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RESEARCH**

APPLICATION NUMBER:

203214Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO

NDA: 203214

Submission date: 10/21/2011

Drug: tofacitinib

Sponsor: Pfizer Inc.

Indication: Treatment of Adult Patients with Moderately to Severely Active Rheumatoid Arthritis (RA) and Inadequate Response to One or More Disease-Modifying-Anti-Rheumatic Drugs (DMARDs)

Reviewing Division: Division of Pulmonary, Allergy and Rheumatology Products

Background Comments:

The pharmacology/toxicology reviewer and team leader in the Division of Pulmonary, Allergy and Rheumatology Products reviewed the nonclinical information for tofacitinib and found it adequate to support approval from a pharmacology/toxicology perspective for the indication listed above.

Discussion:

Genotoxicity

Tofacitinib was assessed in five genotoxicity studies. All of these studies were negative except for an in vitro cytogenetic study in peripheral human lymphocytes. The study was positive for chromosomal aberrations with a 3-hour incubation in the presence of metabolic activation.

Carcinogenicity

The applicant conducted two carcinogenicity studies: a 2-year study in rats and a 6-month study in Tg.rasH2 mice. These studies were reviewed by the division and the Executive Carcinogenicity Assessment Committee. The Committee found that the studies were acceptable and that there were no drug-related neoplasms in mice. The Committee concurred that the following were drug related neoplasms in rats: interstitial cell tumors in the testis of males; benign thymomas in the thymus of females; and malignant hibernomas in females (a rare tumor not meeting statistical significance, but seen at a higher than usual incidence). The Committee noted that the mechanism of action studies support the hibernomas as being pharmacologically plausible.

In addition to the neoplasms noted in the rodent studies, lymphoma was observed in 3 high dose (10 mg/kg/day) animals in the 39 week monkey study. All three animals were also positive for lymphocryptovirus. Immune suppression was the predominant toxicity finding in all repeated-dose animal studies as would be expected from the pharmacologic activity of tofacitinib. The occurrence of

lymphoma in monkeys is likely related to immune suppression and viral re-emergence.

Developmental and Reproductive Toxicity

The primary and secondary reviewers initially recommended that the male rat fertility study be repeated because they considered the design of the study inadequate, albeit, for different reasons. After further assessment of the study, it was determined that the study was adequate and no further assessment of male fertility is considered necessary at this time.

Tofacitinib produced external, skeletal and visceral malformations in rabbits and external and skeletal malformations in rats. The NOAEL for these findings occurred at exposures in rats that are substantially higher (50-100 times) than those achieved in humans; however, the NOAEL for these findings in rabbits occurred at exposures that are within 10 times the maximum human exposure.

A pre/postnatal study in rats showed reduced pup viability and weight gain with a NOAEL substantially greater than the maximum human exposure. Given these findings, the applicant and the primary and secondary pharm/tox reviews recommend pregnancy category C with appropriate wording describing the potential risk to the fetus.

Established Pharmacologic Class

Tofacitinib is an inhibitor of the Janus-associated kinases. One possible appropriate Established Pharmacologic Class term would be "kinase inhibitor". This term has been used for several other moieties that target a variety of kinases. Other more specific terms may also be appropriate such as "Janus-associated kinase inhibitor". This term has not been used previously as an Established Pharmacologic Class.

Conclusions:

I concur with the Division pharmacology/toxicology recommendation that this NDA can be approved. No additional nonclinical studies are recommended. The overall risk of genotoxicity may be relatively low considering the results of all the studies; however, description of the results of the chromosomal aberration assay in labeling seems appropriate. The results of the carcinogenicity studies as described by the Executive Carcinogenicity Assessment Committee should be conveyed in labeling. Including a description of the lymphomas observed in monkeys also seems appropriate. Use of a pregnancy category of C seems warranted.

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/s/

PAUL C BROWN
10/18/2012

**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: 203214
Supporting document/s: SD-000
Applicant's letter date: Oct 21, 2011
CDER stamp date: Oct 21, 2011
Product: Xeljanz (tofacitinib)
Indication: Rheumatoid Arthritis
Applicant: Pfizer Labs
Review Division: Division of Pulmonary, Allergy, and
Rheumatology Products (DPARP)
Reviewer: Molly E. Shea, Ph.D.
Supervisor/Team Leader: Molly E. Shea., Ph.D.
Division Director: Badrul Chowdhury, M.D., Ph.D.
Project Manager: Philantha Bowen

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

This review is a secondary labeling review of NDA 203214 for tofacitinib, a Janus associated kinase inhibitor. Reference is made to Dr. Lawrence (Steve) Leshin's primary review dated July 3, 2012 that provides recommendations for changes to Pfizer's proposed labeling for sections 8.1, 8.3, 8.4, 12.1, and 13.1. Dr. Leshin's recommended changes to the labeling for sections 8.3, 8.4, 12.1 and 13.1 are inconsistent with 21 CFR § 201.00, 201.56, 201.57, and 314 recommendations. Additionally, the Established Pharmaceutical Class for Sections 11 and 12.1 was not determined. Therefore, a secondary review of the label is being conducted herein. This review supersedes Dr. Leshin's labeling recommendations.

The Established Pharmaceutical Class of tofacitinib is Janus-associated Kinase inhibitor (JAK inhibitor).

With the assumption that the clinical 5 mg BID oral dose of tofacitinib is supported by the clinical safety and efficacy data, the 5 mg BID dose is being used to determine exposure ratios in the relevant nonclinical sections of the label. Clinical AUC data were provided for the 5 mg BID dose by the Clinical Pharmacology reviewer (see below).

Clinical Human Pharmacokinetic

	C _{max} , ng/mL		AUC ₀₋₁₂ , ng/mL*hr	
	Day 1	Day 14	Day 1	Day 14
5 mg BID	48.3	50.9	161	154

These data were provided as AUC values from 0-12 hours. Therefore, the AUC₀₋₂₄ hours is **308 ng*h/mL**.

Based on the doses at which toxicities were observed and the No Observed Adverse Effect Levels (NOAELs) identified in the reproductive toxicity assays (fertility, EFD in rat and rabbit, and PPND in rat), in the 39-week monkey general toxicity study, and the two-year rat carcinogenicity study, the following exposure ratios were determined using the animal: human values based on the AUC_{0-24 h} observed at 5 mg BID.

Animal:Human Exposure Ratios

Study	Toxic Dose and NOAEL Dose	AUC _{0-24 h} (ng*h/mL)	Exposure Ratio
Fertility			
Females	Toxic: 10 mg/kg/day NOAEL: 1 mg/kg/day	5620 412	18 1
Males	Toxic: None NOAEL: 100 mg/kg/day	- 67500	- 220
EFD			

Study	Toxic Dose and NOAEL Dose	AUC _{0-24 h} (ng*h/mL)	Exposure Ratio
Rat	Toxic: 100 mg/kg/day NOAEL: 30 mg/kg/day	73800 29400	240 95
Rabbit	Toxic: 30 mg/kg/day NOAEL: 10 mg/kg/day	6350 1470	20 5
PPND*			
Rat	Toxic: 50 mg/kg/day NOAEL: 10 mg/kg/day	36900 7380	120 24
Carc			
39-week Monkey	Toxic Dose for lymphoma: 5 mg/kg twice daily NOAEL: 1 mg/kg twice daily	2890 524	9 2
2-yr Rat	Toxic: 30 mg/kg/day NOAEL: 10 mg/kg/day	12600 3880	40 13

*- No toxicokinetic data from study. Data were extrapolated from rat EFD.

Using these calculated exposure ratios, the Nonclinical sections of the label were updated.

Section 8.1 was revised to include CFR recommended label structure and to provide more detailed descriptions of the rat and rabbit teratogenic findings (b) (4)

Section 8.3 was revised to include CFR recommended language.

Changes recommended in Section 8.3 and 8.3 by Dr. Leshin are not recommended.

No animal data were included in Section 10, which is in agreement with CFR recommendations.

Section 12.1 Mechanism of Action was revised by Clinical Pharmacology with support from nonclinical reviewers, Dr. Luqi Pei and Dr. Leshin. This reviewer agrees with these recommendations. This section has not yet been added to the review team label but it should read as follows:

(b) (4)

(b) (4)

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Section 13.1 was revised to (b) (4) include more detailed information regarding the carcinogenicity, mutagenesis and fertility impairment.

These changes are captured in the track-changes version below.

1.3.3 Labeling

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/s/

MOLLY E SHEA

09/21/2012

I concur.

INTEROFFICE MEMO

TO: NDA 203214 Original submission
Xeljanz (Tofacitinib)

FROM: Molly E. Shea, Ph.D.
Pharmacology/Toxicology Supervisor
Division of Pulmonary, Allergy and Rheumatology Products

DATE: July 27, 2012

Addendum to Nonclinical Supervisory Review July 21, 2012

The July 21, 2012 nonclinical supervisory secondary review recommended that Pfizer conduct a properly designed and analyzed fertility assessment in adult male rats as recommended in ICH5(R2) to be completed as a post-marketing requirement (PMR). The sponsor was informed of this PMR request.

The basis for the male fertility PMR request included the primary reviewers (Dr. Leshin primary review dated July 3, 2012) identification that the study was deficient and the Supervisor's misinterpretation of the primary reviewers summary of the completed male fertility study design. On July 26, 2012, the Nonclinical Supervisor reviewed study 05GR051 *Oral fertility and embryonic development study of CP-690-550-10 (tofacitinib) in male and female rats*. Upon review, the Nonclinical Supervisor concluded that the study design and methodology were in line with the recommended ICH-S5A Guideline and the study results were in agreement with the sponsor's conclusion that tofacitinib had no adverse effect on male rat fertility up to oral doses of 100 mg/kg/day. Therefore, the male rat fertility study is adequate and the PMR request is unfounded and is rescinded. The label should accurately reflect the completed reproductive battery outcomes.

There are no outstanding nonclinical issues for NDA 230214 that require additional studies prior to or post-approval.

Molly E. Shea, Ph.D.
Pharmacology/Toxicology Supervisor

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MOLLY E SHEA
07/27/2012

INTEROFFICE MEMO

TO: NDA 203214 Original submission
Xeljanz (Tofacitinib)

FROM: Molly E. Shea, Ph.D.
Pharmacology/Toxicology Supervisor
Division of Pulmonary, Allergy and Rheumatology Products

DATE: July 21, 2012

Nonclinical Supervisory Recommendation:

NDA 203214 is recommended for approval from the nonclinical perspective pending labeling revisions.

Nonclinical Supervisory Post-Marketing Requirement Recommendation:

Conduct a properly designed and analyzed fertility assessment in adult male rats as recommended in ICH5(R2).

Basis of PMR Recommendation:

Based on the overall nonclinical NDA evaluation, Dr. Leshin concluded that characterization of the nonclinical toxicity profile of tofacitinib was complete with the exception of having an adequate male fertility reproductive toxicology study (see Dr. Leshin's review submitted into DARRTS on July 3, 2012). Dr Leshin recommended approval of the NDA from the nonclinical perspective. Dr. Leshin recommended 2 post-marketing requirement (PMR) nonclinical studies as follows:

- 1) Conduct a properly designed and analyzed fertility assessment in adult male rats as recommended in ICH5(R2).
- 2) Conduct a properly designed and analyzed fertility assessment in juvenile male and female rats as recommended in ICH5(R2).

A secondary review for the necessity of these studies was completed. From the nonclinical supervisory perspective, one PMR is needed to address the deficient male reproductive fertility study. The sponsor completed a male fertility study in rats that were dosed for 63 days but were not mated until 1 month post-dosing. Tofacitinib has a short half-life (ranges from 0.6 to 2.8 hours) and clearance of the drug from the system is rapid. Therefore, Male rats were not sufficiently exposed to drug one month post-dose, potentially allowing recovery from any adverse consequences of tofacitinib on male fertility. A valid male reproductive toxicity study is needed and the study results are recommended to be included in

the label. The lack of a valid study was determined to not be critical for approval of tofacitinib from the nonclinical perspective. This was determined based on the following: 1. labeling can state that male fertility has not adequately been evaluated and 2. the potential benefit of the drug outweighs the risk when physicians and patients are made aware of this deficiency via proper labeling.

Dr. Leshin's recommendation for a second PMR to conduct a fertility assessment in juvenile male and female rats is not supported by the Nonclinical Supervisor. Reproductive toxicology studies including fertility, embryo-fetal development, and peri-post natal studies, should all be conducted in sexually mature animals. Further, the peri- post-natal study assesses development including sexual maturation. Although toxicity studies conducted in juvenile animals studies may reveal an impact on fertility (delayed sexual maturation or direct toxicities on developing reproductive organs), the intent of the male fertility assessment in sexually mature animals is to identify a change in fertility when adults are exposed to drug. Therefore, the second recommended PMR is unfounded.

General Nonclinical Supervisory Review:

Pfizer Incorporated (Pfizer) submitted their New Drug Application (NDA) 203214 on October 21, 2011 for Xeljanz (tofacitinib tablets) as a chronic treatment of adult patients with moderately to severely active rheumatoid arthritis (RA) who have had an inadequate response to one or more disease-modifying anti-rheumatic drugs (DMARDs). The proposed clinical daily oral treatment for RA is 5 mg BID [REDACTED] (b) (4)

[REDACTED] Tofacitinib is proposed as monotherapy or in combination with methotrexate or other nonbiologic DMARDs treatment.

The nonclinical program for tofacitinib included pharmacology, safety pharmacology, pharmacokinetic studies, toxicology studies with durations up to 6 months in rats and 9 months in monkeys, reproductive toxicology, genetic toxicology, carcinogenicity assays in mice and rats, and a photosafety study. Dr. Lawrence Leshin completed the primary review for this package. The following summarizes tofacitinib's toxicological profile and the Nonclinical Supervisory conclusions and recommendations, some of which differ from Dr. Leshin's review.

Tofacitinib is a first-in-class Janus associated kinase (JAK) inhibitor. Tofacitinib's mechanism of action includes inhibition of JAK1, JAK2 and JAK3 with less inhibitory activity against TyK2, all members of the JAK family. Inhibition of these kinases decreases inflammatory cytokine release which in turn decreases lymphocyte activation and proliferation.

Tofacitinib had no adverse effect on cardiovascular, respiratory, renal or gastrointestinal systems in stand alone safety pharmacology studies. Mice orally administered tofacitinib showed a reduction in spontaneous activity at high (100 mg/kg) doses suggesting potential neurotoxicity. However, this observation

coincided with general toxicity observations and no promotion or inhibition of seizures was observed.

Pharmacokinetic assessments of tofacitinib in rat, rabbit, dog and monkeys demonstrated rapid oral absorption with a short half-life (ranged from 0.6 to 2.8 h for all species). Tofacitinib was minimally to moderately protein bound in mice (67%), rats (85%), dogs (80%), monkeys (65%) and humans (62%). After oral administration to rats, tofacitinib was observed to be both renally (~50%) and fecally (~50%) excreted. In rabbits and monkeys, tofacitinib was mainly excreted in the urine (~56%) with less fecal excretion (~30%) after oral administration. Cytochrome P450 isoforms CYP3A4/3A5 and CYP2C19 were the main enzymes metabolizing tofacitinib in an in vitro human liver microsome assay.

General toxicology studies were completed in Sprague-Dawley rats (single-dose, 2-week, 6-week and 6-month oral toxicology studies) and Cynomolgus monkeys (single-dose, 2-week, 1-month, and 39-week oral toxicology studies). In the 6-month oral rat toxicity study where animals were dosed with 0, 1, 10 or 100 mg/kg/day, the target organs of toxicity included: lymph nodes (lymphocyte depletion and atrophy), thymus (lymphocyte depletion and atrophy), spleen (lymphocyte depletion and atrophy), bone marrow (cellular decrease), changes in hematology (decreased white blood cells and red blood cells), adrenal gland (cortical vacuolation), gastrointestinal tract (stomach, duodenum, jejunum), liver (increased liver enzymes and hypertrophy), and lung (histiocytosis thought to be a result of immunosuppression). In general, the longer the duration of the toxicity study in rats the more severe the toxicities were (e.g., shorter term studies showed lymphoid depletion but the chronic study resulted in atrophy of the lymph organs). Based on the 6-month oral toxicity study in rats with atrophy of lymph organs, GI effects and lung histiocytosis, the NOAEL was identified as the low-dose (1 mg/kg/day) that has an associated $AUC_{0-24\text{ h}}$ of 765 and 742 ng*h/mL for males and females, respectively.

In the 39-week oral monkey toxicity study where animals were treated with 0, 0.25, 1, and 5 mg/kg BID (for a total daily dose of 0.5, 2, and 10 mg/kg), the toxicities included: severe immunosuppression resulting in infections and a decrease in immunosurveillance resulting in lymphomas in 3 animals orally treated with 10 mg/kg/day (5 mg/kg BID) and hematological changes (decreased white blood cells, lymphocytes, red blood cell, hematocrit and hemoglobin). The target organs of toxicity included: spleen (viral inclusions and lymphocyte hyperplasia), thymus (lymphocyte hyperplasia), lymph nodes (lymphocyte hyperplasia), bone marrow (erythroid hyperplasia), and stomach (viral inclusions, inflammation-secondary infections). Shorter duration studies in the monkey resulted in general lymphocyte depletion of the lymph organs, where longer duration of treatment resulted in lymphocyte hyperplasia in these organs. The lymphomas were positive for Epstein-Barr virus encoded small RNA 1 and EBNA-2 which indicates that these lymphomas are associated with lymphocryptovirus infection. The Nonclinical Supervisor identified the NOAEL as

the mid-dose (2 mg/kg/day or 1 mg/kg BID), which differed from the primary reviewers recommendation of no NOAEL. The Supervisory conclusion was based on the observed hematology and lymph related findings were expected pharmacological effects. These findings were observed at the low-dose and mid-dose but were not considered dose-limiting. The dose-limiting toxicities were considered severe immunosuppression resulting in lymphomas in monkeys at the high-dose 10 mg/kg/day (5 mg/kg BID). The 2 mg/kg/day (1 mg/kg BID) NOAEL dose is associated with an AUC_{0-24 h} of 397 and 652 ng*h/mL for males and females, respectively.

Juvenile oral toxicology studies were completed in Sprague-Dawley rats (1-month) and Cynomolgus monkeys (39-week study). In the rat doses of 0, 1, 10 and 100 mg/kg/day were orally administered. Only the immune system and related organs were examined for toxicities. No new findings or more severe toxicities were observed in juvenile animals compared to those observed in adult rat toxicity studies. The target organs were changes in hematology (decreased WBCs, RBCs, and lymphocytes), thymus (lymphoid depletion), spleen (decrease in lymphocytes), and lymph nodes (lymphoid depletion). No toxicokinetic evaluation was completed for this study.

In the 39-week juvenile monkey study, animals were orally dosed with 0, 0.5, 2 and 10 mg/kg/day delivered as 0.25, 1, and 5 mg/kg BID (the same doses as the adult chronic monkey study). The major organ systems were evaluated in this study but a standard histopathology was not completed. This is considered acceptable for the purposes of this study. There were no lymphomas observed in this study, no premature deaths and no drug-related effects on the cardiovascular system (no changes in ECG). Compared to the adult chronic monkey study, the potential new finding in the juvenile monkey study was an increase in inflammatory cell foci in the heart at the high-dose. The target organs were the hematologic system (decreased WBC, RBCs and lymphocytes), spleen (lymphocyte hyperplasia), bone marrow (lymphoid follicle), lymph node (lymphocyte hyperplasia), and heart (inflammatory cell foci). As these findings were similar to those observed in the adult chronic toxicity study and these findings (hematological and lymph system) are the expected pharmacological mechanism that are not considered dose-limiting, the dose limiting toxicity was determined to be the inflammatory cell foci of the heart. Therefore, the Nonclinical Supervisor identified the NOAEL as 2 mg/kg/day (1 mg/kg BID) which is associated with an AUC_{0-24 h} of 424 ng*h/mL.

A standard genetic toxicology battery was completed for tofacitinib (bacterial reverse mutation assay, an *in vitro* chromosome aberration assay with human lymphocytes, and an *in vivo* rat micronucleus assay). Tofacitinib was negative for genetic toxicology in the bacterial reverse mutation assay and the *in vivo* rat micronucleus assay. However, tofacitinib showed a statistically significant increase in chromosome aberrations in cultured human lymphocytes in the 3-hour test with metabolic activation, but not in the absence of the addition of

induced liver enzymes. The concentration at which the positive response occurred, $\geq 1700 \mu\text{g/mL}$, corresponded to 48% mitotic suppression. Additional follow-up assays were conducted, *in vitro* CHO/HGPRT assay to assess for mammalian gene mutations and an *in vivo* rat hepatocyte unscheduled DNA synthesis assay which were both negative. Based on the weight of evidence approach, tofacitinib is considered negative for genotoxic potential. However, labeling is recommended to relay the outcomes of each of these studies.

The carcinogenic potential of tofacitinib was assessed in a 2-year study in rats and in a 6-month study in rasH2 transgenic mouse. In the rat study, the findings were sex specific and included interstitial cell adenomas in testis in males, benign thymomas in females and malignant hibernomas in females. Tofacitinib was not carcinogenic in rasH2 transgenic mice. Concurrence was obtained from the Executive Carcinogenicity Assessment Committee on these studies on March 6, 2012. Lymphomas were observed in the 39-week general toxicology study in adult cynomolgus monkeys (see above). These findings are recommended to be included in the label.

Pfizer submitted a reproductive toxicology battery (male and female rat fertility studies, embryofetal development rat and rabbit studies and a peri/post-natal rat reproductive toxicology study). In the female fertility assay, tofacitinib increased post-implantation loss and a reduced pregnancy rate due to reduced numbers of corpora lutea, implantation sites, early resorptions and pre- and post-implantation loss.

The fertility assessment for males was not adequate (see above for recommended PMR). Males were administered tofacitinib for a 63 days, dosing was stopped and males were without exposure to drug for 1 month. Due to the short half-life of the drug (0.6 to 2.8 hours), all drug is systemically cleared quickly. Males were allowed to mate 1 month post-dose, therefore, no drug is present in males. No adverse effects on mating or pregnancy rate were observed in untreated females. The duration of treatment at the time of mating was insufficient for drug exposure for a complete spermatogenic cycle. Adult male rats should be exposed to drug for an adequate assessment of potential effects of drug on male fertility. Therefore, the sponsor is requested to conduct a valid male fertility assessment as a post-marketing requirement.

Tofacitinib was teratogenic in both rats and rabbits. Therefore, Pregnancy Category C is recommended. In rats, postimplantation loss, consisting of early and late resorptions and consequently a reduced number of viable fetuses, and decreased uterine weight was observed. Teratogenic effects included anasarca and membranous ventricular septal defect and numerous skeletal malformations. In rabbits, teratologic findings included thoracogastroschisis, omphalocele, microstomia, microphthalmia, membranous ventricular septal defects, absent gallbladder, and multiple skeletal malformations.

Tofacitinib had no effect on pup delivery, standard landmarks of development, growth, sexual, or behavioral development in the peri-post-natal rat reproductive toxicology study. Tofacitinib was found in breast milk of lactating rats.

Tofacitinib was not phototoxic nor was it a skin sensitizer. Dr. Leshin reviewed the nonclinically relevant sections of the sponsor's proposed labeling. Recommendations to the labeling were made in the primary review dated July 3, 2012 in DARRTS. The labeling will require revisions with completion of an addendum to the primary and secondary reviews. There are no outstanding nonclinical issues that would affect tofacitinib's approval. Therefore, from the nonclinical perspective NDA 203214 for tofacitinib is recommended for approval pending labeling revisions to the nonclinical sections of the label.

Molly E. Shea, Ph.D.
Pharmacology/Toxicology Supervisor

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MOLLY E SHEA
07/21/2012

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PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number: **203214**
Supporting document/s: SD-000, SD-9
Applicant's letter date: Oct 21 2011, Feb 16 2012
CDER stamp date: Oct 21 2011, Feb 16 2012
Product: **Xeljanz (Tofacitinib)**
Indication: **Rheumatoid Arthritis**
Applicant: **Pfizer Inc.**
Review Division: Division of Pulmonary, Allergy and
Rheumatology Products
Reviewer: L. Steven Leshin, D.V.M., Ph.D.
Supervisor/Team Leader: Molly Shea, Ph.D.
Division Director: Badrul Chowdhury, M.D., Ph.D.
Project Manager: Philantha Bowen

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1 Executive Summary

1.1 Introduction

This application is for a first-in-class Janus associated kinase (JAK) inhibitor, tofacitinib (CP-690550), for the treatment of patients with rheumatoid arthritis (RA). Tofacitinib is a small molecule new molecular entity to be administered orally.

