg/mL.
c Approximate density (1.19 g/mL for (b) (4) and 1.18 g/mL for (b) (4)) provided by sponsor.
SC Subcutaneous

Results: One (b) (4) female was found dead on Day 3. Histopathology identified a large abscess within the pelvic cavity along with mucosal/submucosal necrosis within the urinary bladder. No other mortality occurred. Clinical findings, body weights, weight changes, food consumption, and organ weights showed no effects attributable to incisional and subcutaneous administration. Slightly high neutrophil counts and fibrinogen concentrations were seen in most of the (b) (4) females at Day 5. Gross signs of irritation after dose administration were more frequent at incision versus subcutaneous sites. Principal findings included edema and erythema. There was an increased incidence of this finding in the (b) (4) groups. Necropsy findings included gelatinous incision sites in (b) (4) females and one (b) (4) female at Day 5 and dark or red raised areas, sores, and/or scabs at incision and/or subcutaneous sites in some (b) (4) rats at Days 5, 9, and 16. Histopathology findings on Day 5, 9 and 16 included degeneration and necrosis of cutaneous muscle fibers, accompanied by regeneration, reactive fibroplasia, and chronic inflammation. These findings were more severe in animals given (b) (4) compared to saline control. On Day 30, epithelialization was complete, with occasional evidence of a very small degree of thickening (acanthosis). Within the deeper tissues, including dermis and cutaneous muscle, inflammation had mostly subsided, and healing was represented by fibrous tissue, mostly in the form of mature collagen. In most cases, there was some degree of fibrosis in the cutaneous muscle, and in some instances, it was evident that the continuity of the muscle had been permanently disrupted by fibrous scarring.

Overall, there was no evidence of significant systemic toxicity following incisional and subcutaneous administration of (b) (4). Most treatment-related effects were seen at the site of the injection/application. The healing process at incisional sites appeared to be completed by Day 30.

2.6.6.9 Discussion and Conclusions

APF530 is a new formulation containing 2% granisetron. Granisetron is an approved drug and nonclinical safety data for granisetron has been reviewed under the NDAs mentioned before. Since, APF530 is a new formulation containing a novel excipient vehicle (b) (4) the sponsor was asked to conduct nonclinical studies with this novel excipient, (b) (4), as per the CDER Guidance entitled "Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients". APF530 is intended to be used for the prevention of acute and delayed nausea and vomiting associated with initial and repeat courses of moderately and highly emetogenic cancer chemotherapy. The recommended dosage of APF530 is a single 10 mg SC dose administered approximately 30 minutes before the start of single-day chemotherapy or it may be given once every 7 days, and only on the day chemotherapy is given. Based on its intermittent dosage, 3-month toxicology studies were recommended in both a rodent and non-rodent species by the appropriate route of administration as per the above Guidance (Section C: Potential Excipients Intended for Intermediate Use). The sponsor conducted ADME, acute, subacute/subchronic (28- and 90-day) toxicology, genotoxicity, and reproductive toxicology studies with (b) (4). Toxicology studies were conducted in both rats and dogs using subcutaneous route, the intended route of administration in humans using the maximum feasible dose (MFD).

In ADME studies in rats, the highest mean concentrations of radioactivity (^{14}C-(b) (4)) were observed at the injection site and in the liver, bone marrow, urinary bladder, plasma, small intestine and kidneys at 24 hours postdose. The major route of excretion of radioactivity was *via* the urine. The biodisposition of (b) (4) is dependent on the hydrolysis of the polymer. Based on the size and hydrophobic nature of the polymer, intact polymer is not expected to be absorbed systemically. TEG-POE polymer is biodegradable, and is expected to undergo hydrolysis in an aqueous environment. Under *in vivo* conditions (i.e. large amounts of water), the polymer vehicle is expected to undergo hydrolysis yielding a spectrum of breakdown products (b) (4). The findings of the radiolabel studies indicated that the excreted radioactivity was associated primarily with TEG, and was consistent with *in vivo* hydrolysis of (b) (4). Hydrolysis of the (b) (4) polymer vehicle is species independent and, therefore, no novel metabolites are expected to be formed in humans. The data in humans suggested that the majority of the polymer vehicle would be eliminated over the seven day period with low levels of hydrolysis occurring during the initial one to two days following dosing, peak elimination would occur approximately one to two days following the Tmax for granisetron (i.e. at 48 to 72 hours), and low to negligible levels of hydrolyzed polymer excreted over at least the next three to four days.

In toxicology studies, the major organ toxicity associated with APF530 appeared to be restricted to the injection site (inflammatory reactions). Sequelae secondary to the inflammatory reactions at the site of injection were more pronounced in the rat compared to the dog. In the 90-day toxicology study (once every 24 hours) in rats, lymphoid hyperplasia in the draining lymph nodes, splenic hematopoiesis and bone marrow

myeloid hyperplasia were observed. However, similar doses did not produce histopathological changes in the lymph nodes, bone marrow and spleen when administered subcutaneously (once every 72 hours) in the 28-day toxicology study in rats.