A full nonclinical program of pharmacology, pharmacokinetic, and toxicology studies were submitted to support the clinical program. These studies are reviewed herein.

1.2 Brief Discussion of Nonclinical Findings

Pharmacology

Tofacitinib (CP-690550 or (b) (4)) were the code names used in all the nonclinical studies and CP-690550 is the term used throughout this review to refer to tofacitinib) is an inhibitor of the Janus associated kinases (JAK) family. CP-690550 is a more potent inhibitor of JAK1, JAK2 and JAK3 kinase activity than TyK2 members in this kinase family. JAKs are involved in myeloid and erythroid cellular development and function by interrupting the signaling pathway from cytokine receptor to STAT (signal transducers and activators of transcription) through its inhibition of JAK activity intracellularly associated with cytokine receptors. Thus CP-690550 inhibition of JAK activity decreased the cellular response to cytokine signaling (IL-2, -4, -6, -10, -15, and -21, (IC₅₀ potencies ≤500 nM for the cellular forms, i.e., JAK homo or hetero-dimers) resulting in the reduction of the synthesis and secretion of additional cytokines and other inflammatory mediators.

Secondary pharmacodynamic studies evaluated the binding and potency of CP-690550 on a broad panel of receptors, ion channels, and enzymes that demonstrated a lack of significant binding or activity to these compounds. It was highly specific to the JAK family and did not affect the activity of other classes of kinases. There were a few compounds with which CP-690550 had significant activity (inhibition of >50%) in these screens. These were the MT₃ (Melatonin 3) receptor (K_i 5.2 μM) and VEGFR1 (vascular endothelial growth factor receptor 1 (K_i 3.7 μM), CaMK2α (calmodulin dependent protein kinase 2α (K_i 12 μM)), and Lyn A Kinase (K_i 2.3 μM). There were indications in various studies, usually at high doses, CP-690550 may interact with VEGFR1, evident by instances of hemorrhage associated with lymphoid organ atrophy (lymph nodes, thymus), as well as liver and lung, possibly associated with weakening the vascular structural integrity, due to cellular depletion, anoxia and organ atrophy. In this regard, tissue distribution studies (Report DM2004-690550-041) found that [¹⁴C]CP-690550 radioactivity was detected in vessel walls up to 504 hour after oral administration of 10 mg/kg (454 μCi/kg), the last timepoint examined.

In two animal models of arthritis, collagen-induced arthritis model in male DBA/1J mice and adjuvant (*Mycobacterium byturium*)-induced arthritis model in female Lewis rats, CP-690550 demonstrated efficacy by attenuating paw arthritis scores (swelling, edema

and paw volumes). Based on studies of the collagen-induced arthritis in mice the applicant demonstrated that suppression of the inflammatory response did not require continuous exposure to CP-690550 for effectiveness. The specific mechanism for preventing rheumatic changes in bone and cartilage were not addressed in detail, other than to associate CP-690550 inhibition of numerous interleukin compounds' signaling pathway that is blocked by tofacitinib. The two pharmacology studies, the rat adjuvant-induced arthritis model (Report 160531) and mouse collagen-induced arthritis (Report 160243) investigated CP-690550 for histological effects of cartilage damage, bone resorption, inflammation, pannus and CD68 and CD3 cellular infiltrates (rat) or F4/80 and CD3 cellular infiltrates (mice). However both of these studies were flawed at least with regards to interpretation of the effects on cartilage. At the time of the initial CP-690550 treatment (actually 4 hours after the start of treatment CP-690550 or vehicle), approximately half the animals in the first timepoint group had no evidence of cartilage damage (ankle and wrists). This limited not only this groups but sequent timepoint evaluations since any effect in reduction of arthritic disease is confounded with possibility the animal never had signs of disease. At this time, it is not known if CP-690550 has or does not have activity on cartilage, the studies did not allow either conclusion.

The toxicology studies do support the safety of CP-690550 on normal bone and joint structure.

- Adult monkey study 9 month toxicology study: no effect on sternum histopathology
- Rat 6 month toxicology study: no effect on sternum and distal femur and stifle (knee) joint histopathology.
- Rat (2 year) carcinogenicity study no effect on sternum or femur examined microscopically, or joints (stifle) examined macroscopically in aged rats.
- Juvenile monkey study, 9 month treatment starting at month 13-14 of age, no effect on sternum histopathology, and as an exploratory non-GLP aspect of this study, radiographic analysis of radius and tibia length found no effect on growth of bones.
- Embryofetal developmental studies indicating numerous bone abnormalities and delay ossification in rats and rabbits. These only assess macroscopic changes, there was no histopathology which is standard and acceptable practice.
- No effect on rat growth and behavior in postnatal studies.

Thus CP-690550 produced no pathologies on bone and cartilage in juvenile or adult, including aged healthy animals, supporting CP-690550 safety. In contrast, detrimental bone and possibly cartilage effects were evident in studies of fetal development.

Safety pharmacology studies of CP-690550 found no effect on cardiovascular assessments that included, in vitro hERG channel current, or in situ electrophysiological characteristics of cardiac Purkinje fibers, or in vivo assessment of cardiovascular effects in rats. Monkey EKG studies were incorporated into the long term toxicology studies, but there was insufficient information provided to adequately analyze the cardiovascular and EKG parameters. Neurobehavioral assessments in mice found a

reduction in spontaneous activity and signs of general toxicity. There were no significant effects on respiratory characteristics, kidney function, or gastrointestinal transit time.

Pharmacokinetics

Pharmacokinetic parameters were determined for all of the species used in the toxicology studies mouse, rat, rabbit, and cynomolgus monkey. CP-690550 was rapidly absorbed T_{max} values of 0.3 to 1.5 hours. The bioavailability was highly variable in the rat (11 to 129%) and this may be due to issues with early assay development. In the dog and monkey bioavailability was 43% and 48%, respectively, and values of 42.9 to 43.3 were attained in one rat study. For comparison, the human bioavailability is about 75% (refer to the Clinical Pharmacology Review of Dr. Jain). The clearance in the rat (29-42 mL/min/kg), and monkey (18 mL/min/kg) were moderate to high with a low to moderate volume of distribution (rat: 1.4-1.6 L/kg, monkey 1.7 L/kg). The plasma elimination half lives were rapid, ranging from 0.6 to 2.8 hours. The percentage of CP-690550 bound to plasma proteins varied with species, mouse 33%, dog 20%, monkey 35%, and human 39%, with the rat dependent on tofacitinib (ranging from 31% bound at low CP-690550 concentrations to 91% bound at high concentration). It was determined that CP-690550 bound mostly to albumin, and that α 1-acid glycoprotein did not have appreciable binding. CP-690550 distributed approximately equally between red blood cell and plasma compartments indicating red blood cells were not accumulating it.

In the toxicology studies, plasma concentrations, both C_{max} and AUC, increased with increasing dose. It is noteworthy that only the highest administered dose in the rat and monkey toxicology studies provided for a detectable plasma level over a 24 hour period, with the low dose detectable only up to approximately 6 hours per day and 8 to 12 hours for mid doses. Thus the effects observed at lower doses during and at the end of the study occurred in the absence of continuous tofacitinib exposure. In the rat studies the entire daily dose was administered at one time, while in the monkey studies the daily dose was administered as a split dose either 2 or 3 times per day, evenly distributed temporally. In monkeys there was no effect of sex on CP-690550 levels, but in rats females tended to have 2 to 3-fold higher AUC levels than males in the low and mid dose groups. There was no systemic accumulation of CP-690550 during the duration of the studies. Comparison of CP-690550 exposures between juvenile and adult monkeys in separate toxicological studies (Reports 09GR248 and 2003-0301, respectively) receiving the same dosages found reasonably similar C_{max} and AUC exposures.

Tissue distribution studies were conducted in pigmented rats with [14 C]CP-690550 (Report DM2004-690550-041). There was rapid distribution to all tissues except the brain, testis and adipose tissue at the first timepoint 30 min after oral dosing. For most tissues there was a gradual decline in radioactive levels over the following days, however at the last timepoint 504 hours, blood vessels and ocular tissue containing melanin were the only tissues with detectable activity. Ophthalmologic examinations during toxicology studies in rats and monkeys (Reports 77435 and 2003-0301, respectively) found no effects of CP-690550 treatments. A study in lactating rats found milk to plasma ratios of CP-690550 of approximately 2:1 indicating the concentrating

potential in active mammary glands (Report 103847). In vitro studies found that P-glycoprotein but not breast cancer resistance protein is an efflux transporter for CP-690550 (Reports 060532 and 175813, respectively). CP-690550 inhibited the human hepatic uptake transporter OATP 1B1 in an in vitro study of human HEK293 cells (Report 192119) and inhibited human OCT2 mediated uptake of creatinine (Report 13323).

Metabolism and excretion studies were conducted with radioactive [^{14}C]CP-690550 in mice (Report 140653), rats (Report DM2005-CP690550-055), rabbits (Report DM2005-CP690550-064), and monkeys (Report DM2005-CP690550-052) and compared with studies in humans (Report DM2005-CP690-049, and refer to the Clinical Pharmacology Review of Dr Jain for Report A3921010 for further details about the human results). In the rat and monkey elimination was primarily through urine with the unmetabolized drug comprising approximately 10% of total radioactivity recovered in urine in the monkey and male rat, and 30% in the female rat. The circulating levels of unmetabolized drug ranged from 58-60% in the rat and 31-49% in the monkey as a percentage of total blood radioactivity. In vitro CYP 450 phenotyping studies indicated CP-690550 was primarily metabolized by CYP3A4 and 2C19 (Report DM2007 (b) (4) 001). The primary metabolic pathways were due to oxidation of the pyrrolopyrimidine ring (forming metabolite M9), oxidation of the piperidine ring (forming M6 and M18), *N*-demethylation (forming M1), oxidation of the piperidine ring side chain (forming M2), and glucuronidation (forming M20). In comparison, more than 65% of the circulating radioactivity in humans was unmetabolized CP-690550 and all human metabolites were present at <10% of total circulating activity. While all the human metabolites were not present in a single animal species, they were identified in at least one animal species studied. The Applicant indicated that all metabolites have or are predicted to have ≤ 10 -fold the potency of CP-690550 for Janus kinase (JAK) 1/3 inhibition, but there was no empirical data or references to supporting this claim in the nonclinical submissions.

General Toxicology

General toxicology studies were conducted in Spague-Dawley rats and cynomolgus monkeys. A more detailed summary is provided in the Integrative Summary and Safety Evaluation, Section 11, of this review. The pivotal GLP toxicological studies in rats were 6-weeks (Report 01-2063-06) and 6-months (Report 02-2063-20) duration repeated dosing studies administered once daily doses of CP-690550 at 0 (0.5% carboxymethylcellulose vehicle), 1, 10, or 100 mg/kg/day. In the monkey, there were two pivotal studies, a 1-month repeated oral administration study with a 1-month recovery, and a 9-month repeated oral administration study. The doses administered in the monkey were 0 (0.5% carboxymethylcellulose vehicle), 10, 50, and 100 mg/kg/day as split doses TID (0, 3.33, 16.67, and 33.33 mg/kg, 7 hours apart) in the 1-month study, and 0, 0.5, 2, and 10 mg/kg/day as split doses BID (0, 0.25, 1, and 5 mg/kg/day, 12 hours apart) in the 9-month duration study. In the one month study in monkeys, the doses of 50 and 100 mg/kg/day resulted in secondary infections of open wounds, or gastrointestinal erosions or ulcers resulting in all animals in the 100 mg/kg/day group being euthanized by day 12, and 3 animals in the 50 mg/kg/day group being euthanized

throughout the dosing phase. In the 9 month study monkeys one female in the high dose group was euthanized on day 214. A lymphoma had infiltrated the gastric wall resulting in ulceration and erosion. At the 9 month scheduled necropsy, 2 additional animals with lymphomas were identified, one male and another female both in the 10 mg/kg/day dose group. Of the 3 lymphomas, 2 were identified as B cell origin, 1 was T cell origin, and all were in animals positive for lymphocryptovirus.

In both the rat and monkey pivotal studies, the major effects of oral administration of CP-690550 were hematologic findings included decreases in white blood cell parameters (reductions in white blood cell counts (rat, monkey), lymphocyte counts (rat, monkey), eosinophil counts (rat), basophil counts (rat), large unstained cells (rat), and an increase in neutrophils (rat) and monocytes (rat). and signs of anemia (decreases in red blood cell count (rat, monkey), hemoglobin (rat, monkey), and hematocrit (rat, monkey) with regeneration (increase in reticulocytes in monkeys). There were associated changes in lymphoid organs that included atrophy of major lymph nodes, thymus and spleen, and reductions in bone marrow cellularity (reduction in the myeloid:erythroid ratio in monkeys). Most hematological effects were reversible or partly reversible within the 1 month recovery period, but reversibility of anatomic effects were often incompletely assessed. For rats, doses ≥ 10 mg/kg/day, the lower dose generally required a longer treatment period to reach similar effects in the high dose group. Analysis of lymphocyte subset populations by cell surface markers found dose-dependent decreases in all the evaluated lymphocytes subpopulations, helper T cells, cytotoxic T cells and a small subset of NK cells, total T cells, total B cells, and NK cells. However in the 9-month study in monkeys the reduction in total white cells did not always follow a dose-response relationship. By week 38, the low dose lymphocyte population was reduced to 73-74% of controls, the high dose 57-60% of control, with the mid dose intermediate and more variable (63-79% of control). It cannot be determined if these small differences are of clinical importance.

Clinical chemistry findings in the 6 month study in rat included increased total protein, mainly due to an increase in albumin and this was reflected in an increase in A/G ratio. Calcium levels were also increased in association with albumin concentrations. The observed changes in clinical chemistry parameters were likely related to dehydration, possibly do to excessive salivation from drug administration. The applicant's reported changes in glucose, triglycerides, and alkaline phosphatase were within normal variation and not toxicologically significant.

In both species, either lymphoid depletion or atrophy in lymphoid tissues was prominent in the lymph nodes (inguinal, ileo-femoral and mesenteric, gut associated lymphoid tissue), spleen, and thymus. Notably in the monkey, lymphoid hyperplasia involving either lymph nodes, spleen, or gut associated lymphoid tissue was also present in almost all treated animals, particularly all high dose animals. The findings of lymphoid depletion or atrophy was associated with reduced organ weights and macroscopic observations of small size. In monkeys, there was an increase in mononuclear cell infiltration in a number of organs that exceeded control levels. In the rat studies, but not monkeys, pulmonary histiocytosis with interstitial inflammation occurred at the high

dose. In both species, there was a dose-related increase in liver weight (when expressed as % body weight) corresponding with hepatocellular hypertrophy in males and females.

In the rat, systemic exposure was approximately 2-3.5-times greater in females than males at doses of 1 and 10 mg/kg/day, but was similar in females and males at 100 mg/kg/day. There was no sex difference in the monkey. There was no systemic accumulation of drug over the course of the studies. The lack of histopathology evaluation of all animals and lack of recovery animals from observed effects, particularly anatomic pathology, hindered the identification of a NOAEL.

Juvenile Animal Studies

Studies were conducted in the rat and monkey. The rat studies consisted of a dose-range finding study and a 1-month repeated oral dosing study in which the emphasis was on hematological and immunological effects, rather than a complete toxicological evaluation. The monkey study was a GLP 39-week repeated oral dosing study with 6-month recovery, which was a complete toxicological assessment and included additional immunotoxicological assessments. These studies are summarized more completely in the Integrative Summary and Safety Evaluation, Section 11. Another juvenile study was conducted in rats to assess effects on reproduction and fertility which is summarized in the reproductive and developmental toxicology section below.

Juvenile Rat

In the 1-month repeated dose study (Report 01-2063-09) CP-690550 was administered orally once daily to juvenile rats during PND 21-49 at dose levels of 0, 1, 10 and 100 mg/kg/day. A 2-month recovery period followed with animals sacrificed on PND 111. CP-690550 administration produced dose dependent reductions and time dependent reductions in hematological parameters similar to the adult studies although not as severe (reduction in red blood cell counts and reticulocytes). White blood cell count was reduced in a dose-dependent manner by CP-690550 administration which was attributed to dose-dependent reductions in lymphocytes, eosinophils, and basophils. These effects were generally reversible by the end of the recovery period. CP-690550 decreased the mean percentage of lymphocyte subpopulations which were sometimes dose- or time-dependent with further reduction on day 30 (PND 50) compared to day 15 (PND 35). and included reductions in total T cells, cytotoxic T cells, natural killer cells, and NKT cells. There were no effects on B lymphocytes.

Thymus and spleen weights were reduced in a dose dependent manner due to decreased cellularity from histopathology. Decreased cellularity were also noted in the lymph nodes examined (mesenteric lymph node, inguino-femoral lymph node and mandibular lymph node), and these reductions were reversible by the end of the 2-month recovery period.

The effects on hematology and lymphoid organ weights were similar to those observed in adult rats (see Reports 01-2063-06 and 02-2063-20). Since similar doses were

administered in the adult and juvenile studies and generally similar magnitude of effects were observed, the juvenile and adult rat appear to have similar sensitivity to CP-690550. The LOAEL for this juvenile rat study is 100 mg/kg/day, based on the findings of reversibility of CP-90550-related effects and lack of clinical signs of adverse effects or mortality. Since the study focused on hematological aspects of CP-690550 and lacked analysis of serum chemistry, urinalysis, complete anatomical pathology analysis, toxicokinetic, this study would not support clinical studies in pediatric populations.

Juvenile Cynomolgus Monkey

Juvenile cynomolgus monkeys, 13-14 months of age, were administered CP-690,570 twice daily at a total daily dose of 0, 0.5, 2 and 10 mg/kg/day for 39 weeks (Report 09GR248). A 6-month recovery period followed the dosing phase for all doses except for the lowest dose 0.5 mg/kg/day group. There were no effects of CP-690550 on mortality, body weights or weight gain, clinical chemistry, coagulation parameters, bone growth, and cardiovascular and ECG parameters. The mid and high doses resulted in reduced thymus (50% of control) and spleen (66% of control) weights, with full recovery in females and partial recovery in males. There were no histopathological correlates to these changes. Compared to effects in the control group, CP-690550 treatments resulted in an increased incidence in inflammatory cell foci of the heart at the high dose, an increase in ulcers of the tail at the mid and high doses, and in increase in lymphoid hyperplasia at the mid and high doses. A full histopathology assessment was not conducted due to the lack of findings in previous toxicology studies of adult monkeys at these doses. In light of the fact that some of the males in the adult study were actually sexually immature, and that in both and rat monkey similar target organs and effects were found, the lack of a full histopathological assessment was appropriate for the submitted study. It is also important to note the proposed label was for treatment of adults, not a pediatric use, and they did not mention pediatric development of CP-690550 with regards to the juvenile animal studies.

There were CP-690550 dose-related changes in both red blood cell and white blood cell parameters. Red blood cell, hemoglobin and hematocrit were 84-86% of control values at the high dose. Total white blood cells were not altered, but total lymphocytes were reduced. Immunophenotyping indicated that most lymphocyte subsets were reduced (NK cells 9% of predose levels; CD4+ T cells 52%, CD8+ T cells 49%, CD4+ naïve T cells 42%, CD8+ naïve T cells 32%, memory central CD8+ T cells 48% and memory effector CD8+ T cells 69%). There was full or partial recovery of lymphocyte subsets over the 6 month recovery phase with females demonstrating full recovery or more complete recover than males. Bone marrow analysis found no changes in cellular morphology or evidence of substantial erythroid or myeloid cellular lineage, although at the high dose there was a reduction in M:E ratio (77% of control value) due to an increase in erythroid precursors. Additional studies to address immunotoxicity included a T-cell dependent antibody response study using keyhole limpet hemocyanin (KLH). CP-690550 reduced the anti-KLH IgM response and completely inhibited of the anti-KLH IgG response at the high dose. The response to KLH was restored when tested during the recovery phase in previously untested monkeys. The response to a second KLH challenge was evaluated in vitro from peripheral blood mononuclear cells (PBMC)

collected at necropsy. There was no effect of CP-690550 to suppress the PBMC proliferative response or the response to a mitogen, concanavalin A. The NOAEL was 0.5 mg/kg/day, corresponding to AUC₀₋₂₄ of 62 ng-h/mL at week 36 (2 x AUC₀₋₁₂ of 31.1 ng-h/mL), which is approximately 0.11-fold of the systemic exposure as the maximally recommended human dose of 10 mg bid.

Genetic Toxicology

The genetic toxicology of CP-690550 was assessed in a bacterial reverse mutation (Ames) assay, an *in vitro* chromosome aberration assay with human lymphocytes, and an *in vivo* rat micronucleus assay. CP-690550 caused a statistically significant increase in chromosome aberrations in cultured human lymphocytes (Report 01-2063-10) in the 3-hour test with metabolic activation, but not in the absence of the addition of induced liver enzymes. The dose at which the positive response occurred, $\geq 1700 \mu\text{g/mL}$, corresponded to 48% mitotic suppression. The results suggest a metabolite may have clastogenic activity. Additional follow-up assays were also conducted, *in vitro* CHO/HGPRT assay (Report 01-2063-16) to assess for mammalian gene mutations and an *in vivo* rat hepatocyte unscheduled DNA synthesis assay (Report 01-2063-17), which were both negative. Since the reason for these two follow-up assays was to confirm the clastogenic finding in the chromosomal aberration assay with added metabolic enzymes, neither assay was an optimal follow-up, being assays for mutagenic, rather than clastogenic signals.

Overall, the evidence indicated no mutagenic or clastogenic activity of CP-690550 and absence of an increase in liver tumors in the rat and mouse carcinogenicity studies support the conclusion that the positive chromosomal aberration finding in the presence of metabolic enzymes was likely due to a more generalized drug toxicity resulting in false positive rather than a specific carcinogenic mechanism.

Carcinogenicity

Lymphomas were observed in the 9-month general toxicology study in cynomolgus monkeys (Report 2003-0301). They occurred in 3 of 8 adult monkeys dosed orally with CP-690550 at 5 mg/kg BID (10 mg/kg/day) for 39 weeks. They were not present at the lower dose corresponding to an exposure margin of 1X for the maximal recommended human dose of 10 mg BID. In a 9-month study in juvenile monkeys (Report 09GR248), 13-14 month of age at the start of dosing, dosed with the regimen and dose levels as in the adult study, no lymphomas or lymphoid hyperplasia was evident. The lymphomas in the adult monkey study were associated with lymphocryptovirus and thought to occur due to CP-690550 mediated immune suppression allowing for viral reactivation. The juvenile monkeys were selected for positive exposure to lymphocryptovirus, but did not develop lymphomas. It's possible the juvenile animals due to the relatively shorter interval from colony vaccinations had a "more robust" immunologic defence despite the presence of immunosuppressive doses of CP-690550. These lymphoma findings in the non-human primate support the human clinical trial occurrences of lymphoproliferative disease (refer to the Clinical Review of Dr. Nikolov) that they were due to CP-690550,

likely through immunosuppression providing a window for reemergence of opportunistic infectious agents.

The carcinogenic potential of CP-690550 was assessed in a 2-year duration carcinogenicity study in rats (Report 07GR439) and in a 6-month duration study in rasH2 transgenic mouse (Report 08GR481). In the rat study, the findings were sex specific and included benign Leydig cell tumors, benign thymomas in females and hibernomas (malignancy of brown adipose tissue). The lowest AUC exposure at which malignancy occurred was in males due to sex differences in systemic exposure. The absence of malignancies occurred at the 10 mg/kg/kg dose, and based on male rat AUC values corresponded to an exposure margin of 7-fold the maximal recommended human dose of 10 mg BID. The systemic CP-690550 exposure in comparison to human exposure at the MRHD are based on values obtained at 26 weeks in the 2 year rat study and were reasonable similar to values in the 6-month rat general toxicology study (Report 77435).

CP-690550 was not carcinogenic in rasH2 transgenic mice with exposures at 32-times the maximal recommended human dose of 10 mg BID the highest administered dose. While appropriate for the mechanism of carcinogenicity the assay was developed to detect, it may be insufficient to determine carcinogenicity due to immunosuppression, the demonstrated mechanism of action of CP-690550. It's also notable that in the 2-year (lifetime) rat carcinogenicity study, lymphomas did not develop.

Overall, results from both the genetic toxicology and carcinogenic studies indicate a low risk of direct drug-induced carcinogenicity for patients, due to the large exposure margins relative to the therapeutic dose. However, this cannot be applied for immunosuppression-associated malignancies, since they occurred in the monkey toxicology study and in clinical trials.

Reproductive and Developmental Toxicology

Fertility in female rats was reduced with CP-690550 administration (Report 05GR051). An increase in postimplantation loss occurred with at exposure 14-times the maximal recommended human dose of 10 mg BID and a reduction in pregnancy rate due to reduced numbers of corpora lutea, implantation sites, early resorptions and pre- and post-implantation loss occurred at an exposure 125-times MRHD. The NOAEL for postimplantation loss was 1 mg/kg/day producing an exposure margin of 1.3-times the maximal recommended human dose of 10 mg BID.

The fertility assessment for males was atypical (Report 05GR051). In this study, males were administered CP-690550 for at least 63 days, but matings occurred after 1 month of treatment, with no effect on pregnancy assessment in the untreated female partners. The duration of treatment at the time of mating was insufficient for drug exposure for a complete spermatogenic cycle. However after 63 days of treatment, there was no effect on sperm motility or concentrations, but sperm morphology was not assessed.

However, the fertility assessment for males occurred after 1 month of treatment, an insufficient duration of drug exposure for a complete spermatogenic cycle and therefore does not meet regulatory requirements for safety of male fertility. Therefore, at least the study in adult males should be repeated and conducted in accordance with ICH5(R2), refer to Note 12 in Section 4.1.1. In studies with cynomolgus monkeys (Report 2003-0301) there were too few animals that were sexually mature to assess effects on mature testis and epididymis, and although spermatogenic staging was not evaluated, there were no obvious histopathological changes in sexual organs in males attributed to CP-690550 treatment.

In juvenile rats, a fertility study (Report 05GR051) was similarly inappropriately designed and conducted. The same doses of CP-690550 administered as in the study with sexually mature animals, were administered to females from days 21 to 55 of age and to males from days 21 to 70 days of age, i.e. up to expected puberty and through expected puberty, respectively. There was no effect in females on indicators of sexual development and subsequent estrous cyclicity and for males, there was no effect on mating, sperm motility or sperm concentrations. However, the mating assessment for males and females occurred weeks after CP-690550 treatment had ended, allowing for recovery of any potentially adverse effects on fertility. Therefore, this study does not meet regulatory requirements for safety of either male or female fertility evidenced by mating studies. The aspects of the study characterizing estrous cyclicity and spermatozoa are acceptable. Since this study was optional, although useful to provide safety for the treatment of a pediatric population, the mating aspects of this study should be repeated and conducted in accordance with ICH5(R2), refer to Note 12 in Section 4.1.1.

Embryo-fetal development studies were conducted in rats and rabbits with CP-690550 doses administered during the period of organogenesis. In rats orally administered 30, 100, or 300 mg/kg/day, maternal toxicity was observed at doses ≥ 100 mg/kg/day and was associated with postimplantation loss, consisting of early and late resorptions and consequently a reduced number of viable fetuses, and decreased uterine weight. Teratogenic effects were observed at 100 mg/kg/day consisting of anasarca and membranous ventricular septal defect and numerous skeletal malformations. The NOAEL for maternal and developmental toxicity in this study was 30 mg/kg/day, corresponding to an exposure margin of 53-times MRHD based on a systemic exposure AUC comparison. In rabbits, doses of ≥ 30 mg/kg/day produced no evidence of maternal toxicity, but resulted in numerous teratologic findings including thoracogastroschisis, omphalocele, microstomia, microphthalmia, membranous ventricular septal defects, absent gallbladder, and multiple skeletal malformations associated with a minimal exposure margin of 14-times MRHD. The NOAEL margin for the rabbit was 2.7-times MRHD.

A postnatal study in rats (Report 08GR095) found no effect on pup delivery, standard landmarks of development, growth, sexual, or behavioral development at doses up to 50 mg/kg/day administered to dams from late gestation through lactation (up to 21 days after delivery resulting in a NOAEL of 76-times the maximal recommended human dose

of 10 mg BID. In a study with lactating rats, a single oral dose of CP-690550 increased CP-690550 in secreted milk approximately 2-fold greater than serum concentrations, indicating that CP-690550 can be concentrated in actively secreting mammary glands.

Embryo-fetal development studies rats (Report 09GR353) and rabbits (Report 05-2063-25) indicate that there is a risk for early embryo loss, and, if pregnancy is maintained, a risk for life-threatening embryo-fetal malformations. The rabbit was the more sensitive species to tofacitinib since the NOAEL dose corresponded to an exposure margin of only 2.7-fold greater than the MRHD. Since this exposure difference is relatively small and the sensitivity of human fetal development for tofacitinib is unknown, it was recommended to avoid use of tofacitinib during pregnancy.

Special Toxicology Studies

CP-690550 was not a skin sensitizer in the local lymph node assay (Report 07GR202) a measure of its ability to induce hypersensitivity reactions. CP-690550 lacked evidence of phototoxic potential when tested in the *in vitro* neutral red uptake assay (Report 07AM087). In an *in vivo* assessment of phototoxicity (Report 10GR350) there was insufficient intensity of simulated solar radiation to adequately evaluate for the wavelengths of CP-690550 absorption at the low end of the UVB spectrum (around 290 nm). These wavelengths weakly penetrate through skin layers and are unlikely to penetrate into the dividing basal cell layer. The lack of any signal for serious adverse skin effects such as melanoma in the safety database (email consultation with Dr Nikolov) provided sufficient evidence that CP-690550 is not phototoxic.

1.3 Recommendations

1.3.1 Approvability Yes

1.3.2 Additional Non Clinical Recommendations

- 1) Conduct a properly designed and analyzed fertility assessment in adult male rats as recommended in ICH5(R2).
- 2) Conduct a properly designed and analyzed fertility assessment in juvenile male and female rats as recommended in ICH5(R2).

Reasons for the above recommendations

- 1) The fertility assessment for males was inappropriately designed. In that study, males were administered tofacitinib for at least 63 days, but matings occurred after 1 month of treatment, an insufficient duration of drug exposure for a complete spermatogenic cycle. Therefore, at least the study in adult males should be repeated and conducted in accordance with ICH5(R2), refer to Note 12 in Section 4.1.1.
- 2) The study in juvenile rats was inappropriately designed. In this study both males and females were administered CP-690550 from weaning on day 21 until near or at puberty in the female, and in the male through expected puberty for an appropriate duration of exposures in both sexes. However, they were not mated until a few weeks after drug dosing stopped, allowing for recovery of any potentially adverse effects on fertility. The study in juvenile animals was not required, but if done correctly, could be incorporated into labeling to provide safety for treatment of pediatric patients with regards to reproductive maturation and fertility. These differ from classic segment 3 postnatal studies in that in the segment 3 studies the juvenile animals are not dosed with drug, only the dams are dosed, and the juvenile are followed to see if there are generational effects of the drug. The study in juvenile rats incorporates drug administration directly to the juvenile animals. This is an optional study, but if conducted, should also be designed and evaluated as recommended in ICH5(R2)

1.3.3 Labeling

Section 8

Applicant's proposed Label

7 Pages Of Draft Labeling Have Been Withheld As b4 (CCI/TS) Immediately Following This Page

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number (Optional)

CAS 540737-29-9

2.1.2 Generic Name

Tofacitinib

2.1.3 Code Name

CP-690550-10 (the citrate salt form; in circulation and in PK/TK assessments, the free form "CP-690550" is present)

(b) (4) an early code name, used in the early nonclinical ADME studies

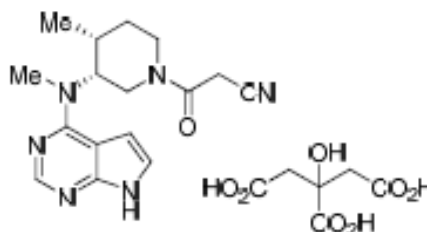
2.1.4 Chemical Name

IUPAC: 3-((3*R*,4*R*)-4-methyl-3-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino)piperidin-1-yl)-3-oxopropanenitrile, 2-hydroxypropane-1,2,3-tricarboxylic acid

2.1.5 Molecular Formula/Molecular Weight

C₁₆H₂₀N₆O • C₆H₈O₇ (citrate salt) / 504.49 Daltons (citrate salt)

2.1.6 Structure



CP-690550-10 has 2 chiral centers at C₃ and C₄, giving 4 possible stereoisomers. The absolute configuration at the 3-position is the *R* configuration. The absolute configuration at the 4-position is the *R* configuration. There is only one crystalline form of CP-690550-10 designated as Form A. No other polymorphs have been observed during development.

2.1.7 Pharmacologic class

kinase inhibitor (of the Janus Kinase family of kinases), immunosuppressant

2.2 Relevant INDs, NDAs, BLAs and DMFs

INDs currently or previously under development by the applicant:

IND	Indication
(b) (4)	(b) (4)
70,903	Treatment of Rheumatoid Arthritis
(b) (4)	

[REDACTED] (b) (4)

2.3 Drug Formulation

A commercial dosage formulation for CP-690550-10 was developed as an immediate release film-coated tablet to deliver 5 mg and 10 mg.

Table 1: Composition of CP-690550-10 Tablets, 5 and 10 mg

(from Applicant Table 2.3.P.1-1)

Component	Function	Reference to Standard	Theoretical Unit and/or Formula	
			5 mg tablet	10 mg tablet
CP-690,550-10	Active	Pfizer	(b) (4)	(b) (4)
Microcrystalline Cellulose ²	(b) (4)	NF, Ph. Eur., JP	(b) (4)	(b) (4)
Lactose Monohydrate		NF, Ph. Eur., JP		
Croscarmellose Sodium		NF, Ph. Eur., JP		
Magnesium Stearate		NF, Ph. Eur., JP		
Total Finished Tablet ³			206.00 mg	

Note: NF = National Formulary; USP = United States Pharmacopeia; Ph. Eur. = European Pharmacopeia; JP = Japanese Pharmacopeia

(b) (4)

2.4 Comments on Novel Excipients

There are no novel excipients.

2.5 Comments on Impurities/Degradants of Concern

The impurities are toxicologically qualified based on findings described in previous reviews (DARRTS: March 9 2012 and April 16, 2012) for product quality consults..

2.6 Proposed Clinical Population and Dosing Regimen

The proposed label indicates tofacitinib is to be used for the treatment of adult patients with moderately to severely active rheumatoid arthritis who have had an inadequate response to one or more disease-modifying anti-rheumatic drugs (DMARDs).

The proposed dosing regimen is a starting dose of 5 mg BID and that some patients may benefit from an increase to 10 mg BID. Tofacitinib may be used as monotherapy or in combination with methotrexate or other nonbiologic DMARDs.

2.7 Regulatory Background

The tofacitinib development program for rheumatoid arthritis was developed under IND 70903 which was submitted Oct 13, 2004 and received Oct 14 2004.

End of Phase 2A questions were submitted by the applicant on Oct 16 2006 to address future Phase 2B and Phase 3 clinical trials, and although no meeting was held, responses were sent to the applicant on Jan 12, 2007. An End of Phase 2 meeting package was submitted Oct 15 2008 (SD-155) for a Dec 16, 2008 meeting with one question concerning the nonclinical support for the Phase 3 program and NDA. It was noted in the meeting minutes (DARRTS Jan 12 2009) that the current studies support the Phase 3 program for the NDA, the rat embryo-fetal study did not use a sufficiently high dose and would need to be repeated. Except for this, the nonclinical information would support an NDA.

However, upon review of the NDA, the male fertility assessment was determined to be inadequate based on the study design. It is unclear why this was missed in earlier reviews, but the wording in the study report placed the emphasis on the end of the treatment period results. Mating which should have occurred at the end of the treatment period, occurred in the middle of the treatment period, providing an insufficient duration of drug exposure in males. This study will be submitted for inclusion as a postmarketing requirement should the drug be approved.

A Pre-NDA briefing document was submitted Jan 3 2011 for the meeting on Feb 16 2011. There was just one question concerning the adequacy and completeness of the nonclinical program. The need for the previously mentioned embryo-fetal study in a second species was reiterated, but otherwise the program appeared adequate. In the NDA submission, this repeat study incorporating higher dose levels was submitted.

With submission of the NDA, consults were submitted (Nov 28 2011 and Dec 12 2011) for toxicological assessments of drug product and drug substance impurity specifications. The chemistry consult was completed and comments were provided to the Review team on March 9 2012 and April 16 2012.

Special Protocol Assessments for the rat and mouse carcinogenicity studies were reviewed and feedback provided after discussion with the Executive Carcinogenicity Advisory Committee (ECAC) for the rat study on May 2, 2007 and the mouse study on Nov 7, 2008 (both reviewed under IND (b) (4)). During the NDA review cycle, a meeting with the ECAC was held on March 6 2012, to discuss the results of the carcinogenicity studies and the conclusions were sent to the applicant on March 8 2012.

The Advisory Committee Meeting was held on May 9, 2012. The findings of lymphomas in chronic monkey toxicology studies provided coincided with the clinical occurrence of lymphoproliferative disease noted in clinical trials.

3 Studies Submitted

3.1 Studies Reviewed

Table 2: List of Submitted Studies

Study Number	Type of Study
Pharmacology	
Mechanism of Action	
083053	Activity of CP-690550 in Kinase Selectivity Panel Assays
D08AI0333	Potency Determination for CP-690550 Inhibition of JAK1, 2, 3 and TyK2 Kinase Activity
D08AI0334	Characterization of Mechanism of Inhibition of JAK1, 2, 3 and TyK2 Kinases by CP-690550
D08AI0337	Comparison of CP-690550 on JAK3 and JAK2 signaling in human peripheral blood mononuclear cells and human whole blood
D08AI0338	In vitro activity of CP-690550: Effects on cytokine-dependent STAT phosphorylation within lymphocyte subsets in human whole blood
113015	In vitro activity of CP-690550: Effects on cytokine-dependent STAT phosphorylation with leukocyte populations in human whole blood
142305	Effects of CP-690550 on IL-23 and IL-21 Dependent STAT3 Phosphorylation in the KIT225 T Cell Line
7570532	<i>In Vitro</i> Pharmacology: Pfizer Tier 0 Profile - Study of CP-690550-10 -
7571347	<i>In Vitro</i> Pharmacology and ADME-Tox: Pfizer Tier 1 Profile - Study of CP-690550-10 -
Animal Model Studies, proof of concept	
Rat Adjuvant Induced Arthritis	
100214	Evaluation of Arthritis, Peripheral Blood Neutrophil Counts, Plasma Cholesterol, and Plasma Cytokines in the Rat Adjuvant-Induced Arthritis (AIA) Model with Oral CP-690550 Administration Initiated Prior to Disease Development
102743	Evaluation of Arthritis, Peripheral Blood Neutrophil Counts, Plasma Cholesterol, and Cytokines in the Rat Adjuvant-Induced Arthritis (AIA) Model with Oral CP-690550 Administration Following Arthritis Development
112613	CP-690550: Transcriptional Profiling in the Rat Adjuvant Induced Model of Rheumatoid Arthritis
120754	Evaluation of Pfizer Compound CP-690550 on In Vivo Reverse Cholesterol Transport (RCT) Using Stable Isotopes in AIA Rats with Chronic Inflammation

141740	Effect of CP-690550 on Inflammatory End Points in Rat Adjuvant-Induced Arthritis Model
155854	Characterization of CP-690550 Effects on Lipid Regulation in Rat Adjuvant-Induced Arthritis Model also Amendment 1
160531	Therapeutic Efficacy, Assessed by Histopathology and Immunohistopathology, of CP-690550 in a Rat Adjuvant-Induced Arthritis (AIA) Model
Mouse Collagen-Induced Arthritis Model	
135046	CP-690550: Transcriptional Profiling in the Mouse Collagen-Induced Arthritis Model of Rheumatoid Arthritis
141423	Characterization of inflammatory end points after a single dose of CP-690550 in mouse collagen-induced arthritis
150736	Preclinical Pharmacokinetic-Pharmacodynamic Relationships of CP-690550, a JAK Inhibitor, in the Mouse Collagen-Induced Arthritis Model; Relationship to Human Rheumatoid Arthritis
151443	Effect of Oral Administration of CP-690550 Dosed Prophylactically in Mouse Collagen-Induced Arthritis Model
153609	In Vivo Potency and Selectivity of CP-690550 – Effect on STAT Phosphorylation within Lymphocyte Subsets in mouse whole blood
160243	Therapeutic Efficacy, Assessed by Histopathology and Immunohistopathology of CP-690550 in a Mouse Collagen-Induced Arthritis Model
160531	Therapeutic Efficacy, Assessed by Histopathology and Immunohistopathology, of CP-690550 in a Rat Adjuvant-Induced Arthritis (AIA) Model
165255	Characterization of Mechanism of Action with CP-690550 in Mouse Collagen-Induced Arthritis Model
Other	
NP-02-005	Examination of the Effects of CP-690, 550 on EPO-Induced Increases in Reticulocytes in Cynomolgus Monkeys
Safety Pharmacology	
General, Neurobehavioral, Cardiovascular, Kidney, Gastrointestinal and Binding Pharmacology	
general pharmacology - evaluation Zhou, 2001	General Pharmacology Evaluation of CP-6909550
Cardiovascular	
48879-104	Effect of CP-690550-10 on HERG-Encoded Potassium Current in Stably Transfected HEK-293 Cells
11GR018	Effect of CP-690550-10 on Cloned hERG Potassium

	Channels Expressed in Human Embryonic Kidney Cells
CP690550-10/CG/001/00	Effects of CP-690550-10 on action potentials recorded from dog isolated Purkinje fibers in vitro
11GR001	Safety Pharmacology – Cardiovascular assessment of CP-690550 in telemeterized conscious female rats
AA2761	Safety Pharmacology – Blood Pressure, Heart Rate, and Cardiac Rhythm Effects of CP-690550-15 In Cynomolgus Monkeys

Pharmacokinetics	
Analytical Methodology	
PDMK2007-036VL; PDMK2007-036VLAM1 PDMK2007-036VLAM1	Method Validation Report for the Determination of CP-690550 in Mouse Serum Using LC-API/MS/MS Amendment 1
DM2005-690550-056	Method Validation Report for the Determination of CP-690550 in Rat Serum Using LC-API/MS/MS
DM2004-690550-054	The measurement of CP-690550 in rabbit serum.
AR690B AR690B.A1 AR690B.A2 AR690B.A3	Method Validation Report for the Determination of CP-690550 in Cynomolgus Monkey Serum Using LC-API/MS/MS Amendment 1 Amendment 2 Amendment 3
DM2004-690550-043	Assay Characterization for CP-690550 Using HPLC with MS/MS Detection in Monkey Plasma with Solid Phase Extraction
Absorption	
DM2004-690550-048	Pharmacokinetics of CP-690550 in Sprague-Dawley Rats Following Intravenous and Oral Administration
DM2005-690550-062	Pharmacokinetics of Total Radioactivity and Unchanged Drug in Plasma of Sprague-Dawley Rats After Oral Administration of [¹⁴ C]CP-690550-10
DM2001-690550-015	Pharmacokinetics Of CP-690550 In Sprague-Dawley Rats Following Intravenous And Oral Administration
DM2001-690550-014	Pharmacokinetics Of CP-690550 In Beagle Dogs Following Intravenous And Oral Administration
DM2001-690550-013	Pharmacokinetics Of CP-690550 In Monkeys Following Intravenous And Oral Administration
Distribution	
DM2004-690550-041	Tissue Distribution of CP-690550 (Pyrrolo[2,3-d]pyrimidine) in Long-Evans Male Rats
055956	Blood to plasma concentration ratio of CP-690550 in

	rat, monkey and human whole blood
DM2001-690550-018	Plasma Protein Binding of CP-690550 in Mouse, Rat, Dog, Monkey and Human
DM2002-690550-025	Protein Binding of CP-690550 in Human Serum Albumin and α 1-Acid Glycoprotein
Metabolism	
In Vivo Metabolism/Excretion	
140653	Radiolabelled Mass Balance, and Metabolic Profiles of [14 C]CP-690550 in CByB6F1-Tg(HRAS)2Jic(homozygous wild type) Mice
DM2005-690550-055	Radiolabelled Mass Balance and Metabolic Profiles of [14 C]CP-690550 (ring labeled) in Sprague-Dawley Rats
DM2005-690550-064	Identification of Urinary and Circulating Metabolites of CP-690550 in Female New Zealand White Rabbits after Oral Administration of [14 C]CP-690550-10
DM2004-690550-052	Radiolabelled Mass Balance and Metabolic Profiles of [14 C]CP-690550 (ring labeled) in Cynomolgus Monkeys
DM2004-0690550-049	Metabolic Profile and Routes of Excretion of [14 C]CP-690550 in Healthy Male Subjects
In Vitro Metabolism	
DM2004-690550-046	Identification of <i>In Vitro</i> Metabolites of CP-690550 in Human Liver Microsomes and Recombinant Cytochrome P-450 isoforms
DM2007-690550-067	Identification of Human Cytochrome P450 Isoforms Responsible for In Vitro Metabolism of CP-690550
Excretion	
103847	Lacteal Excretion of CP-690550 Following Administration of a Single Oral Dose to Rats
095019	Proposed mass balance model for CP-690550 in human
DM2001-690550-020	Effect of CP-690550 on Human Drug Metabolizing Enzymes <i>In Vitro</i>
DM2007- (b) (4) -001	An investigation of the potential for (b) (4) to induce CYP3A4 and CYP1A2 in human hepatocytes
094719	Drug-drug interaction assessment for CP-690550 using SIMCYP® modeling
095440	In vitro inhibition of OATP 1B3 by CP-690550
XT088024/ (b) (4)	In vitro studies in MDCK MDR1 cells to identify compound (b) (4) as substrate for p-glycoprotein
060532	The in vitro study of p-glycoprotein inhibition by (b) (4) (CP-690550) in Caco-2 cells
175813	CP-690550: BCRP substrate evaluation
135323	In Vitro Renal Transport Inhibition by CP-690550

192119	In vitro inhibition of OATP 1B1 by CP-690550
General Toxicology	
Single Administration Toxicology	
Rat	
01-2063-07	CP-690550-10 Acute Oral Toxicity Study in Sprague-Dawley Rats
09GR453	Single Dose Intravenous Toxicity Study of CP-690550 in Rats with a 14-Day Recovery
Monkey	
00-2063-04	CP-690550-10 Single Day Oral Toxicity Study in Cynomolgus Monkeys
Repeated Dose Toxicology	
Rat	
00-2063-03	Two Week Exploratory oral Toxicity Study with CP-690550-10 in Sprague-Dawley Rats
01-2063-06	CP-690550-10 Six Week Oral Toxicity Study with One Month Recovery in Sprague-Dawley Rats
02-2063-20	A 6-Month Oral Toxicity Study of CP-690550-10 in Rats
Juvenile Rat	
09GR249	Oral Dose Range-Finding Study of CP-690550 in Juvenile Rats
10GR307	1-Month Oral Toxicity Study of Tasocitinib (CP-690550) in Juvenile Rats with a 2-Month Recovery
Monkey	
00-2063-05	Two Week Oral Exploratory Toxicity Study in Cynomolgus Monkeys with CP-690550-10
01-2063-09	CP-690550-10: One Month Oral Toxicity Study with a One Month Recovery Period in Cynomolgus Monkeys
2003-0301	CP-690550-10: 39-Week Oral Toxicity Study in the Monkey
Juvenile Monkey	
09GR248 Final report submitted to SD-9, Feb 16 2012	Tofacitinib (CP-690550) 39-week oral (gavage) administration toxicity study in the juvenile cynomolgus monkey with a 26-week recovery phase
Genetic Toxicology	
01-2063-11	CP-690550-10, Microbial Reverse Mutation Assays
01-2063-16	Mammalian mutation assays (forward mutation assay, HGPRT locus)
01-2063-10	Genetic Toxicology Report, CP-690550: In Vitro Cytogenic Assays
01-2063-12	In Vivo Rodent Micronucleus, Oral Route in Rats: CP-690550

01-2063-17	CP-690550 In Vivo/In Vitro Unscheduled DNA Synthesis in Rat Primary Hepatocyte Cultures at 2 Timepoints
Carcinogenicity	
RAT	
07GR439	2-Year Oral Gavage Carcinogenicity and Toxicokinetic Study with CP-690550 in Rats
Follow-up Investigational Studies	
11GR016	Investigative Study with Rat Primary Leydig Cells
11GR015	Final Report Amendment 1 An Investigative Study with Rat Brown Adipocytes Treated with Ovine Prolactin and CP-690550
10GR431	14-Day Oral Investigative Study of the Effects of CP-690550 on Brown Adipose Tissue in Female Sprague-Dawley Rats
11GR383 Submitted to SD-9 Feb 16 2012	14-Day Oral Investigative Study of CP-690550 on Plasma Norepinephrine in Female Sprague Dawley Rats
MOUSE	
Dose-Finding Studies	
07KL023	7-Day oral escalating Dose Toxicokinetic Study of CP-690550 in rasH2 Wild-Type Mice
07GR160	4-Week Dose Range-finding Oral Gavage Toxicity and Toxicokinetic Study with CP-690, 550 in Model 001178-T (hemizygous CB6F1/Jic-TgrasH2@Tac) Mice
Carcinogenicity	
08GR481	6-Month Oral Gavage Carcinogenicity Study with CP-690550 in Model 001178-T (hemizygous), CB6F1/Jic-TgrasH2@Tac Mice and Model 001178-W(homozygous wild type), CB6F1/Jic-TgrasH2@Tac Mice for Toxicokinetic Exposures
Reproduction and Developmental Toxicology	
Fertility and Early Embryonic Development	
05GR051	Oral fertility and embryonic development study of CP-690550-10 in male and female rats
Embryonic Fetal Development	
Rabbit	
04-2063-23	Oral dose range-finding study of CP-690550 in pregnant New Zealand white rabbit
05-2063-25	Oral embryo-fetal development study of CP-690550-10 in rabbits
Rat	
04-2063-22	Oral dose range-finding study of CP-690550 in pregnant rats

05-2063-24	Oral embryo-fetal development study of CP-690550-10 in rats
09GR353	Oral embryo-fetal development study of CP-690550-10 in rats
Prenatal and Postnatal Development	
08GR095	Oral (Gavage) Developmental and Perinatal/Postnatal Reproduction Toxicity Study of CP-690550-10 in Rats, Including a Postnatal Behavioral/Functional Evaluation
09GR250	Fertility Study of Tofacitinib (CP-690550) in Juvenile Rats
Special Toxicology	
07GR202	Murine local lymph node assay with CP-690550
09GR482	1-Day in vitro blood compatibility study of CP-690550 with human blood
07GR202	Murine local lymph node assay with CP-690550
07AM087	Determination of the phototoxic potential of CP-690550-10 in the 3T3 neutral red uptake (NRU) phototoxicity assay.
B65935	Primary Skin Irritation Study in Rabbits (4 Hour Semi-Occlusive Application)
B65946	Primary Eye Irritation Study in Rabbits
10GR350	Phototoxicity Study to Determine the Effects of Seven Days of Oral (Gavage) Administration of CP-690550 on Eyes and Skin in Pigmented Rats
CP-690550-PDM-006	Ocular 4-Day Ocular Exposure, Rabbits

Studies Not Reviewed

Regulatory decisions concerning the transgenic mouse carcinogenicity protocol were based on previous reviews of the following studies submitted to IND (b) (4) therefore they were not reviewed here.