(b) (4) was negative in the Ames test, the mouse lymphoma assay, and the *in vivo* rat bone marrow micronucleus test. APF530 was also negative in the standard battery of genotoxicity studies. Overall, neither (b) (4) nor APF530 exhibited any mutagenic or clastogenic activity in any of the assays and, therefore, are nongenotoxic.

(b) (4) was not teratogenic in rats and rabbits and did not appear to have adverse effects on fertility and early embryonic development in male and female rats. In addition, in Segment III pre- and postnatal development study in rats, (b) (4) did not cause any significant adverse effect on pre- and postnatal developmental parameters.

APF530 has been characterized for its impurity profile. Related substances present in APF530 are 1) carried in as an impurity in the drug substances (RM36/Granisetron), 2) degradant of RM36 due to manufacturing process, (b) (4) degradants associated with the stability. In approaching and developing a rationale for setting the initial acceptance criteria for the APF530 specification, the following categories of information was utilized: in-vivo qualified lots: non-clinical and clinical, stability data, European Pharmacopoeia Granisetron HCl monograph reference limits and ICH Q3B(R2) Impurities in New Drug Products guidelines. The following table (from the sponsor's submission) shows the specifications of the Drug Product.

Table 2 : APF530 Final Drug Product (FDP)

Approved current APF530 FDP Specification (file located in Folder 3.2.P.5.1)

Test Identity	Acceptance Criteria	SOP #
Description	(b) (4)	P-347
Identity by IR		P-374
Granisetron by HPLC		P-391
Granisetron Related Substances by HPLC		P-392
MMD by GPC		P-399, P-400
Dynamic Viscosity @ 37°C		P-326
(b) (4)		(b) (4)
In Vitro Release of Granisetron @ 37°C		PMT-025 PMT-026 PMT-027 PMT-028 PMT-029 P-413
Particulate Matter by Light Obscuration		AP-QC-035 AP-EQP-018 USP <788>
Sterility		USP <71>
Endotoxin by LAL		USP <85>

APF530 Final Drug Product (FDP) Specification continued.

The specification acceptance criteria for individual and total impurities were based on the material qualified in the non-clinical and clinical studies. The known impurities qualified during the studies are (b) (4). All the impurities, except for (b) (4), demonstrated levels below the qualification threshold (b) (4) per the ICH guidelines. The following lists the highest total impurities tested for the studies: Non-clinical: (b) (4)%. The highest known impurities for the non-clinical studies are: (b) (4)%. The highest known impurities tested in the clinical studies are: (b) (4) %. The highest unknown impurity amount for the non-clinical and clinical studies was (b) (4)%. Lot #146-005-002 and batches of APF530 manufactured thereafter, the unknown impurity values decreased to (b) (4)% as a result improved manufacturing process. The following amount of total impurities was qualified in the corresponding animal studies: Rats: (b) (4) Dogs: (b) (4); Humans: (b) (4). Overall, individual impurities in the drug product are qualified at specified levels. The highest amount of total impurities tested in animals and humans were (b) (4)% and (b) (4)%, respectively. The proposed specification level of (b) (4)% for total impurities in the drug product was not qualified in the nonclinical studies. The sponsor should be asked to qualify the proposed total impurity specification level of (b) (4)% or reduce the specification level to (b) (4)%.

Overall, the constituents of APF530, granisetron and the (b) (4) (novel vehicle excipient), have been adequately characterized in general toxicology, genetic toxicology and reproductive toxicology studies and support approval of APF530. In addition,

APF530 has been fully evaluated in genetic toxicology studies as well as single and repeat dose toxicology studies. In conclusion, this 505(b)(2) application contains adequate studies for the marketing approval of APF530 for the intended patient population at the recommended doses. Nonclinical studies conducted with APF530 provided adequate assurance of safety for its proposed use. Therefore, from a nonclinical standpoint, this NDA may be approved.

2.6.6.10 Tables and Figures

Tables and figures have been incorporated in the appropriate sections of this review.

2.6.7 TOXICOLOGY TABULATED SUMMARY

None

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: This 505(b)(2) NDA application contains adequate nonclinical studies for the marketing approval of Sustol® for the intended patient population at the recommended doses and the nonclinical studies conducted with APF530 provided adequate assurance of safety for its proposed use. Therefore, from a nonclinical standpoint, this NDA may be approved.

Unresolved Toxicology Issues: The proposed specification level of (b) (4)% for total impurities in the drug product was not qualified in the nonclinical studies. The sponsor should be asked to qualify the proposed total impurity specification level of (b) (4)% or reduce the specification level to (b) (4)%. In addition, the sponsor should be asked to provide information regarding the genotoxic potential of the impurities.

Recommendation: From a nonclinical standpoint, this NDA is recommended for approval.

Suggested labeling: The draft labeling of Sustol® generally conforms to the format specified under 21CFR 201.57(c)(14) Requirements for PLR (Physician's Labeling Rule) Prescription Drug Labeling. However, the recommended changes should be incorporated as mentioned under "Recommendations on Labeling" in the "Executive Summary" section.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

None

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22445	ORIG-1	AP PHARMA INC	APF530

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

/s/

TAMAL K CHAKRABORTI
02/03/2010

SUSHANTA K CHAKDER
02/04/2010
I concur with the contents and recommendations of the review