07KL023	7-Day oral escalating Dose Toxicokinetic Study of CP-690550 in rasH2 Wild-Type Mice
07GR160	4-Week Dose Range-finding Oral Gavage Toxicity and Toxicokinetic Study with CP-690, 550 in Model 001178-T (hemizygous CB6F1/Jic-TgrasH2@Tac) Mice

The following publications were referenced in the NDA. They were consulted but not formally reviewed.

Reference Publications

Brees DJ, et al. (2008). Pharmacological Effects of Nicotine on Norepinephrine Metabolism in Rat Brown Adipose Tissue: Relevance to Nicotinic Therapies for Smoking Cessation. *Toxicologic Pathology*, 36: 568-575.

Chan E and Swaminathan R (1990). Role of prolactin in lactation-induced changes in brown adipose tissue *Am J Physiol* 258:R51-6.

Cook JC, et. al., (1999). Rodent Leydig cell tumorigenesis: a review of the physiology, pathology, mechanisms, and relevance to humans. *Crit Rev Toxicol.* 29(2):169-261.

Koch S, et. al., (2011). Signal transduction by vascular endothelial growth factor receptors. *Biochem J.* 437:169-183.

Morton D. et. al., (2002). The Tg rasH2 Mouse in Cancer Hazard Identification. *Toxicologic Pathology* 30(1):139-146.

Takaoka M, et. al., (2003). Interlaboratory Comparison of Short-Term Carcinogenicity Studies Using CB6F1-rasH2 Transgenic Mice. *Toxicologic Pathology* 31(2):191–199.

Viengchareun S, et. al., (2008). Prolactin Receptor Signaling Is Essential for Perinatal Brown Adipocyte Function: A Role for Insulin-like Growth Factor-2. *PloS One* 2(e1535):1-13.

3.3 Previous Reviews Referenced

Pharmtox reviews exist for the following INDs:

IND	(b) (4)
IND	(b) (4)
IND	(b) (4)

4 Pharmacology

4.1 Primary Pharmacology

The pharmacological aspects of CP-690550 are reviewed by Dr. Pei which is appended in Section 12 of this review.

CP-690550 is an inhibitor of the Janus associated kinase (JAK) family, with inhibitory actions more specific for JAK1, JAK2 and JAK3 kinase activity than for TyK2. Based on accumulating knowledge of the cellular locations and actions of JAKs, CP-690550 is able to influence myeloid and erythroid cellular development and function by interrupting the signaling pathway from cytokine receptor to STAT (signal transducers and activators of transcription) through its inhibition of JAK activity intracellularly associated with cytokine receptors. Thus tofacitinib inhibition of JAK activity decreased the cellular response to cytokine signaling (IL-2, -4, -6, -10, -15, and -21, (IC₅₀ potencies ≤500 nM for the cellular forms, i.e., JAK homo or hetero-dimers) resulting in the reduction of the synthesis and secretion of additional cytokines and other inflammatory mediators. The applicant demonstrated that CP-690550 inhibition of JAK activity decreased the cellular response to cytokine signaling (IL-2, -4, -6, -10, -15, and -21) which resulted in the reduction of the synthesis and secretion of additional cytokines and other inflammatory mediators.

In two animal models of arthritis, collagen-induced arthritis model in male DBA/1J mice and adjuvant (*Mycobacterium byturium*)-induced arthritis model in female Lewis rats, tofacitinib demonstrated efficacy by attenuating paw arthritis scores (swelling, edema and paw volumes). Based on studies of the collagen-induced arthritis in mice the applicant demonstrated that suppression of the inflammatory response did not require continuous exposure to CP-690550 for effectiveness.

The specific mechanism for preventing rheumatic changes in bone and cartilage were not addressed in detail, other than to associate CP-690550 inhibition of numerous interleukin compounds' signaling with the reduction in synthesis and secretion of various inflammatory compounds.

The two animal models used to demonstrate CP-690550 efficacy in inflammatory swelling and edema, rat adjuvant-induced arthritis (Report 160531) and mouse collagen-induced arthritis (Report 160243) were investigated for effects of CP-690550 on histological effects of cartilage damage, bone resorption, inflammation, pannus and CD68 and CD3 cellular infiltrates (rat) or F4/80 and CD3 cellular infiltrates (mice). To supplement the pharmacology review conducted by Dr Pei, these studies will be described in more detail below. At this time, the clinical radiographic evidence for prevention of disease progression is inadequate to address this claimed benefit (Refer to the Clinical Review by Dr. Nikolov). Nonclinical toxicology studies also do not contribute substantially to understanding the proposed mechanistic effects of CP-690550 on cartilage or bone, other than to support CP-690550 safety toward these

tissues as no detrimental effects or pathology was found in association with bone and cartilage from healthy animals, or in joints from aged rats (carcinogenicity Report 07GR439). It should be noted that without special stains, only fairly gross observation of cartilage dimensions and general structure are evaluated in these studies, and special stains were not used in the toxicology studies to examine type of cartilage or matrix, as there was no initial findings to cause further investigations.

There was some concern raised that tofacitinib would not be effective on cartilage injury. The two animal models studies were suppose to provide support for the histological effectiveness of CP-690550 to prevent further injury and possibly demonstrate reduction in severity of injury (i.e. beginnings of tissue repair). However both of these studies were flawed with regards to interpretation of the effects on cartilage. At the time of the initial CP-690550 treatment (actually 4 hours after the start of treatment), approximately half the animals in the first timepoint group had no evidence of cartilage damage. Since there were few animals at each treatment timepoint of this factorial design (n=5 to 8), one cannot distinguish an effect of CP-690550, or no effect of CP-690550, from an animal that may have never had cartilage injury. The applicant's analysis does not account for this. The conclusion reached is that at least for cartilage effects, there is no data yet that demonstrates CP-690550 has any effect nor can one conclude CP-690550 has no effect. More appropriately designed studies are necessary to address CP-690550' role on cartilage.

Study Title: Therapeutic Efficacy, Assessed by Histopathology and Immunohistopathology, of CP-690550 in a Rat Adjuvant-Induced Arthritis (AIA) Model

Study no.:	160531
Study report location:	4.2.1.1
Conducting laboratory and location:	Pfizer Global Research and Development
Date of study initiation:	unknown, report signed March 22, 2010
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	CP-690550-10 (citrate salt of CP-690550), Lot GR00905, Purity

Key Findings:

The applicant reports in the rat adjuvant (mycobacterium butyrium)--induced arthritis model there was reduction in both inflammation and osteoclast-mediated bone resorption at 7 days into daily CP-690550 therapy without changes in joint cartilage. However, the data in this study indicated that adjuvant induced arthritis did not cause cartilage damage of ankle at the time of the initiation of CP-690550 treatments, 16 days from the injection of adjuvant, in 8 of 14 (57%) of the animals and there was no cartilage damage in the digits in 100% of the animals. Since it was a factorial study design and each animal was not followed in time through the treatments, its not possible to determine for later times if the animals were part of the 57% that never developed

cartilage damage of the ankle or were part of the 43% that did develop damage. Therefore, it is not possible to attribute any cartilage score to the effect of CP-690550 treatment. These cartilage results differ from the inflammation score in which all animals, except 1 had inflammation (score of >0). Ankle bone resorption scores were also problematic similar to that for cartilage. For this type of study, a much greater number of animals would be needed to establish an statistically significant effect (this study was improperly analyzed by simple t statistics). Alternatively, a more robust model of bone and cartilage injury should be used.

Methods

Arthritis was induced in young female rats with three 50 uL injections of 15 mg/mL suspension of heat-killed Mycobacterium butyrium in squalene oil into the base of the tail. On day 15, post immunization, rats were weighed, paw volume measured and rats were randomized into groups (n=7-8/gr) so paw volume was equalized across all treatment groups.

Treatments of either vehicle or CP-690550 (6.2 mg/kg oral once daily) from day 16 through day 22. Subsets of rats were sacrificed 4 hours post dose on day 16, 20, 23 and 24 hours post dose on day 17. The left rear paws were processed for histopathological analysis and stained with hematoxylin and eosin for general evaluation or toluidine blue for specific assessment of cartilage changes. Immunohistochemical stains were used to identify CD3+ T cells, and ED-1/CD68 macrophages. Paws were evaluated via histopathology and scored for inflammation and osteoclast-mediated bone resorption, cartilage damage, and CD3 and CD68 cellular infiltrates.

Results

Only results pertaining to cartilage injury and bone resorption are presented below to illustrate the deficiencies with applicants conclusions.

Table 3: Summary of Rat AIA Study (Incidence and Tissue Injury Score for Cartilage Damage and Bone Resorption, Report 160531)

	No Damage (score =0)		All Scores	
	Vehicle	CP-690550	Vehicle	CP-690550
Ankle Cartilage Damage				
first measurement 16, +4 h = Baseline	3 of 7 (43%)	5 of 7 (71%)	scores: 0, 0, 0, 5, 4, 4, 1	scores: 0, 0, 0, 0, 0, 4, 4
	<i>Reviewer Comment:</i> 16 days after induction of arthritis, a large number of animals had no histological evidence of cartilage damage. The scoring was defined for inflammation, bone resorption, and cellular infiltration (assess by CD68 and CD3 immunohistochemistry), but not cartilage histopathology.			
d 17, (1 day of treatment)	3 of 5 (60%)	3 of 5 (60%)	scores: 0, 0, 0, 3, 1, 1	scores: 0, 0, 0, 3, 4
d 20, (4 days of treatment)	4 of 7 (57%)	5 of 7 (71%)	scores: 0, 0, 0, 0, 4, 4, 4	scores: 0, 0, 0, 0, 0, 3, 1

d 23 (7 days of treatment)	1 of 4 (25%)	2 of 5 (40%)	scores: 0, 4, 1, 3	scores: 0, 0 1, 1, 1
	<i>Reviewer Comment:</i> The t statistical analysis ignores the variability from the experimental design. Animals that may never had any signs of cartilage injury are confounded with those that might have had injury reduced by CP-690550. It also appears that cartilage injury may be a slower process in this model.			
Digits, cartilage damage				
first measurement 16, +4 h = Baseline	7 of 7 (100%)	7 of 7 (100%)	0 of 7	0 of 7
d 17, (1 day of treatment)	5 of 5 (100%)	5 of 5 (100%)	0 of 5	0 of 5
d 20, (4 days of treatment)	7 of 7 (100%)	7 of 7 (100%)	0 of 7	0 of 7
d 23 (7 days of treatment)	4 of 4	4 of 4*	0 of 4	0 of 4
* one animal was not examined, no reason provided				
Bone resorption				
first measurement 16, +4 h = Baseline	1 of 7	2 of 7	6 of 7 scores 0, 5, 4, 3, 3, 4, 3	5 of 7 scores 0, 0 4, 4, 5, 5, 2
d 17, (1 day of treatment)	1 of 5	1 of 5	4 of 5 scores 0, 3, 3, 2, 2	4 of 5 scores 0, 4, 3, 4, 3
d 20, (4 days of treatment)	2 of 7	3 of 7	5 of 7 scores 0, 0, 4, 5, 5, 1, 4	4 of 7 scores 0, 0, 0, 1, 4, 4, 2
d 23 (7 days of treatment)	0 of 4	4 of 5	4 of 4 scores 5, 3, 4, 3	1 of 5 score 0, 0, 0, 0, 1
	<i>Reviewer Comment:</i> For bone resorption, while there is the same confounding issue described above, the slightly greater incidence of damage and less incidence of 0 scores in the first and subsequent measurements, provides a greater degree of confidence that the result is real and not random, although again the analysis by t-statistics is inappropriate for this design.			
Scoring for Bone Resorption 0 = normal 1 = minimal (small areas of resorption in the medullary trabecular or cortical bone, not readily apparent on low magnification, and rare osteoclasts) 2 = mild (increasing areas of resorption in medullary trabecular or cortical bone, not readily apparent on low magnification, with osteoclasts more numerous) 3 = moderate (obvious resorption of the medullary trabecular and cortical bone, without full-thickness defects, lesion apparent on low magnification, and osteoclasts more numerous) 4 = marked (full-thickness defects in the cortical bone, marked loss of medullary trabecular bone, numerous osteoclasts)				

5 = severe (full-thickness defects in the cortical bone, severe loss of medullary trabecular bone)

Scoring for Cartilage Injury

Criteria were not provided, but they are likely to be the same as described in a similar study in the collagen-induced arthritis model in mice (Report 160243)

0 = normal

1 = loss of toluidine blue staining with no chondrocyte degeneration/loss and/or matrix disruption

2 = loss of toluidine blue staining with minimal chondrocyte degeneration/loss and/or mild matrix disruption in some affected joints

3 = loss of toluidine blue staining with moderate chondrocyte loss and obvious (depth to deep zone) matrix loss in affected joints,

4 = loss of toluidine blue staining with marked (depth to tide mark) chondrocyte and matrix loss

5 = loss of toluidine blue staining with severe (depth to subchondral bone) chondrocyte loss and matrix loss in affected joints

At best, there may be an effect of CP-690550, but this study was not sufficiently robust to conclude that CP-690550 had either no effect or a positive effect toward reducing cartilage injury.

Study Title: Therapeutic Efficacy, Assessed by Histopathology and Immunohistopathology of CP-690550 in a Mouse Collagen-Induced Arthritis Model

Study no.:	160243
Study report location:	4.2.1.1
Conducting laboratory and location:	Pfizer Global Research and Development
Date of study initiation:	unknown, report signed March 22, 2010
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	CP-690550-10 (citrate salt of CP-690550), Lot GR00905, Purity

Key Findings:

In the mouse, collagen-induced arthritis model, the therapeutic efficacy of CP-690,550 in CIA mice was evaluated via histopathology and immunohistochemistry. The analysis indicates that the effect of CP-690550 on cartilage is confounded with animals that had no cartilage pathology, and therefore no conclusions can be drawn concerning CP-690550 effects on cartilage. It cannot be distinguished if an animal had cartilage damage at the time of CP-690550 treatment and this is reduced by CP-690550 or if the animals never had cartilage damage.

Methods

Arthritis was induced in 8-12 week-old male DBA/1J mice by injection of 50 µg of chick type-II collagen (CII) in complete Freund's adjuvant at the base of the tail on day 0. The mice were boosted on day 21 by injection of 50 µg of chick CII in incomplete Freund's adjuvant at the base of the tail. Naïve or normal mice, untreated, were included to provide baseline information. On day 45, mice were evaluated for signs of arthritis by scoring the severity of each paw using a score of 1 - 3 for each paw (score 1 = redness or swelling of the digits of the paw, 2 = gross swelling of the whole paw or deformity, and 3 = ankylosis of the joints; maximum score of 12/mouse). Subsequently, mice were randomly grouped to equalized severity across all groups prior to treatment. CP-690,550-10 (prepared as aqueous suspension in the vehicle consisting of 5% methylcellulose/ 0.025% Tween-80) was administered at 50 mg/kg BID (100 mg/kg total per day) from day 48 and through day 55. Control animals received vehicle twice daily. Subsets of mice were sacrificed 4 and 12 hours post dose on day 48 and 4 hours post dose on days 49 (24 hrs), and 55 (168 hrs) and paws were proceeded for histopathological analysis of joints. Toluidine blue staining was incorporated for specific assessment of cartilage changes. Also F4/80 and CD3 immunohistochemistry was used to evaluate phenotypes of cellular infiltrates. Tissue was qualitatively scored based on published criteria for inflammation, cartilage damage, pannus and bone resorption.

Results

The information presented below is only for cartilage assessments of the wrists and ankles in the vehicle and CP-690550-treated mice.

Table 4: Summary of Rat CIA Study (Incidence and Tissue Injury Score for Cartilage Damage, Report 160243)

	Not Remarkable (score = X)		All scores	
	Vehicle	CP-690550	Vehicle	CP-690550
Right Wrist Cartilage Damage				
first measurement day 48, +4 h = Baseline	3 of 8 (38%)	2 of 8 (25%)	5 of 8 (62%) scores 4,3,4,2,4	6 of 8 (75%) scores 1,4,5,3,2,3
	<i>Reviewer Comment:</i> 48 days after induction of arthritis, a large number of animals had no histological evidence of cartilage damage.			
day 48, +12 h (day 1 of treatment)	2 of 8 (25%)	1 of 8 (12%)	scores: 0, 0, 3, 1, 5, 3, 3, 3	scores: 0, 4, 5, 4, 4, 4, 2, 2
day 49, +4 h, (day 2 of treatment)	4 of 8 (50%)	4 of 8 (50%)	scores: 0, 0, 0, 0, 3, 5, 5, 4,	scores 0, 0, 0, 0, 4, 5, 5, 3
day 55, +4 h (= 168 h, day 8 of treatment)	2 of 8 (25%)	4 of 8 (50%)	scores: 0, 0, 5, 4, 3, 5, 5, 3	scores 0, 0, 0, 0, 5, 2, 5, 5
Left Wrist Cartilage Damage				
first measurement day 48, +4 h =	2 of 8 (25%)	5 of 8 (62%)	scores: 0, 0, 3, 1, 4, 4, 3, 2	scores: 0, 0, 0, 0, 0, 2, 3, 5

Baseline				
day 48, +12 h (day 1 of treatment)	2 of 8 (25%)	3 of 8 (38%)	scores: 0, 0, 3, 4, 5, 5, 4, 3	scores: 0, 0, 0, 2, 4, 4, 2, 4
day 49, +4 h, (day 2 of treatment)	4 of 8 (50%)	3 of 8 (38%)	scores: 0, 0, 0, 0, 3, 3, 2, 4	scores: 0, 0, 0, 5, 5, 2, 4, 3
day 55, +4 h, (168 h, day 8 of treatment)	2 of 8 (25%)	2 of 8 (25%)	scores: 0, 0, 4, 3, 4, 5, 5, 3	scores: 0, 0, 4, 4, 2, 5, 3, 2
Right Ankle Cartilage Damage				
first measurement day 48, +4 h = Baseline	4 of 8 (50%)	6 of 8 (75%)	scores: 0, 0, 0, 0, 3, 4, 3, 3,	scores: 0, 0, 0, 0, 0, 0, 3, 3,
day 48, +12 h (day 1 of treatment)	4 of 8 (50%)	3 of 8 (38%)	scores: 0, 0, 0, 0, 2, 1, 4, 3	scores: 0, 0, 0, 2, 3, 2, 1, 2
day 49, +4 h, (day 2 of treatment)	4 of 8 (50%)	4 of 8 (50%)	scores: 0, 0, 0, 0, 4, 3, 4, 3	scores: 0, 0, 0, 0, 2, 2, 4, 3
day 55, +4 h (day 8 of treatment)	2 of 8 (25%)	5 of 8 (62%)	scores: 0, 0, 3, 5, 2, 2, 4, 2	scores: 0, 0, 0, 0, 0, 2, 4, 2
Left Ankle cartilage damage				
first measurement day 48, +4 h = Baseline	5 of 8 (62%)	3 of 8 (38%)	scores: 0, 0, 0, 0, 0, 1, 4, 4	scores: 0, 0, 0, 3, 4, 3, 2, 3
day 48, +12 h (day 1 of treatment)	4 of 8 (50%)	5 of 8 (62%)	scores: 0, 0, 0, 0, 3, 4, 1, 2	scores: 0, 0, 0, 0, 0, 2, 4, 2
day 49, +4 h, (day 2 of treatment)	3 of 8 (38%)	3 of 8 (38%)	scores: 0, 0, 0, 4, 4, 4, 3, 3	scores: 0, 0, 0, 2, 2, 2, 4, 3
day 55, +4 h (168 h = day 8 of treatment)	3 of 8 (38%)	3 of 8 (38%)	scores: 0, 0, 0, 3, 3, 3, 2, 3	scores: 0, 0, 0, 3, 3, 3, 4, 5
Scoring for Cartilage Injury Criteria were not provided, but they are likely to be the same as described in a similar study in the collagen-induced arthritis model in mice (Report 160243) 0 = normal 1 = loss of toluidine blue staining with no chondrocyte degeneration/loss and/or matrix disruption 2 = loss of toluidine blue staining with minimal chondrocyte degeneration/loss and/or mild matrix disruption in some affected joints 3 = loss of toluidine blue staining with moderate chondrocyte loss and obvious (depth to deep zone) matrix loss in affected joints, 4 = loss of toluidine blue staining with marked (depth to tide mark) chondrocyte and matrix loss 5 = loss of toluidine blue staining with severe (depth to subchondral bone) chondrocyte loss and matrix loss in affected joints				

4.2 Secondary Pharmacology

Secondary pharmacodynamic studies evaluated the binding and potency of CP-690,550 on a broad panel of receptors, ion channels, and enzymes. CP-690550 lacked significant binding and activity to these compounds. It was highly specific to the JAK family and did not affect the activity of other classes of kinases. There were a few compounds with which CP-690550 had significant activity (inhibition of >50%) in these screens. These were the MT₃ (Melatonin 3) receptor (K_i 5.2 µM) and VEGFR1 (vascular endothelial growth factor receptor 1 (K_i 3.7 µM), CaMK2α (calmodulin dependent protein kinase 2α (K_i 12 µM) and Lyn A Kinase (K_i 2.3 µM).

Of note is that VEGFR1 activity is mediated through tyrosine kinase signaling, and is essential to maintain vascular integrity (Koch et al 2011). In the initial early single dose toxicology studies at dose up to 2000 mg/kg, internal hemorrhage was present in the gastrointestinal tract and pulmonary systems and probably cause of death. In the the 6-month study in the rat at substantially lower doses ≤100 mg/kg, there were infrequent and not dose-related incidences of hemorrhage associated with lymphoid organ atrophy (lymph nodes, thymus), as well as liver and lung. These were unexplained findings in the study reports, but could be attributed to weakening the vascular structural integrity, due to cellular depletion, anoxia and organ atrophy resulting from CP-690550 inhibition of VEGFR1 activity.

4.3 Safety Pharmacology

Safety pharmacology studies assessed the potential acute effects of tofacitinib on the cardiovascular (in vitro and in vivo), central nervous system (CNS), respiratory, renal and gastrointestinal systems.

There was a dose-dependent inhibition of hERG current but at doses considered potentially adverse (30 µM, 9372 ng/mL) there was <4% current inhibition and not likely pharmacologically meaningful. There were no effects on electrophysiological characteristics of cardiac Purkinje fibers at doses up to 10 µM (3120 ng/mL). Monkey EKG studies were incorporated into the long term toxicology studies, but for the study in adult monkeys (Report 2003-0301) there was insufficient information provided to adequately analyze the cardiovascular and EKG parameters. In juvenile monkeys (approximately 1-2 years of age Report 09GR248), there no effects on cardiovascular and EKG parameters at doses of 1, 10 and 100 mg/kg/day of CP-690550. However, in both studies mean heart rates were highly elevated (generally between 200 and 250 bpm) in all animals including controls, which was probably due to procedural stress and inadequate habituation, but also limited the accurate assessment of effects on QT interval. The absence of significant effects of tofacitinib on cardiovascular function at therapeutic and supratherapeutic doses was confirmed in the human tQT study (tested up to 100 mg tofacitinib, refer to the Clinical Review by Dr. N. Nikolov).

Neurobehavioral assessments in mice found a reduction in spontaneous activity and signs of general toxicity at doses of ≥100 mg/kg, and there were no effects on promotion or inhibition of seizure activity at doses up to 32 mg/kg, IP. There were no

significant effects on respiratory characteristics, kidney function, or gastrointestinal transit time with a single dose up to 100 mg/kg.

5 Pharmacokinetics/ADME/Toxicokinetics

ABSORPTION

Early studies were conducted in the animal species expected to be treated in the toxicological studies to support the safety of CP-690550. Thus these early ADME studies were conducted in rats and monkeys. It is noteworthy that these studies were also conducted at a time at which methodology for the quantification of CP-690550 in serum, plasma and urine were being developed. Different formulations were being employed to adjust for tolerability issues, different serum and plasma extraction methods and assay methodology were tried. Often this process relied on fewer than optimal animals, and sample collection. There were times in which data variability could be attributed to these factors, but in total, there was general agreement on the overall ADME characteristic trends between the species and studies. The most variable characteristic which was not satisfactory resolved was the bioavailability of CP-690550 in rats. Since in the pivotal toxicology studies, circulating CP-690550 concentrations were determined and exposures calculated, the bioavailability uncertainty in the rat is not a major concern as it would have been if circulating concentrations were unknown.

The pharmacokinetic parameters of these studies are summarized below for intravenous administration and oral administration of CP-690550.

Table 5: Pharmacokinetic Parameters of CP-690550 in Rats, Dogs, and Monkeys Following a Single Intravenous Administration

Species	Gender	Formulation	Dose (mg/kg)	CL (mL/min/kg)	V _{ss} (L/kg)	t _{1/2} (h)	AUC _(0-∞) (ng•h/mL)
Rat	M	70% water, 20% cremaphor, 10% ethanol or glycerol formal	3 or 10 ^a	62 ± 14	2.6 ± 1.3	0.6 ± 0.1	840 ± 184 ^a
	M	25% SBECD	5	29 ± 11	1.6 ± 0.3	2.8 ± 0.8	3200 ± 1170
	F			42 ± 20	1.4 ± 0.2	1.8 ± 1.3	2730 ± 2200
Dog	M + F	60% 0.1 M meglumine, 40% glycerol formal	3	19 ± 10	1.8 ± 0.8	1.2 ± 0.1	3250 ± 1610
Monkey	M	Glycerol formal	3	18 ± 4	1.7 ± 0.2	2.1 ± 0.4	2850 ± 543

SD = Standard deviation; CL = Systemic plasma clearance; V_{ss} = Apparent volume of distribution at steady state; t_{1/2} = Apparent terminal elimination half-life; AUC_(0-∞) = Area under the concentration-time curve from time 0 to infinity; M = Male F = Female; SBECD = Sulfobutylether-β-cyclodextrin.

^a AUC values for rats receiving 10 mg/kg were normalized to 3 mg/kg.

Table 6: Pharmacokinetic Parameters of CP-690,550 in Rats, Rabbits, Dogs, and Monkeys Following a Single Oral Administration

Species	Gender	Dose (mg/kg)	Formulation	C _{max} (ng/mL or ng eq/mL)	T _{max} (h)	AUC _(0-∞) (ng•h/mL or ng eq•h/mL)	t _{1/2} (h)	F (%)
Rat	M	10	0.5% methylcellulose	261 ± 90	0.5 ± 0.3	462 ± 143	ND	16.5 ^a
	F			670 ± 514	0.5 ± 0.4	1138 ± 427	ND	12.3 ^b
	M	30		619 ± 473	0.25 ± 0.0	940 ± 478	ND	11.2 ^a
	M	100	Water	4390 ± 2490	0.9 ± 0.8	12000 ± 6460	ND	42.9 ^a
	M	10		2400 ± 948	0.3 ± 0.1	2770 ± 837	2.0 ± 1.5	43.3 ^b
	F			3670 ± 1430	0.3 ± 0.0	7030 ± 1780	1.5 ± 0.2	129 ^b
	M	10 ^c		796 ± 133	0.5 ± 0.0	1210 ± 303	ND	ND
	F		0.5% methylcellulose	2390 ± 187	0.5 ± 0.0	4690 ± 194	ND	ND
Rabbit	F	30 ^c	0.5% methylcellulose	16800 ± 1750	0.9 ± 0.3	ND	2.4 ± 0.4	ND
Dog	M + F	5	0.5% methylcellulose	1020 ± 255	0.5 ± 0.4	2330 ± 423	ND	43.0
Monkey	M + F	5	0.5% methylcellulose	791 ± 157	1.1 ± 0.9	2280 ± 338	ND	48.0
	M	5 ^c	0.5% methylcellulose	513	1.5	1240	1.4	ND
	F		0.5% methylcellulose	783	1	1820	1.2	ND

SD = Standard deviation; C_{max} = Maximum plasma concentration; T_{max} = Time to reach C_{max}; AUC_(0-∞) = Area under the concentration-time curve from time 0 to infinity; t_{1/2} = Apparent terminal elimination half-life; F = Bioavailability; M = Male; ND = Not determined; F = Female.

^a Oral bioavailability was calculated from male intravenous data at 3 mg/kg.

^b Oral bioavailability was calculated from gender relevant intravenous data at 5 mg/kg.

^c Radiolabeled study using [¹⁴C]CP-690,550 (crystalline citrate salt); data represent parent CP-690,550 pharmacokinetic parameters.

The mean oral bioavailability was low, 17% in males and 12% in females.(Report DM2001-690550-015). The dog was included in the early pharmacokinetic studies to help with allometric scaling to enable predictions of human pharmacokinetics. Of note is that oral administration of the same formulation (in 0.5% methylcellulose) as administered to rats, resulted in an oral bioavailability of 43%, much higher than for the rat. (Report DM2001-690550-014). In the monkey, the bioavailability was approximately 48% (Reports DM2001-690550-013; DM-2004-690550-052). Due to the apparent consistent responses between the dog and monkey for bioavailability the rat was reevaluated (Report DM2001-690550-048). Intravenous administration of 5 mg/kg CP-690550 to male and female rats resulted in clearance 35.6 mL/min/kg, volume of distribution at steady state 1.49 L/kg, and terminal elimination half-life 2.29 hours and no

sex differences in pharmacokinetic parameters. Oral administration of 10 mg/kg did reveal a small sex difference in C_{max} , 1.5-fold, greater in females 3670 ng/mL than males 2400 ng/mL with no difference in T_{max} (0.31 hours). The oral bioavailability of CP-690, 550 in male and female rats was 43.3 and 129%, respectively. This small sex difference in (approximately 2-fold) at this dose was a fairly consistent findings in the toxicology studies, but there was no obvious sex differences in toxicities in association with these levels. While the male bioavailability of 43% coincides with that in the monkey and the dog, differing from the previous value of 17% in the rat, the calculated value for females exceeded that physically feasible (129%) and contrasts with the previous value of 12% bioavailability. There is no obvious explanation for these varied results in the rat, so the bioavailability of oral administration is still unverified and unsettled.

DISTRIBUTION

Study title: Blood to plasma concentration ratio of CP-690550 in rat, monkey, and human whole blood

Study no.:	055956
Study report location:	Mod 4.2.2.3
Conducting laboratory and location:	Pharmacokinetics, Dynamics and Metabolism, Pfizer, Sandwich, UK
Date of study initiation:	Not indicated, report signed Feb 23 2011
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	CP-690550-10, Lot 053265-108-1B, Purity not provided

Key Study Findings

- The mean blood to plasma concentration ratio of CP-690550 at concentration of 1 μ M, (~312 ng/mL of active moiety) was 1.2 for the rat, monkey and human. indicating minimal uptake into blood cells.

Method

Blood to plasma concentration ratio of CP-690550 at a concentration of 1 μ M (equivalent to 312 ng/mL) in rat, monkey and human blood from pooled sex sources. Rat, monkey, and human (n=3/species) fresh whole blood was collected and pooled by species into heparinized tubes on the day of the experiment. A hematocrit between 40-60% was deemed acceptable to proceed with the experiment. CP-690550 was added to aliquots of rat, monkey and human whole blood resulting in a final incubation concentration of 1 μ M. Whole blood samples containing CP-690550 were incubated at 37°C for approximately 3 hours. Aliquots of whole blood from each initial sample were collected for analysis at 60 and 180 minutes (n=3 per time point). Each aliquot was diluted with acetonitrile to lyse the cells. The supernatant was directly analysed by LC-MS/MS. Plasma was also obtained and analysed.

Results

The mean blood to plasma concentration ratio of CP-690550, at a concentration of 1 μM , was 1.2 for all three species, rat, monkey and human.

Table 7: Whole Blood / Plasma Concentration Ratio

Species	CP-690550 Concentration (μM)	Mean Whole Blood/Plasma Concentration Ratio (Cb/Cp)	n
Rat	1	1.2	3
Monkey	1	1.2	3
Human	1	1.2	3

Mean Whole Blood/Plasma Concentration Ratio calculated from unrounded values.

Abbreviations: Cb = Concentration in whole blood; Cp = Concentration in plasma; n = Number of separate determinations on different days.

Study title: Plasma Protein Binding of CP-690550 in Mouse, Rat, Dog, Monkey and Human

Study no.:	DM2001-690550-18
Study report location:	Mod 4.2.2.3
Conducting laboratory and location:	Department of Pharmacokinetics, Dynamics and Metabolism Pfizer Global Research and Development Groton, Connecticut 06340
Date of study initiation:	Not indicated, report signed Oct 1, 2001
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	CP-690550-10 (citrate salt of CP-690550) (Lot 47685-220-1, Purity not provided)

Key Study Findings

- In a study of plasma protein binding, the percentage of CP-690550 bound was independent of its concentration, and the mean percent unbound fraction of CP-690550 (free CP-690550) was 67% for mice, 84.7% for rats, 79.9% for dogs, 64.8 for monkeys, and 61.5% for humans.

Method

Plasma protein binding was determined using plasma prepared from freshly collected blood. For mouse, rat, dog and monkey, the plasma was pooled. For human, the plasma was segregated by individual (n=5). CP-690550 was added to plasma at nominal final concentrations of 156, 1250 and 2500 ng/mL, incubated for ~10 minutes at ~37°C in a shaking waterbath, then ultrafiltered to determine protein binding. Initial spiked plasma samples and ultrafiltrate samples were assayed for CP-690550 by LC/MS/MS. The unbound fraction of drug (f_u) was calculated as $f_u = C_{\text{ultrafiltrate}}/C_{\text{plasma}}$.

Results

The plasma protein binding was independent of CP-690550 concentration for mouse, dog, monkey and human at the concentrations examined. The unbound fraction of CP-

690550 increased in rat plasma with increasing CP-690550 concentration. The amount of nonspecific binding was not indicated.

Table 8: Mean % Unbound Fraction of CP-690550 in mouse, rat, dog, monkey and human.

	mouse	rat	dog	monkey	Human (mean of n=5)
Concentration (ng/mL)					
156	60.7	69.1	81.1	74.8	57.9
1250	69.0	91.3	76.4	56.0	63.2
2500	71.9	93.7	82.3	63.5	63.3
Average	67.2	84.7	79.9	64.8	61.5

Study title: Protein Binding of CP-690550 in Human Serum Albumin and α_1 -Acid Glycoprotein

Study no.: **DM2002-690550-025**
 Study report location: Module 4.2.2.3
 Conducting laboratory and location: Department of Pharmacokinetics,
 Dynamics and Metabolism
 Pfizer Global Research and Development
 Groton, Connecticut 06340
 Date of study initiation: Not provided, report signed 6/3/2002
 GLP compliance: No
 QA statement: No
 Drug, lot #, and % purity: CP-690550-10 (citrate salt form of CP-690550), Lot 43798-2-1H, Purity not provided

Key Study Findings

- CP-690550 bound to human serum albumin (49%, unbound 51%) at slightly lower than in binding studies in human plasma (unbound 61.5%), indicating most the binding in blood is due to binding to albumin.
- CP-690550 did not bind to α_1 -acid glycoprotein.

Methods

Protein binding was determined using freshly prepared human serum albumin (HAS) at 40 mg/mL or α_1 -acid glycoprotein (AAG) at 0.75 mg/mL in Dulbecco's Phosphate Buffered Saline. CP-690550 was added at nominal final concentrations of 156, 1250 and 2500 ng/mL. Samples were incubated for ~10 minutes at ~37°C in a shaking waterbath, then protein binding was determined by the ultrafiltration method. The initialed spiked plasma samples and the ultrafiltrate samples were assayed for CP-690550 by LC/MS/MS. The unbound fraction of drug (fu) was calculated as $f_u = C_{ultrafiltrate}/C_{initial}$.

Results

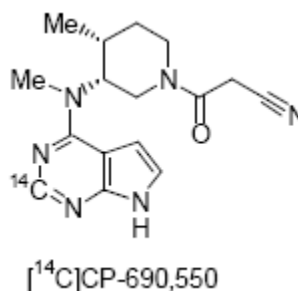
CP-690550 bound to HSA (49%, unbound 51%), but not to AAG. CP-690550 binding to HAS was independent of the initial concentration. The values for binding to HAS were similar to the values obtained in binding studies with human plasma (unbound 61.5%) described in report DM2001-690550-018. The amount of nonspecific binding was not indicated.

Table 9: Mean % Unbound Fraction of CP-690550

	human serum albumin	α_1 -acid glycoprotein
Concentration (ng/mL)		
156	51	119
1250	52	110
2500	49	120
Average	51	116

Study title: Tissue Distribution of CP-690550 (Pyrrolo[2,3-d]pyrimidine) in Long-Evans Male Rats

Study no.: DM2004-690550-041
Study report location: Module 4.2.2.3
Conducting laboratory and location: Department of Pharmacokinetics,
Dynamics and Metabolism
Pfizer Global Research and Development
Pfizer Inc
Groton, Connecticut 06340
Date of study initiation: Not indicated, report signed Feb 6 2004
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: [¹⁴C]CP-690550-10 (citrate salt form of
CP-690550), Lot 56871-167-2, Purity
>99%,
Specific Activity 14.3 mCi/mmol (45.7
mCi/mg).

**Key Study Findings**

- The distribution of [¹⁴C]CP-690550 in blood and 57 tissues of Long-Evans male rats was studied up to 504 hr following oral administration of 10.0 mg/kg (454 µCi/kg).
- Maximum concentrations of [¹⁴C]CP-690550 occurred at 0.5 hr for 43 tissues, 1 hr for 10 tissues and the whole-body, and 12 hr for the uvea.
- [¹⁴C]CP-690550 across the blood-brain barrier by 0.5 hr and persisted in all CNS tissues for at least 4 hr.
- [¹⁴C]CP-690550 distributed into and was eliminated from 47 of 57 tissues by 24 hr following oral administration.
- By 72 hr, only the intervertebral discs, liver, vessel walls, kidneys, and ocular tissues impregnated with melanin contained measurable levels of [¹⁴C]CP-690550. By 168 and 504, only vessel walls and ocular tissues impregnated with melanin still had measurable concentrations of [¹⁴C]CP-690550.

Methods

The distribution of [^{14}C]CP-690550 into tissues of Long-Evans male rats was studied up to 504 hr following oral administration of 10.0 mg/kg (454 $\mu\text{Ci/kg}$) dissolved in 0.5% methylcellulose (0.96 mg/mL or 43.7 $\mu\text{Ci/mL}$). One rat was euthanized by CO_2 asphyxiation at 0.5, 1, 2, 4, 8, 12, 24, 72, 168, and 504 hr postdose. All rats were prepared for whole-body autoradioluminography immediately following euthanasia. The lower limit of quantification (LLOQ) was 1.56 nCi/g or 0.034 $\mu\text{g eq/g}$. Cryosection Quality Control Samples (CQCS) were prepared from rats administered [^{14}C]glucose at four concentrations of 14.5 (0.32 $\mu\text{g eq/g}$), 38.3 (0.84 $\mu\text{g eq/g}$), 113 (2.5 $\mu\text{g eq/g}$), and 225 (4.9 $\mu\text{g eq/g}$) nCi/g, sacrificed at similar times up to 168 hrs postdose. A second set of CQCS was prepared for the single rat euthanized at 504 hr. Drug radioequivalents ($\mu\text{g eq/g}$) were calculated by averaging tissue concentrations measured at different sectioning levels and/or from replicate cryosections obtained from the same sectioning level.

Results

Distribution

Drug radioequivalents distributed into 56 tissues by 0.5 hr following an oral dose of [^{14}C]CP-690550. Tissue concentrations of [^{14}C]CP-690550 were highest at 0.5 hr for 43 tissues, at 1 hr for 10 tissues and the whole-body, and at 12 hr for melanin-containing ocular tissues impregnated with melanin.

By 8 hr, nondetectable levels (LLOQ of 0.034 $\mu\text{g eq/g}$) were present in lymph nodes, subcutaneous adipose, coagulating gland, prostate, and all CNS tissues except for the pituitary. By 12 hours additional tissues with nondetectable radioactivity included gastric mucosa, mesentery adipose, and vitreous body. By 72 hr [^{14}C]CP-690550 radioequivalents were present only in intervertebral discs, liver, vessel walls, kidneys, and melanin-containing ocular tissues. By 168 and 504 hr, only vessel walls and ocular tissues impregnated with melanin had measurable concentrations of [^{14}C]CP-690550 radioequivalents.

The $t_{1/2}$ for tissues in the Long-Evans male rat ranged from 0.8 for pelvic bone marrow to 432 hr for blood vessel walls.

Blood: [^{14}C]CP-690550 was detected in blood up to 12 hr after administration. The C_{max} at 0.5 hr was 1.92 $\mu\text{g eq/g}$, and levels were 0.08 $\mu\text{g eq/g}$ at 12 hr, and below the LLOQ by 24 hours. The mean $\text{AUC}_{0-\text{Tlast}}$ was 4.22 $\mu\text{g eq-hr/g}$. The mean $t_{1/2}$ for blood was 2.5 hr. These are all based on $n=1/\text{timepoint}$.

Brain and pituitary: [^{14}C]CP-690550 was detectable in the CNS by 0.5 hr and persisted in all central nervous system (CNS) tissues for at least 4 hr. The cerebral to systemic blood ratio at C_{max} was 0.05, indicating very little uptake into CNS, the procedure is not sufficiently refined to distinguish between intravascular and neuronal parenchyma, actual penetration past the blood-brain barrier. The pineal gland and pituitary, areas lacking a blood-brain barrier, had concentrations of [^{14}C]CP-690550 radioequivalents that were 18.9 and 22.4 –fold greater than those observed for the cerebrum at 0.5 hr. At

8 and 12 hr, [^{14}C]CP-690550 was present only in the pituitary, but reached levels below the LLOQ by 24 hr.

Eye: The lens of the eye was devoid of [^{14}C]CP-690550 at all sampling times. The uvea appeared to have a disproportional distribution of [^{14}C]CP-690550 into its three substructures (e.g. choroid, ciliary body, and iris). The radioactivity in the ciliary body was 1.6 to 1.9-fold higher than that of choroid and iris, respectively up to 12 hr postdose. The uvea, iris, choroid, and ciliary body had $t_{1/2}$ values of 132, 147, 171, and 193 hr, respectively. The vitreous body contained approximately 33 to 111-fold less [^{14}C]CP-690550 compared to the uvea.

Table 10: [¹⁴C]CP-690550 Radioequivalents in Tissues of Male Long-Evans Rats

Table 2. Mean Concentrations^a of Drug Radioequivalents (µg equivalents/g) In Tissues From Long-Evans Male Rats Administered A Dose (10 mg/kg) of [¹⁴C]CP-690,550 (pyrrolo[2,3-d]pyrimidine).

TISSUE	0.5	1	2	4	8	12	24	72	168	504
Gastric Mucosa	2.77	----- ^b	0.70	0.36	0.15	-----	-----	-----	-----	-----
Intervertebral Disc	4.06	1.50	1.19	0.92	-----	0.55	0.20	0.21	-----	-----
Liver	8.65	6.55	3.14	1.92	0.55	0.51	0.23	0.07	-----	-----
Lung	1.99	1.45	0.64	0.34	0.08	0.11	-----	-----	-----	-----
Lymph Node	-----	1.43	0.59	0.34	-----	-----	-----	-----	-----	-----
Muscle	1.81	1.31	0.54	0.31	0.07	0.06	-----	-----	-----	-----
Myocardium	2.21	1.52	0.69	0.39	0.09	0.10	-----	-----	-----	-----
Skin	2.09	1.36	1.18	0.37	0.08	0.10	-----	-----	-----	-----
Spleen	1.96	1.50	0.68	0.35	0.10	0.09	-----	-----	-----	-----
Vessel Wall	3.32	2.35	0.96	0.58	0.19	0.26	0.10	0.06	0.07	0.04
Whole Body	8.86	9.67	8.48	6.08	6.17	3.67	0.30	-----	-----	-----
<u>ADIPOSE</u>										
Mesentery	0.16	0.17	0.08	0.05	0.04	-----	-----	-----	-----	-----
Multilocular	1.48	1.04	0.37	0.24	0.08	0.10	-----	-----	-----	-----
Subcutaneous	0.31	0.22	0.13	0.05	-----	-----	-----	-----	-----	-----
<u>BLOOD</u>										
Hepatic	2.24	1.39	0.63	0.33	0.07	0.07	-----	-----	-----	-----
Myocardial	1.90	1.30	0.57	0.29	0.07	0.09	-----	-----	-----	-----
Systemic	1.89	1.29	0.57	0.29	0.08	0.08	-----	-----	-----	-----
Testicular	1.62	1.21	-----	0.34	-----	-----	-----	-----	-----	-----
Vena Cava	1.95	1.33	0.55	0.30	0.06	0.10	-----	-----	-----	-----
<u>BONE MARROW</u>										
Femur	1.37	1.08	0.54	0.27	0.07	0.09	-----	-----	-----	-----
Humerus	1.38	1.06	0.42	0.28	-----	-----	-----	-----	-----	-----
Pelvis	1.65	1.04	0.42	-----	-----	-----	-----	-----	-----	-----
Tibia	1.34	1.07	0.45	0.26	-----	0.08	-----	-----	-----	-----
Vertebral	1.07	0.91	0.38	0.25	-----	-----	-----	-----	-----	-----
<u>CENTRAL NERVOUS SYSTEM</u>										
Cerebellum	0.09	0.07	0.06	0.04	-----	-----	-----	-----	-----	-----
Cerebrum	0.10	0.08	0.05	0.04	-----	-----	-----	-----	-----	-----
Medulla Oblongata	0.07	0.07	0.05	0.03	-----	-----	-----	-----	-----	-----
Mesencephalon	0.09	0.10	0.07	0.04	-----	-----	-----	-----	-----	-----
Olfactory Bulb	0.06	0.08	0.06	0.04	-----	-----	-----	-----	-----	-----
Pineal Gland	1.89	1.11	0.50	0.31	-----	-----	-----	-----	-----	-----
Pituitary	2.24	1.51	0.76	0.48	0.77	0.20	-----	-----	-----	-----
Spinal Cord	0.07	0.08	0.05	0.04	-----	-----	-----	-----	-----	-----
Thalamus	0.14	0.12	0.06	0.04	-----	-----	-----	-----	-----	-----

TISSUE	0.5	1	2	4	8	12	24	72	168	504
<u>GLANDS</u>										
Adrenal	3.53	2.83	1.24	0.81	0.14	0.24	0.05	-----	-----	-----
Buccal	2.56	1.53	0.67	0.39	0.09	0.12	-----	-----	-----	-----
Harderian	2.96	2.17	0.97	0.51	0.08	0.13	-----	-----	-----	-----
Lacrimal: Exobital	4.33	2.47	0.85	0.42	0.11	0.10	-----	-----	-----	-----
Lacrimal: Intraorbital	2.75	2.51	1.02	0.53	0.09	0.11	-----	-----	-----	-----
Pancreas	2.16	1.51	0.73	0.41	0.12	0.11	-----	-----	-----	-----
Parotid	2.50	1.77	0.80	0.45	0.11	0.11	-----	-----	-----	-----
Salivary: Sublingual	2.56	1.78	0.75	0.45	0.11	0.12	-----	-----	-----	-----
Salivary: Submaxillary	2.54	1.78	0.73	0.44	0.10	0.11	-----	-----	-----	-----
Thymus	1.85	1.36	0.60	0.31	0.07	0.09	-----	-----	-----	-----
Thyroid	3.20	2.78	0.90	0.65	0.21	0.23	-----	-----	-----	-----
<u>OCULAR</u>										
Choroid	3.70	5.64	6.51	5.02	4.87	7.49	3.28	2.17	0.99	0.41
Ciliary Body	6.06	9.61	10.62	8.96	7.56	11.84	4.48	3.25	2.22	0.68
Iris	3.45	4.02	5.74	3.55	4.51	7.96	3.55	3.56	0.49	0.56
Uvea	4.68	6.03	7.38	5.48	5.76	7.77	5.79	3.05	1.00	0.48
Vitreous Body	0.08	0.18	0.15	0.08	0.05	-----	-----	-----	-----	-----
<u>UROGENITAL</u>										
Coagulating Gland	1.72	1.40	-----	0.29	-----	-----	-----	-----	-----	-----
Epididymis	1.50	1.34	0.72	0.36	0.14	0.06	-----	-----	-----	-----
Kidney	6.37	5.53	3.91	1.26	0.22	0.28	0.09	0.04	-----	-----
Preputial	3.80	3.21	1.86	1.08	0.39	0.32	-----	-----	-----	-----
Prostate	1.02	1.33	-----	0.38	-----	-----	-----	-----	-----	-----
Renal Cortex	7.29	6.49	3.07	1.53	0.27	0.32	-----	-----	-----	-----
Renal Medulla	8.48	8.78	2.39	1.30	0.19	0.33	-----	-----	-----	-----
Renal Pelvis	-----	-----	20.66	5.44	0.31	0.33	-----	-----	-----	-----
Seminal Vesicle	1.43	1.37	0.92	0.54	0.07	-----	0.06	-----	-----	-----
Testis	0.57	0.74	0.52	0.30	0.06	0.05	-----	-----	-----	-----
Urine, Bladder	-----	43.93	-----	26.33	-----	1.98	0.10	-----	-----	-----

*The lower limit of quantitation (LLOQ) was 0.034 µg equivalents/g.

^bTissue drug radioequivalents were not determined because drug radioequivalents decline below the LLOQ, the tissue was indistinguishable from surrounding tissues, or the tissue was not sampled.

Table 11: Toxicokinetic Parameters for [¹⁴C]CP-690550 Tissue Distribution

Table 3. Pharmacokinetics^a of [¹⁴C]CP-690,550 (pyrrolo[2,3-d]pyrimidine) in Long-Evans Male Rats.

Tissue	AUC _(0-T_{last}) (μg eq·hr/g)	t _{1/2} (hr)	C _{max} (μg eq/g)	T _{last} (hr)	T _{max} (hr)
Gastric Mucosa	5.37	2.8	2.77	8	0.5
Intervertebral Disc	26.1	27	4.06	72	0.5
Liver	34.5	22	8.65	72	0.5
Lung	4.57	2.6	1.99	12	0.5
Lymph Node	2.67	1.6	1.43	4	1
Muscle	4.03	2.3	1.81	12	0.5
Myocardium	5.00	2.5	2.21	12	0.5
Skin	5.46	2.4	2.09	12	0.5
Spleen	4.75	2.5	1.96	12	0.5
Vessel Wall	38.7	432	3.32	504	0.5
Whole Body	98.5	3.6	9.67	24	1
<u>ADIPOSE</u>					
Mesentery	0.55	5.4	0.17	8	1
Multilocular	3.30	3.0	1.48	12	0.5
Subcutaneous	0.57	1.4	0.31	4	0.5
<u>BLOOD</u>					
Hepatic	4.51	2.3	2.24	12	0.5
Myocardial	4.12	2.5	1.90	12	0.5
Systemic	4.11	2.5	1.89	12	0.5
Testicular	3.42	1.6	1.62	4	0.5
Vena Cava	4.13	2.5	1.95	12	0.5
<u>BONE MARROW</u>					
Femur	3.54	2.7	1.37	12	0.5
Humerus	2.39	1.5	1.38	4	0.5
Pelvis	1.82	0.8	1.65	2	0.5
Tibia	3.79	4.3	1.34	12	0.5
Vertebral	2.04	1.6	1.07	4	0.5
<u>CENTRAL NERVOUS SYSTEM</u>					
Cerebellum	0.220	3.6	0.09	4	0.5
Cerebrum	0.220	2.7	0.10	4	0.5
Medulla Oblongata	0.210	2.8	0.07	4	1
Mesencephalon	0.260	2.3	0.10	4	1
Olfactory Bulb	0.220	3.2	0.08	4	1
Pineal Gland	2.83	1.4	1.89	4	0.5
Pituitary	8.32	4.4	2.24	12	0.5
Spinal Cord	0.220	2.8	0.08	4	1
Thalamus	0.290	2.0	0.14	4	0.5

Tissue	$AUC_{(0-T_{last})}$ ($\mu\text{g eq}\cdot\text{hr/g}$)	$t_{1/2}$ (hr)	C_{max} ($\mu\text{g eq/g}$)	T_{last} (hr)	T_{max} (hr)
GLANDS					
Adrenal	10.9	4.0	3.53	24	0.5
Buccal	5.21	2.6	2.56	12	0.5
Harderian	6.66	2.3	2.96	12	0.5
Lacrimal, Exorbital	7.19	3.1	4.33	12	0.5
Lacrimal, Intraorbital	6.94	2.3	2.75	12	0.5
Pancreas	5.22	2.6	2.16	12	0.5
Parotid	5.76	2.5	2.50	12	0.5
Salivary, Sublingual	5.74	2.5	2.56	12	0.5
Salivary, Submaxillary	5.65	2.5	2.54	12	0.5
Thymus	4.23	2.5	1.85	12	0.5
Thyroid	8.30	3.0	3.20	12	0.5
OCULAR					
Choroid	646	171	7.49	504	12
Ciliary Body	1142	193	11.8	504	12
Iris	669	147	7.96	504	12
Uvea	809	132	7.77	504	12
Vitreous Body	0.740	3.8	0.18	8	1
UROGENITAL					
Coagulating Gland	3.75	1.4	1.72	4	0.5
Epididymis	4.62	3.2	1.50	12	0.5
Kidney	23.7	23	6.37	72	0.5
Preputial Gland	12.5	3.1	3.80	12	0.5
Prostate	3.41	----- ^b	1.33	4	1
Renal Cortex	19.4	2.3	7.29	12	0.5
Renal Medulla	19.8	2.4	8.78	12	1
Seminal vesicle	5.88	5.0	1.43	24	0.5
Testis	2.86	2.7	0.74	12	1

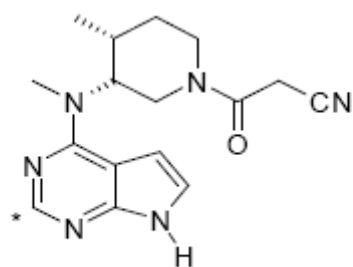
^a $AUC_{(0-T_{last})}$ values were calculated using linear trapezoidal approximation. The $t_{1/2}$ was calculated as $0.693/K_{el}$.

^b The $t_{1/2}$ was not determined (-----) because a definitive elimination phase was not discernible.

METABOLISM

Metabolism studies were conducted in mice (Report 140653), rats (Report DM2005-CP690550-055), rabbits (DM2005-690550-064) and monkeys (Report DM2004-690550-052). These studies used an orally administered radiolabeled [^{14}C]CP-690550. Quantification of the metabolites were carried out by measuring radioactivity in the individual HPLC-separated peaks using a β -RAM operated in the homogeneous liquid scintillation counting mode. The remaining effluent was directed into the flow cell of the β -RAM, providing simultaneous detection of radioactivity and mass spectrometry data.

Figure 1: Structure of [^{14}C]CP-690550



(* = indicates position of radiolabel)

Greater than 87% of the radioactivity administered was recovered contributing to adequacy and acceptability of the studies. In the rat and monkey, the major proportion of circulating radioactivity was parent compound in rat (58.2%-60.5%), monkey (30.8%-48.6%) and human (69.4%). The predominant route of elimination was through urine, with unchanged drug comprising approximately 10% in male rat, 30% in female rat, and approximately 10% in monkey.

The primary metabolic pathways were due to oxidation of the pyrrolopyrimidine ring (M9), oxidation of the piperidine ring (M6 and M18), *N*-demethylation (M1), oxidation of the piperidine ring side chain (M2), and glucuronidation (M20). The other metabolites were due to combinations of these primary metabolic pathways. Metabolites M6, M23 (M6 glucuronide), M26 (hydroxy CP-690,550 metabolite), and M28 (M2 glucuronide) were identified only in monkeys. Metabolites M23, M26 and M28 were the glucuronide conjugates of primary metabolites.

Metabolism and excretion of CP-690,550 was studied in humans (Report DM2004-690550-49 and Clinical Study A3921010) following oral administration of [^{14}C]CP-690,550. Greater than 65% of the circulating radioactivity in humans was due to unchanged CP-690,550. Approximately 94% of the radioactivity was recovered (80% in urine, approximately 30% parent, and 14% in feces, approximately 1% parent). All metabolites were present at <10% of total circulating activity. The metabolites identified in humans were observed in the animal studies, although not all metabolites were detected for each species.

The applicant noted that all metabolites have or are predicted to have ≤ 10 -fold the potency of CP-690550 for Janus kinase 1/3 but there was no data presented to support this statement.

The proposed biotransformation pathways of CP-690,550 for the rat, monkey, and human are presented in the Figure below.

Figure 2: Proposed Biotransformation Pathways for CP-690,550 in Human (H), Monkey (M), and Rat (R) Plasma, Urine, and Feces

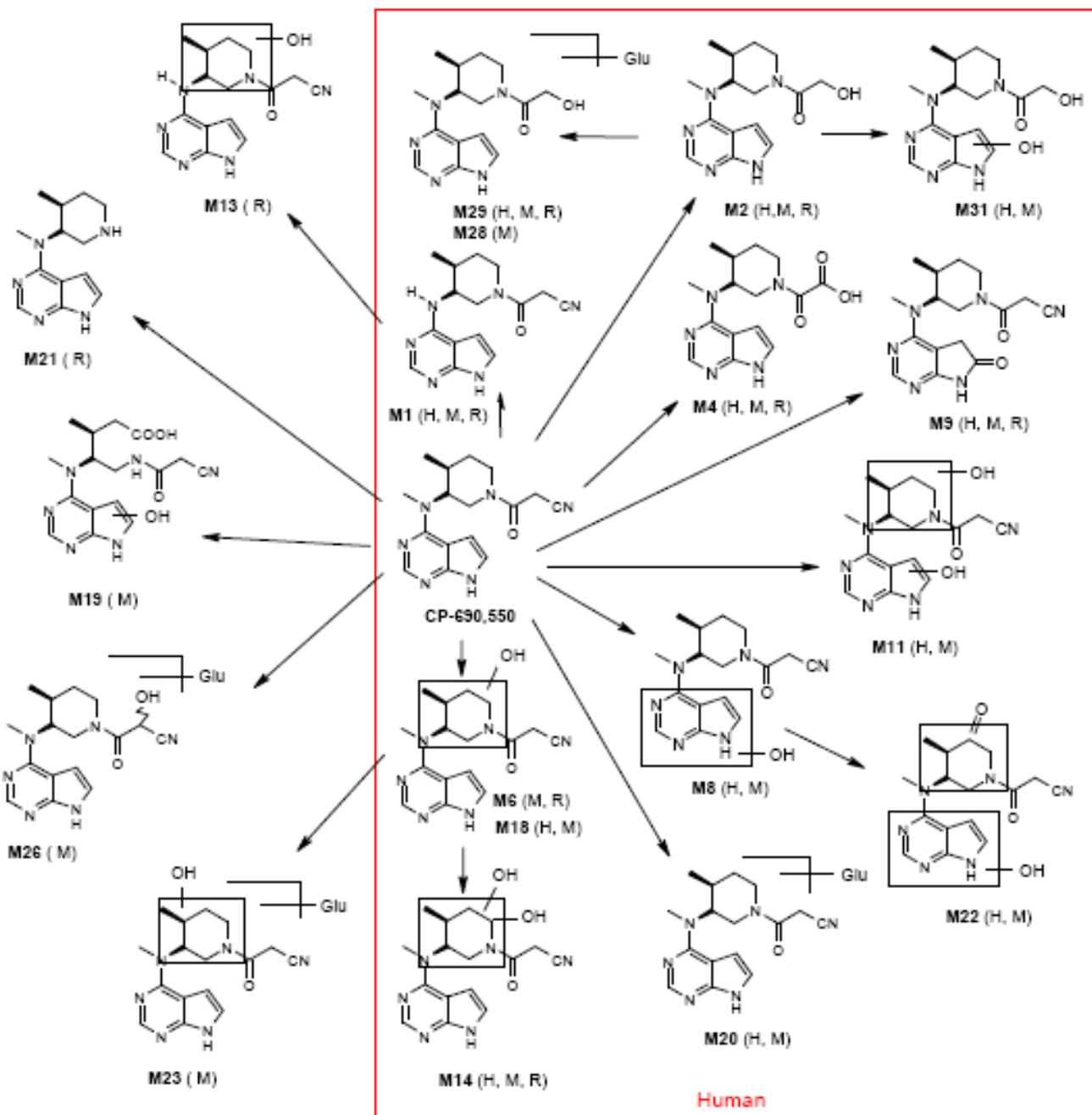
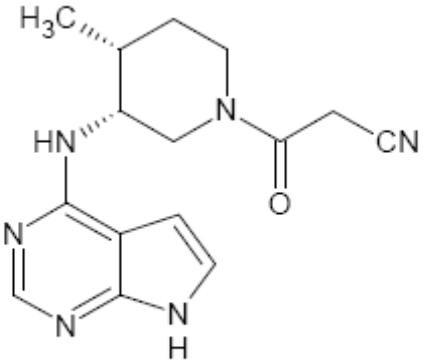
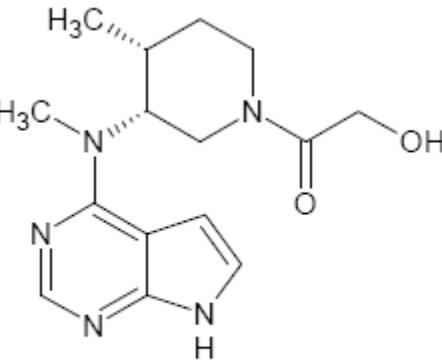
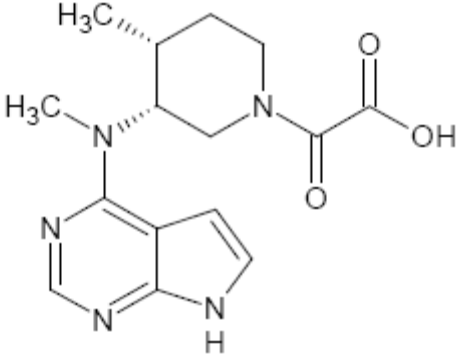
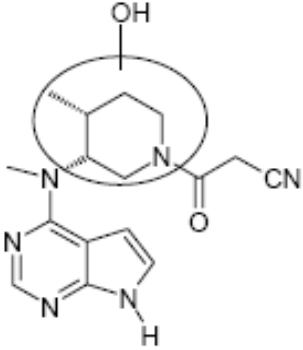
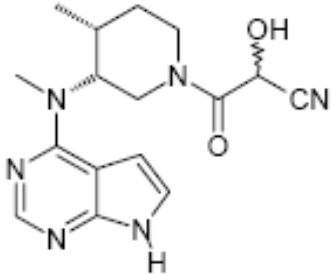
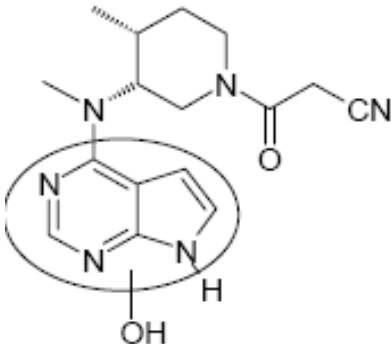
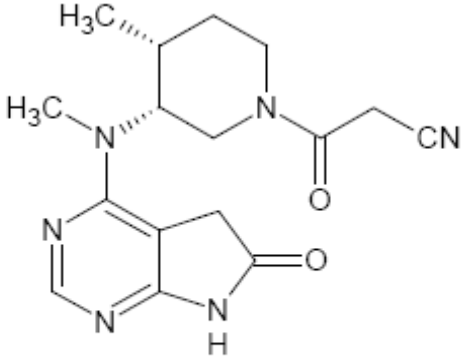
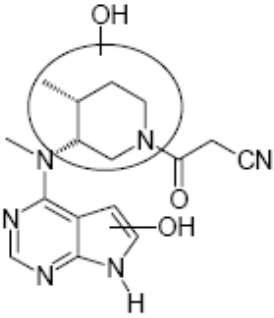
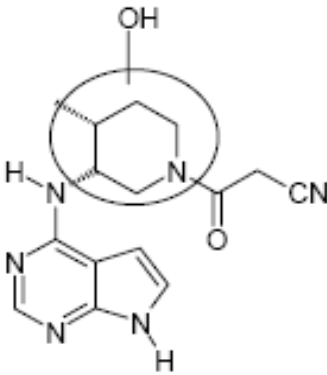
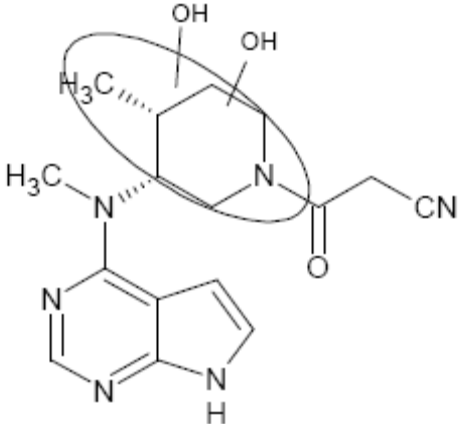


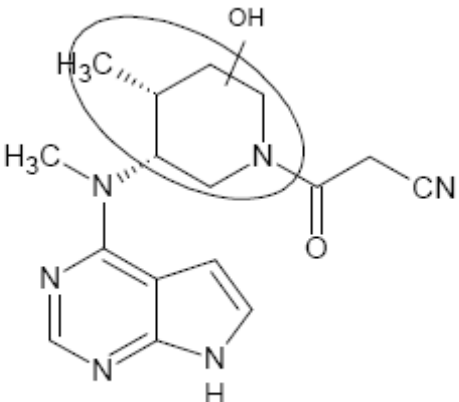
Table 12: Identified Metabolites and Species of Detection

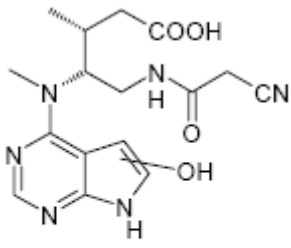
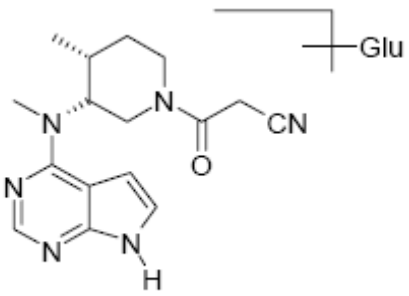
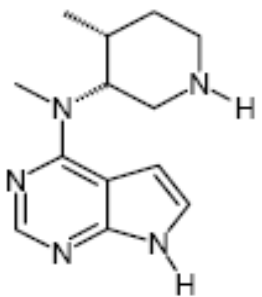
Metabolite Number and Description	Structure
<p>M1: mouse, rat, monkey, human M1 was detected in urine, feces and plasma. In monkey, detected in urine, feces, bile, and plasma. In human detected in urine and plasma. There is loss of methyl group from the nitrogen atom of the pyrrolo[2,3-d]pyrimidine methylamine moiety.</p> <p>M1 was assigned as N-desmethyl-CP-690550.</p>	 <p style="text-align: center;">M1</p>
<p>M2: mouse, rat, rabbit, monkey, human M2 was detected in urine, feces and plasma in rat and mouse; in urine and plasma in rabbit. In monkey and human detected in urine, feces, and plasma. In the rabbit, it could not be resolved radio chromatographically from M26.</p> <p>modification had occurred at the side chain</p> <p>M2 was identified as 2-hydroxy-1-{4-methyl-3-[methyl-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-amino]-piperidin-1-yl}-ethanone.</p>	 <p style="text-align: center;">M2</p>

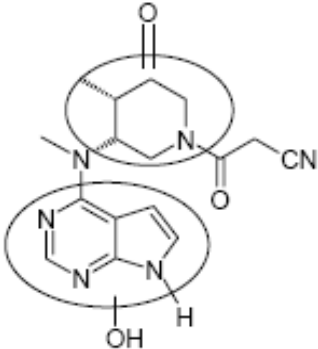
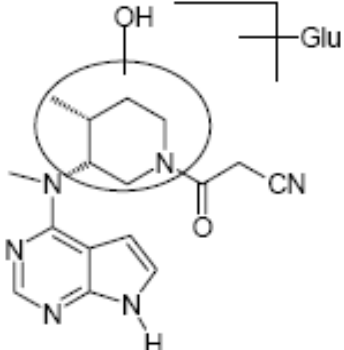
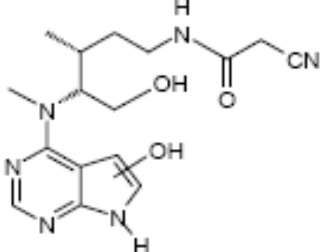
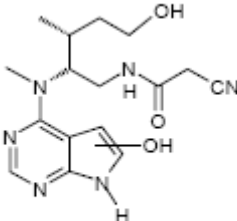
<p>M4: mouse, rat, rabbit, monkey and human Metabolite M4 was detected in urine, feces and plasma. In rabbit and human found in urine and plasma In monkey detected in feces and plasma. In the rabbit. could not resolve radiochromatographically from M28</p> <p>Loss of CO₂, suggested the presence of a carboxyl group.</p> <p>tentatively identified as 2-carboxy-1-{4-methyl-3-[methyl-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-amino]-piperidin-1-yl}-ethanone</p>	 <p style="text-align: center;">M4</p>
<p>M6: rat, rabbit, monkey M6 in rat was detected in urine and feces; in rabbit detected in urine and plasma</p> <p>In monkey, found in plasma, urine, and feces</p> <p>In the rabbit could not be resolved radio chromatographically from M14</p> <p>M6 was identified as 3-{hydroxy-4-methyl-5-[methyl-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-amino]-piperidin-1-yl}-3-oxo-propionitrile.</p>	 <p style="text-align: center;">M6</p>
<p>M6a: rabbit In rabbit, found in urine and plasma hydroxylation had occurred in the piperidine moiety is a regioisomer of M6</p> <p>M6a was tentatively identified as 3-</p>	

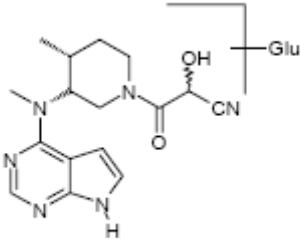
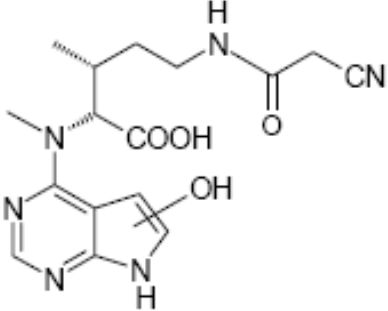
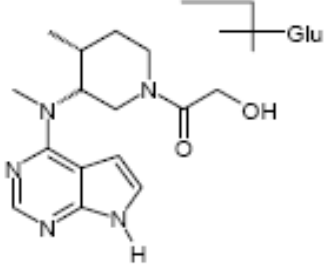
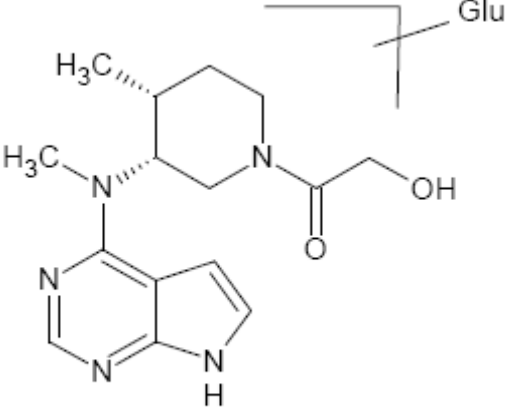
<p>{hydroxy-4-methyl-5-[methyl-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-amino]-piperidin-1-yl}-3-oxopropionitrile</p>	
<p>M7a and M7b: monkey detected only in bile</p> <p>M7a and M7b were tentatively identified as 2-hydroxy-3-{4-methyl-3-[methyl-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-amino]-piperidin-1-yl}-3-oxo-propionitrile</p>	 <p>M7a, M7b</p>
<p>M8: rabbit, monkey, human In rabbit, found only in plasma</p> <p>In monkey and human detected only in urine</p> <p>M8 was tentatively identified as 3-{3-[(hydroxy-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-methyl-amino]-4-methylpiperidin-1-yl}-3-oxo-propionitrile</p>	 <p>M8</p>
<p>M9: mouse, rat, rabbit, monkey, human Metabolite M9 was detected in urine and feces in mouse and rat In rabbit found only in urine and plasma. In monkey and human detected in urine, feces and plasma. An oxygen atom had been added to the pyrrolopyrimidine moiety and hydroxylation occurred on the carbon next to nitrogen.</p> <p>M9 was identified as 3-{(3R,4R)-4-methyl-3-[methyl(6-oxo-6,7-dihydro-5H-pyrrolo[2,3-d]pyrimidin-4-yl)amino]piperidin-1-yl}-3-oxopropanenitrile.</p>	 <p>M9</p>

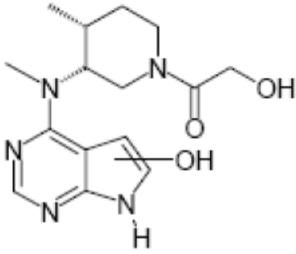
<p>M11: rabbit, monkey, human In rabbit found in both urine and plasma</p> <p>In monkey and human, detected in urine, plasma and feces</p> <p>M11 was tentatively identified as 3-{hydroxy-5-[(hydroxy-7Hpyrrolo[2,3-d]pyrimidin-4-yl)-methyl-amino]-4-methyl-piperidin-1-yl}-3-oxopropionitrile.</p>	 <p style="text-align: center;">M11</p>
<p>M13: rat, rabbit Metabolite M13 was detected only in male urine, plasma and feces; in rabbit found in urine and plasma</p> <p>Loss of a methyl group from the nitrogen atom of the pyrrolo[2,3-d]pyrimidine methylamine moiety and loss of H₂O from piperidine moiety.</p> <p>M13 was identified as 3-[hydroxy-4-methyl-5-(7H-pyrrolo[2,3-d]pyrimidin-4-ylamino)-piperidin-1-yl]-3-oxo-propionitrile.</p>	 <p style="text-align: center;">M13</p>
<p>M14: mouse, rat, rabbit, monkey, human</p> <p>Metabolite M14 was detected in urine, feces and plasma. In rabbit found in urine and plasma. In monkey, detected in urine, plasma, feces and bile. In human detected in urine and feces</p> <p>Dihydroxylation had occurred on the piperidine moiety.</p> <p>In rabbit was not resolved</p>	 <p style="text-align: center;">M14</p>

<p>radiochromatographically from M6. M14 was tentatively identified as 3-{dihydroxy-4-methyl-5-[methyl-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-amino]piperidin-1-yl}-3-oxo-propionitrile.</p>	
<p>M14a: rabbit found in plasma,</p> <p>Mass spectrum was identical to that of metabolite M14 but had a different retention time indicating that M14a is a regioisomer of previously identified metabolite M14</p> <p>M14a was tentatively identified as 3-{dihydroxy-4-methyl-5-[methyl-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-amino]piperidin-1-yl}-3-oxo-propionitrile</p>	
<p>M18: mouse, rabbit, monkey, human</p> <p>M18 was detected in urine and feces in mouse; in urine and plasma in rabbit, detected only in feces in monkey and human. The addition of an oxygen atom had occurred on the piperidine moiety.</p> <p>Is a regioisomer of M6.</p> <p>M6 and M18 were tentatively identified as 3-{hydroxy-4-methyl-5-[methyl-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-amino]piperidin-1-yl}-3-oxopropionitrile.</p> <p>M18 was tentatively identified as 3-{hydroxy-4-methyl-5-[methyl-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-amino]piperidin-1-yl}-3-oxo-propionitrile.</p>	 <p style="text-align: center;">M18</p>

<p>M19: rabbit, monkey in monkey, was detected only in female urine and male bile in rabbit found in urine and plasma</p> <p>not resolved radiochromatographically from M6 and M14</p> <p>M19 and M27 are regioisomers</p> <p>Chemical name not provided</p>	 <p>M19</p>
<p>M20: rabbit, monkey, human not resolved radiochromatographically from M29.</p> <p>Found in urine and plasma in rabbit and human. Found in urine, feces, bile and plasma in monkey</p> <p>Is a quaternary N-glucuronide, M20 was tentatively identified as the glucuronide of CP-690550</p>	 <p>M20</p>
<p>M21: rat Metabolite M21 was detected in urine and plasma.</p> <p>Loss of the piperidine ring side chain.</p> <p>M21 was tentatively identified as methyl-(4-methyl-piperidin-3-yl)-(7Hpyrrolo[2,3-d]pyrimidin-4-yl)-amine.</p>	 <p>M21</p>

<p>M22: monkey, human In monkey, M22 was detected in feces and bile In human, was detected only in feces (bile not collected) Chemical name not provided</p>	 <p style="text-align: center;">M22</p>
<p>M23: monkey detected in urine, bile and plasma M23 was tentatively identified as a glucuronide of M6</p>	 <p style="text-align: center;">M23</p>
<p>M24: monkey detected in bile identified as two piperidine ring-opened structures Chemical name not provided</p>	 <p style="text-align: center;">M24</p>
<p>M25:rabbit, monkey Found in only in plasma in rabbit. In monkey detected in bile. Identified as two piperidine ring-opened structures Chemical name not provided</p>	 <p style="text-align: center;">M25</p>

<p>M26: rabbit; monkey not resolved radiochromatographically from M2 Found in urine and plasma</p>	 <p style="text-align: center;">M26</p>
<p>M27: monkey detected in bile</p> <p>M19 and M27 are regioisomers</p> <p>Chemical name not provided</p>	 <p style="text-align: center;">M27</p>
<p>M28: rabbit, monkey</p> <p>in monkey detected in urine and plasma , not resolved radiochromatographically from M4 Found in urine and plasma</p> <p>M28 and M29 were tentatively identified as M2 glucuronides</p>	 <p style="text-align: center;">M28</p>
<p>M29: mouse, rat, monkey, human</p> <p>In mouse, rat and human, M29 was detected in urine and plasma.</p> <p>M29 was tentatively identified as glucuronide of M2.</p>	 <p style="text-align: center;">M29</p>

<p>M31: monkey, human In monkey, detected only in urine and feces. In human, detected only in urine.</p> <p>M31 was tentatively identified as 2-hydroxy-1-{4-methyl-3-[methyl-hydroxy-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-amino]-piperidin-1-yl}-ethanone</p>	 <p style="text-align: center;">M31</p>
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Study title: Radiolabelled mass balance, and metabolic profiles of [¹⁴C]CP-690550 in CByB6F1-Tg(HRAS)2Jic(homozygous wild type) mice

Study no.: 140653
Study report location: Module 4.2.2.4
Conducting laboratory and location: Department of Pharmacokinetics, Dynamics and Metabolism
Pfizer Global Research and Development
Pfizer Inc
Groton, Connecticut 06340

Date of study initiation: Report dated May 6 2009, signed electronically Jan 13 2011
(b) (4) date Sept 14, 2009

GLP compliance: No
QA statement: No
Drug, lot #, and % purity: [¹⁴C]CP-690550-10 (citrate salt), Lot 00701642-047-KTG00A, Purity >99.7%
Specific Activity 5.73 mCi/mmol

CP-690550-10 (citrate salt), Lot 52546-111-5QS, Purity not provided

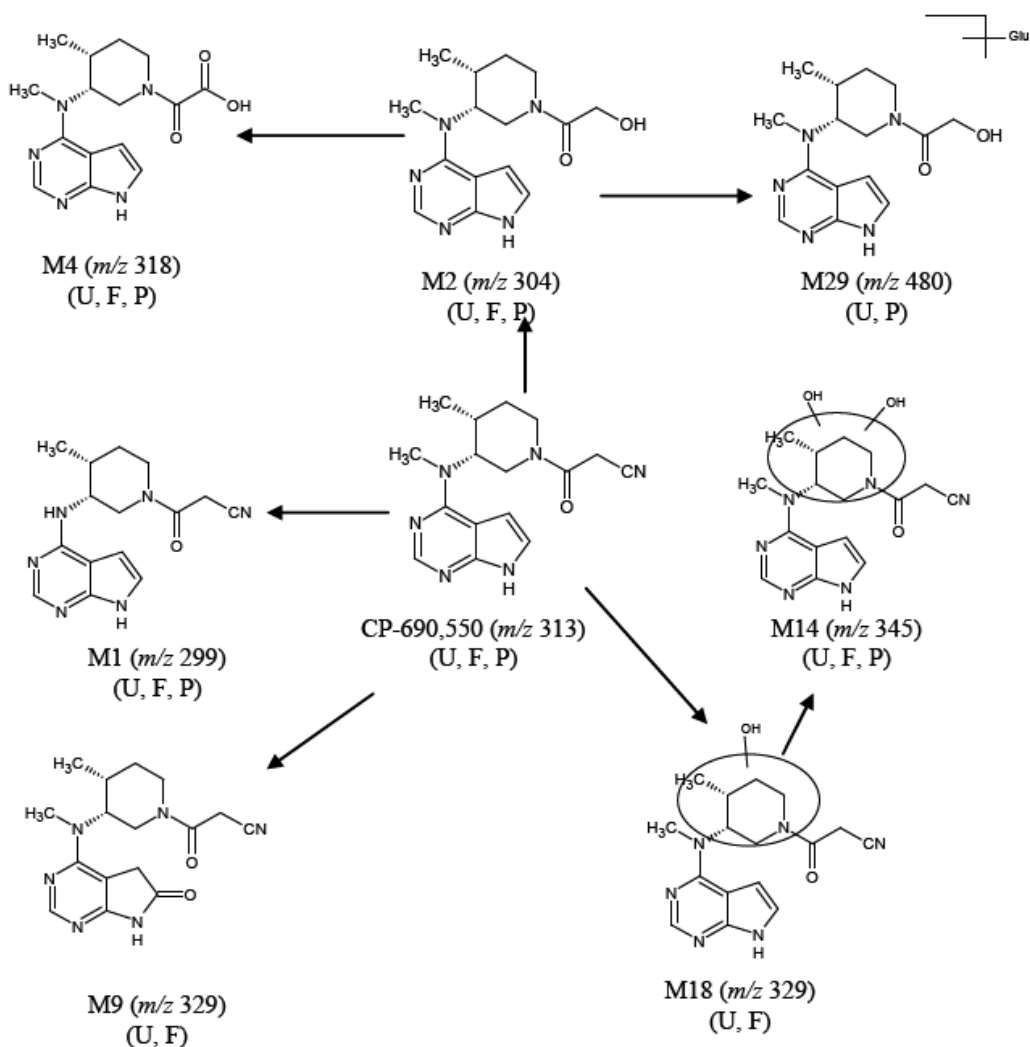
Key Study Findings

- The mass balance, routes of excretion, and metabolic profiles of CP-690550 were investigated in male and female CByB6F1-Tg(HRAS)2Jic(homozygous wild type) mice following a 31 mg/kg (12-18 µCi/animal) oral dose of [¹⁴C]CP-690550-10. The heterozygous mouse variant of this transgenic strain was used in the 6-month carcinogenicity study (Report 08GR481).
- The recovery of administered radioactivity was 87.7 % in the male and 88.3 % in the female.

- Total recovery of the dose in the feces over 96h was 72.1% in the male and 51.2% in the female. The total recovery of the dose in the urine over 96 h was 10.1% in the male and 32.1% in the female.
- CP-690550 made up 3.1% of the dose in urine, 7.6% of the dose in feces, and 12.5% of circulating activity in plasma.
- Seven metabolites were identified by LC/MS/MS.
- The major mouse metabolite excreted in urine was the oxidized/glucuronidated M29 (7.5% of dose).
- The major mouse metabolites excreted in feces were the oxidized M2 (15.2% of dose) and M4/M18 (22.6% of dose).
- The major metabolites in plasma were M29 (33.1% of total radioactivity) and M4 (20.1% of total radioactivity).
- The proposed metabolic pathways of CP-690550 in CByB6F1-Tg(HRAS)2Jic(homozygous wild type) mice is presented below:

Figure 3: Proposed Metabolic Pathway of CP-690550 in the Tg(HRas) Homozygous Mouse

Figure 13. Proposed metabolic pathways of CP-690,550 in CByB6F1-Tg(HRAS)2Jic (homozygous wild type) mice following single oral administration of 31 mg/kg dose of [14C]CP-690,550-10.



U-urine
F-feces
P-plasma

Study title: Radiolabelled Mass Balance and Metabolic Profiles of [¹⁴C]CP-690550 (ring labeled) in Sprague-Dawley Rats

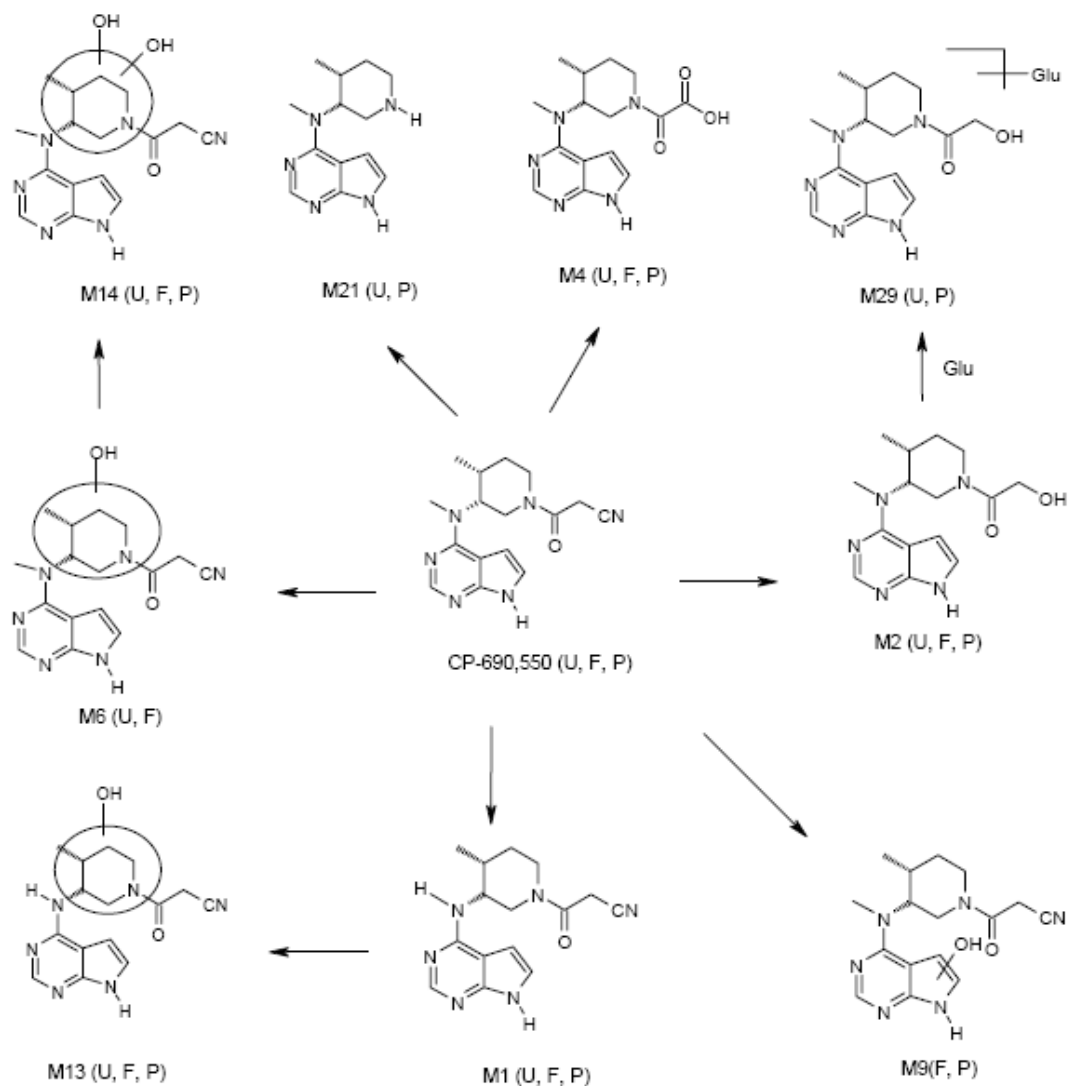
Study no.: DM2005-CP690550-055
Study report location: Module 4.2.2.4
Conducting laboratory and location: Department of Pharmacokinetics,
Dynamics and Metabolism
Pfizer Global Research and Development
Pfizer Inc
Groton, Connecticut 06340
Date of study initiation: Not indicated, report signed May 17,
2005
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: [¹⁴C]CP-690550-10 (citrate salt), Lot
56871-179-1, Purity ≥99
Specific Activity 4.74 mCi/mmol

Key Study Findings

- A single 10 mg/kg dose of [¹⁴C]CP-690550-10 was orally administered to rats to determine CP-690550 metabolism and excretion profiles. The mean total recovery of radioactivity at 168 hours post-dose was 96.2% for males and 97.6% for females.
- Within 48 hours, >75% of the radioactivity was excreted. The mean values of urinary excretion were 48.8% and 54.5% for male and female rats, respectively. Fecal excretion accounted for a mean recovery of 46.6% in males and 42.7% in females.
- There were sex-related qualitative and quantitative differences in the excretion of metabolites.
- The following metabolites were identified in the rat: M1, M2, M4, M5, M6, M9, M13, M21, and M29. M13 was observed only in male rats.
- The major metabolic pathways of CP-690550 included N-demethylation, oxygenation of the piperidine ring, piperidine ring side chain oxygenation and oxygenation of pyrrolopyrimidine ring.

Figure 4: Proposed Metabolic Pathway of CP-690550 in Rats

Figure 16. Proposed Metabolic Pathways of CP-690,550 in Rats

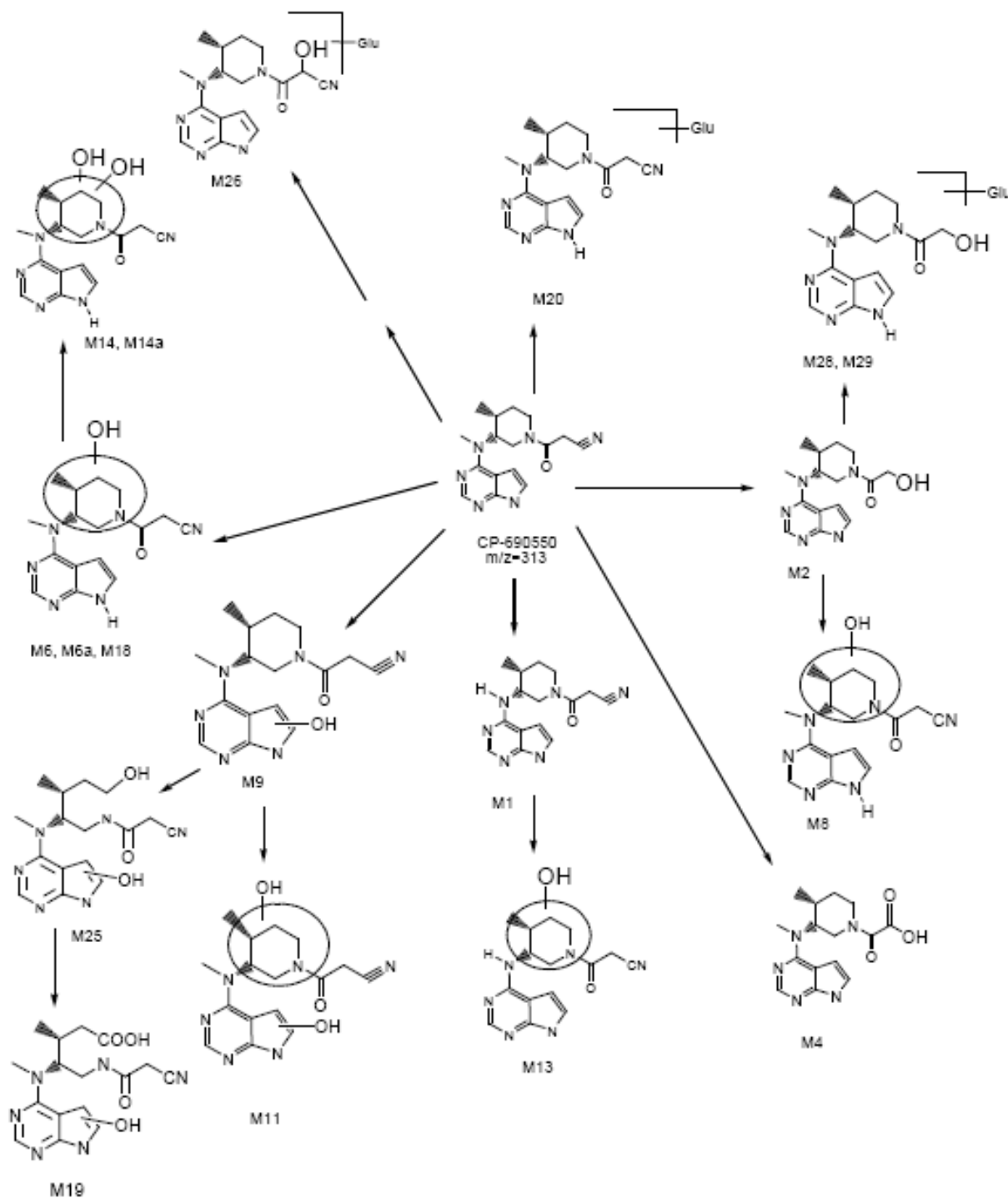


Study title: Identification of Urinary and Circulating Metabolites of CP-690550 in Female New Zealand White Rabbits after Oral Administration of [¹⁴C]CP-690550-10

Study no.:	DM2005–690550–064
Study report location:	Module 4.2.2.4
Conducting laboratory and location:	Department of Pharmacokinetics, Dynamics and Metabolism Pfizer Global Research and Development Pfizer Inc Groton, Connecticut 06340 in-life portion of the study were conducted at Pfizer Inc; (Kalamazoo MI
Date of study initiation:	Not indicated, report signed April 13, 2007
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	[¹⁴ C]CP-690550-10 (citrate salt), Lot 104014-138-1, Purity ≥99%
	Specific activity 0.4082 mCi/mmol

Key Study Findings

- The metabolite profile of CP-690550 was investigated in female New Zealand white rabbits after oral administration of a single oral 30 mg/kg dose of [¹⁴C]CP-690550-10 (37.8 to 38.4 µCi/kg).
- The primary route of excretion in female rabbits was via the urine. The total radioactivity peaked at ~1 h after oral administration.
- The major circulating metabolites of CP-690550 were descyano-carboxy- (M4), M2-glucuronides (M28, M29) and CP-690550- glucuronide (M20). Identified metabolites accounted for >93% circulating radioactivity.

Table 13: Proposed Metabolic Pathway of CP-690550 in New Zealand White Rabbits**Figure 7. Proposed Metabolic Pathways of CP-690,550 in Rabbit.**

Study title: Radiolabelled Mass Balance and Metabolic Profiles of [¹⁴C]CP-690550 (ring labeled) in Cynomolgus Monkeys

Study no.: DM2004-690550-052
Study report location: Module 4.2.2.4
Conducting laboratory and location: Department of Pharmacokinetics,
Dynamics and Metabolism
Pfizer Global Research and Development
Groton, Connecticut 06340
In life study conducted at
(b) (4)
Date of study initiation: Not indicated, Report Signed April 7
2005
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: [¹⁴C]CP-690550-10 (citrate salt), Lot
56871-184-12, Purity ≥99%
Specific Activity 3.4 mCi/mmol

Key Study Findings

- Following oral administration of a single 5 mg active/kg dose of [¹⁴C]CP-690550-10 (54 uCi/kg), the mean total recovery of radioactivity at 168 h post-dose was 87.4% for males and 91.8% for females.
- Most of the radioactivity excreted within 48 h post-dose with the primary route of excretion via the urine, with mean values of 42.6 for males and 55.7% for females. Fecal excretion accounted for a mean recovery of 27.3% in males and 28.7% in females, and approximately 25% in bile.
- The major metabolic pathways of CP-690550 were similar to those found in humans and included oxidation of the pyrrolopyrimidine ring, oxidation of the piperidine ring, piperidine ring side chain oxidation and glucuronidation. A minor metabolic route was due to N-demethylation.

Metabolites

A total of 13 biliary metabolites were identified. The major biliary metabolites were M29), (M26, M23 M25. Unchanged drug accounted for 0.3% of the dose in bile for male monkeys.

In plasma, 12 metabolites were detected. The major circulating metabolites were M2 M28, M29, M23, M11, M20.

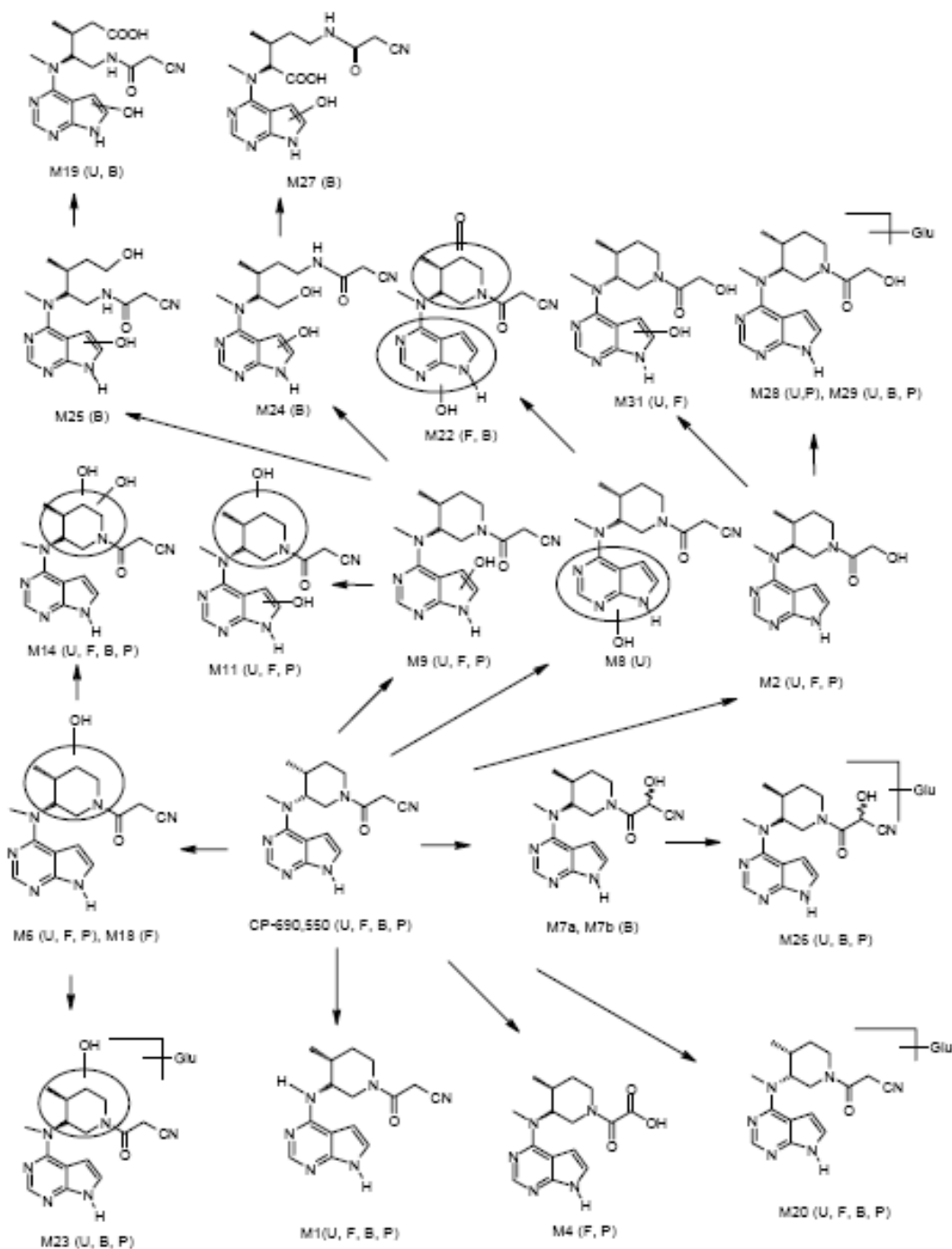
In urine there were 14 metabolites detected. The major metabolites were M9, M2, M28, M29. M9, M23 and M20. Unchanged drug accounted for 6.1% and 10.9% of the dose in urine for male and female monkeys, respectively.

In feces there were 11 metabolites were detected. The major fecal metabolites were M6, M9, M18, M2, M4, and M14. Unchanged drug accounted for 1.5% and 1.9% of the dose in feces for male and female monkeys, respectively.

A total of 13 biliary metabolites were identified. The major biliary metabolites were M29, (M26, M23 M25. Unchanged drug accounted for 0.3% of the dose in bile for male monkeys.

Figure 5: Proposed Metabolic Pathway of CP-690550 in Cynomolgus Monkey

Figure 31. Proposed Metabolic Pathways of CP-690,550 in Monkey



Study title: Metabolic Profile and Routes of Excretion of [¹⁴C]CP-690550 in Healthy Male Subjects

Study no.: DM2004-690550-049
Clinical Study # A3921010
Study report location: Module 4.2.2.4
Conducting laboratory and location: Department of Pharmacokinetics,
Dynamics and Metabolism
Pfizer Global Research and Development
Pfizer Inc
Groton, Connecticut 06340
Date of study initiation: Not indicated, report signed Oct 18 2004
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: [¹⁴C]CP-690550-10 (citrate salt), Lot
61011-122-1, Purity >99%
Specific Activity 1.97 µCi/mg (salt form)

Key Study Findings

- The routes of excretion, biotransformation and pharmacokinetics of CP-690550 were investigated in healthy male subjects (6 healthy male volunteers, between the ages of 18 to 55 years) after oral administration of a single 50 mg dose of [¹⁴C]CP-690550-10 (164 µCi of radiotracer).
- Total radioactivity peaked at ~1 hour after oral administration. The mean terminal phase T_{1/2} values were 3.15 and 3.18 hours for parent drug and total radioactivity, respectively.
- The total recovery of administered radioactive dose over a period of 192 hours was 93.9%, with 80.1% in the urine and 13.8% in the feces.

Metabolites

- In plasma eight metabolites were detected in the radiochromatogram, M1, M2, M29, M4 M9, M11, and M14, M20.
- **The mean metabolite levels found in plasma were each less than 10% of the administered dose.**
- The major metabolic pathways of CP-690550 included oxidation of the pyrrolopyrimidine ring, oxidation of the piperidine ring, piperidine ring side chain oxidation and glucuronidation. A minor metabolic route was due to N-demethylation.

Pharmacokinetics

Plasma concentrations for both CP-690550 and total radioactivity peaked at <2 hour after oral administration. C_{max} values for the parent drug ranged from 331 to 480 ng/mL with a mean value of 397 ng/mL. C_{max} values for the total radioactivity ranged from 545 to 734 ng-equiv/mL with a mean value of 611 ng-equiv/mL. AUC_{0-∞} values for the parent drug ranged from 977-2060 ng·h/mL with a mean value of 1680 ng·h/mL. AUC_{0-∞} values for total radioactivity ranged from 2430-4700 ng-equiv·h/mL with a mean value of 3440

ng-equiv·h/mL. The mean terminal phase half-life ($T_{1/2}$) was estimated to be 3.2 hours for both parent drug and total radioactivity.

Figure 4. Mean plasma concentration time curves for total radioactivity and parent compound in male subjects following oral administration of a single 50 mg dose of [14 C]CP-690,550.

Clinical Study # A3921010; DM Study Number: DM2004-690550-049

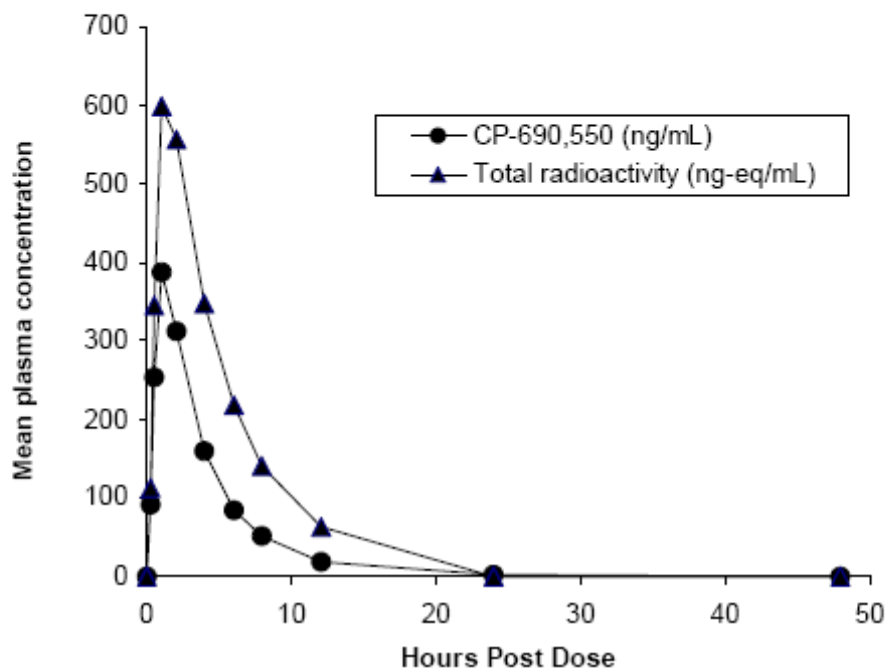


Table 5. Pharmacokinetic Parameters For CP-690,550 in Male Subjects Following Oral Administration of a Single 50 mg Dose of [^{14}C]CP-690,550

Clinical Study # A3921010; DM Study Number: DM2004-690550-049

Subject #	T _{1/2} h	T _{max} h	C _{max} ng/mL	AUC _{0-t} (ng•h/ml)	AUC _{0-∞} (ng•h/ml)
1	3.39	1.00	368	1690	1700
2	3.37	1.00	440	2050	2060
3	3.44	1.00	480	1860	1870
4	3.50	2.00	332	1860	1880
5	3.18	0.50	428	1600	1610
6	2.04	1.00	331	961	977
Mean	3.15	1.08	397	1670	1680
SD	0.56	0.49	62	381	380

Table 6. Pharmacokinetic Parameters For Total Radioactivity in Male Subjects Following Oral Administration of a Single 50 mg Dose of [^{14}C]CP-690,550

Clinical Study # A3921010; DM Study Number: DM2004-690550-049

Subject #	T _{1/2} h	T _{max} h	C _{max} ng-eq/mL	AUC _{0-t} (ng-eq•h/ml)	AUC _{0-∞} (ng-eq•h/ml)
1	3.26	1.00	554	3160	3470
2	3.78	1.00	734	4130	4700
3	2.77	1.00	634	2760	2930
4	3.68	2.00	592	3450	3920
5	2.96	1.00	609	2940	3150
6	2.62	1.00	545	2310	2430
Mean	3.18	1.17	611	3120	3440
SD	0.48	0.41	69	621	798

Metabolites

In plasma eight metabolites were detected in the radiochromatogram, M1, M2, M29, M4 M9, M11, and M14, M20.

The mean metabolite levels found in plasma were each less than 10% of the administered dose.

Table 14: Percentage of Circulating Metabolites of CP690550 in Male Subjects

Table 9. Percentage of Circulating Metabolites of CP-690,550 in Male Subjects Following Oral Administration of a Single 50 mg Dose of [¹⁴C]CP-690,550

Clinical Study # A3921010; DM Study Number: DM2004-690550-049

Metabolites	m/z	Ret. Time (min)	Percent of Dose							
			Subject #							
			1	2	3	4	5	6	Mean	SD
M14	345	8.3	3.2	0.7	1.1	4.5	3.3	6.2	3.2	2.1
M4	318	12.4	4.4	5.3	1.4	5.5	2.6	4.5	3.9	1.6
M20, M11, M29	489, 345, 480	15.1	4.8	8.0	4.7	7.5	5.8	6.6	6.2	1.4
M1, M2	299, 304	17.6	0.8	7.3	7.4	7.9	10.4	10.9	7.4	3.6
CP-690,550	313	20.8	77.5	69.5	77.1	57.4	73.8	61.1	69.4	8.5
M9	329	26.4	1.6	1.2	0.6	0.5	ND	ND	1.0	0.5

ND = Not detected

In urine 10 metabolites were identified by LC-MS/MS. The major urinary metabolites were M9, M4, M2, M29 M11, and M14, and M20).

Table 15: Percentage of Urinary Metabolites of CP690550 in Male Subjects

Table 7. Percentage of Urinary Metabolites of CP-690,550 in Male Subjects Following Oral Administration of a Single 50 mg Dose of [¹⁴C]CP-690,550

Clinical Study # A3921010; DM Study Number: DM2004-690550-049

Metabolites	m/z	Ret. Time (min)	Percent of Dose							
			Subject #							
			1	2	3	4	5	6	Mean	SD
M14	345	11.2	3.1	4.4	2.3	3.7	3.2	4.7	3.5	0.9
M4	318	12.7	8.0	10.2	6.7	9.3	7.9	7.8	8.2	1.2
M20	489	14.9	2.3	3.2	1.7	2.2	1.2	2.6	2.2	0.7
M11, M29	345, 480	15.2	10.0	13.1	7.9	11.2	9.7	12.3	10.6	1.9
M1, M2	299, 304	18.1	3.6	3.1	3.4	3.8	3.8	3.9	3.6	0.3
CP-690,550	313	20.5	33.7	18.2	37.9	28.9	31.2	24.4	28.8	7.1
M31	320	23.3	1.2	1.3	0.8	1.3	1.4	2.4	1.4	0.5
M8	329	24.9	0.8	1.9	0.9	1.7	1.4	1.9	1.4	0.5
M9	329	27.3	17.8	18.3	18.0	21.2	20.2	23.6	19.6	2.2

In feces 7 metabolites were identified by LC-MS/MS. The major fecal metabolites were M9, M18, M4, M2 M11, and M14.

Table 16: Percentage of Fecal Metabolites of CP690550 in Male Subjects

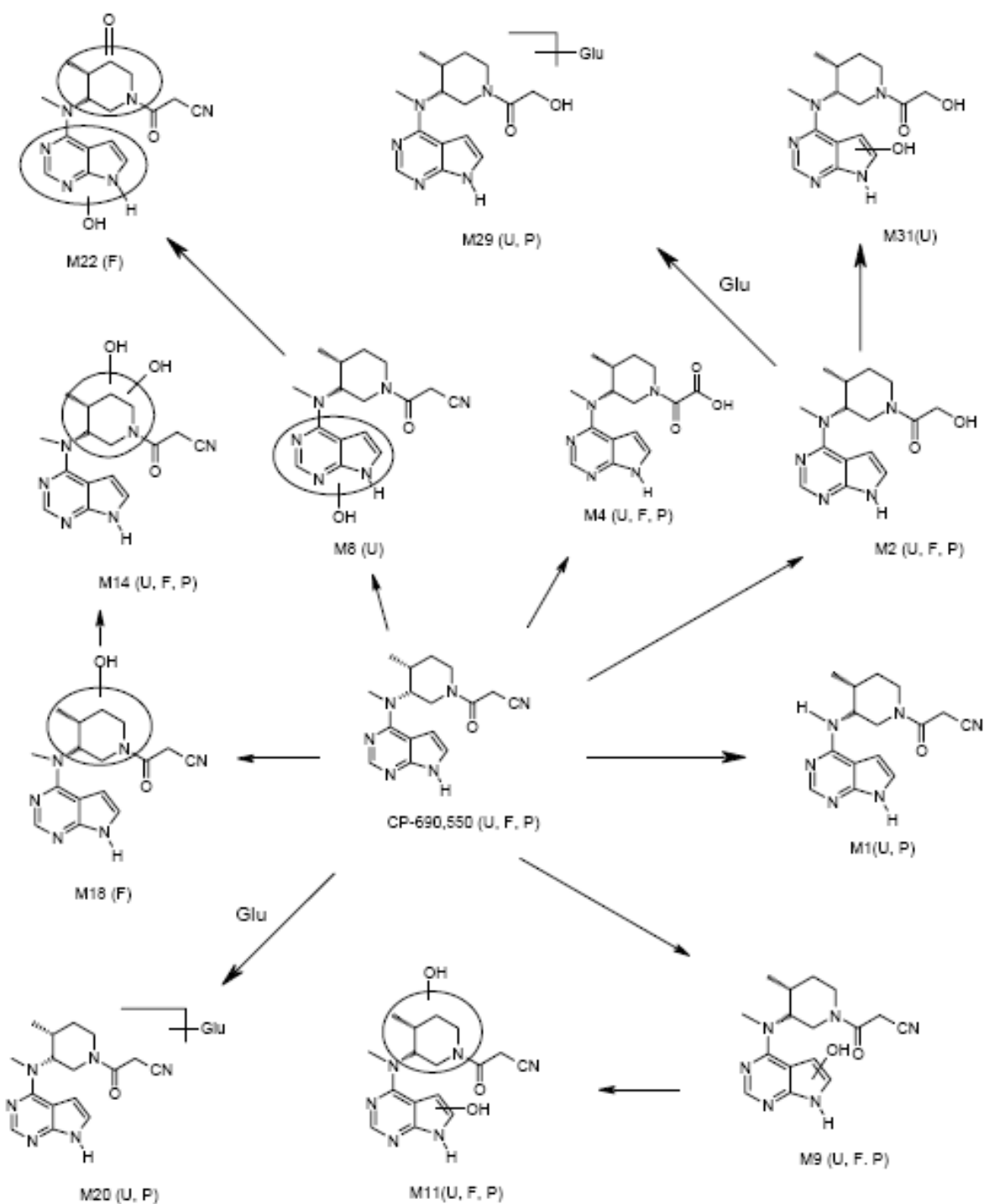
Table 8. Percentage of Fecal Metabolites of CP-690,550 in Male Subjects Following Oral Administration of a Single 50 mg Dose of [¹⁴C]CP-690,550

Clinical Study # A3921010; DM Study Number: DM2004-690550-049

Metabolites	m/z	Ret.Time (min)	Percent of Dose							
			Subject #						Mean	SD
			1	2	3	4	5	6		
M14	345	9.2	2.4	2.1	1.6	1.8	1.8	1.5	1.9	0.3
M18, M4	329, 318	12.6	3.8	2.9	2.1	4.3	4.5	3.0	3.4	0.9
M11	345	14.7	1.7	2.1	1.2	1.3	1.4	1.3	1.5	0.3
M2	304	17.1	0.7	0.4	0.7	0.5	0.5	0.4	0.5	0.1
CP-690,550	313	20.8	0.4	1.1	2.5	1.2	0.3	0.2	0.9	0.8
M9	329	27.0	1.9	1.6	2.0	1.8	1.1	1.0	1.6	0.4
M22	343	31.4	1.6	2.4	1.9	2.1	1.3	1.4	1.8	0.4
Unknown		34.0	3.2	1.8	1.7	2.4	2.0	1.9	2.2	0.6

Table 17: Proposed Metabolic Pathways of CP-690550 in Humans

Figure 23. Proposed Metabolic Pathways of CP-690,550 in Humans



Study title: Identification of in vitro metabolites of CP-690550 in human liver microsomes and recombinant cytochrome P-450 isoforms

Study no.: DM2004-690550-046
Study report location: Module 4.2.2.4
Conducting laboratory and location: Department of Pharmacokinetics,
Dynamics and Metabolism
Pfizer Global Research and Development
Pfizer Inc
Groton, Connecticut 06340
Date of study initiation: Not indicated, Report signed Sept 2 2004
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: [¹⁴C]CP-690550, Lot 56871-179-1, Purity
>99%
Specific Activity 30.3 mCi/mmol,
Labeled at the C-6 of the
pyrrolopyrimidine ring

Key Study Findings

- CP-690550 is mainly metabolized by CYP3A4/3A5 and CYP2C19
- The major oxidative metabolites of CP-690550 included oxidation of the pyrrolopyrimidine ring, oxidation of the piperidine ring, N-demethylation, and piperidine ring side chain oxidation.

Methods

[¹⁴C]CP-690550 was incubated in vitro with human liver microsomes or with commercially obtained recombinant human cytochrome P-450 isoforms (1A2, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4 and 3A5). Each 2 hour incubation contained microsomes (ca. 0.5 µM CYP or expressed CYP isoforms 50 pmol), [¹⁴C]CP-690550 (10 µM), and 100 µL of a 10 mM NADPH solution in 100 mM potassium phosphate pH 7.4. The incubations were stopped with cold acetonitrile, the precipitated and the precipitate microsomal proteins removed by centrifugation, and the supernatants analyzed by HPLC system LC/MS/MS.

Analysis of the metabolites was performed on by LC/MS/MS. The effluent from the HPLC column was split, and about 50 µL/min was introduced into the atmospheric ionization source via a pneumatically assisted electrospray interface. The remaining effluent was directed into the flow cell of the β-RAM. providing simultaneous detection of radioactivity and mass spectrometry data.

Results**Metabolism of CP-690550 in Human Liver Microsomes**

There were 10 metabolites of CP-690550 detected in the radio-chromatogram of human liver microsomes. In all studies, >95% of the initial radioactivity was extracted from the microsomal protein. In incubations devoid of NADPH, no new radioactive peaks were generated.

Metabolism of CP-690550 in Recombinant Cytochrome P450

The relative percentages of metabolites are presented in Table 1. Turnover of CP-690550 was highest in CYP3A4 (83.6%) followed by 2C19 (43.9%), 3A5 (15.9%), 1A2 (9.5%) and 2D6 (8.8%). No obvious turnover was found in the CYP2C9, 2E1 and 2C8 incubations.

Metabolites were identified as described in the previous study (Report 140653) and included the following:

M1, M2, M3, M5, M8, M9, M14, M15, M18, M22, M25

Table 1. Percentage of Metabolites of CP-690,550 in Human Liver Microsomes and Recombinant Human Cytochrome P-450 Isoforms

Metabolite	m/z	RT(min)	Human Microsome	3A4	2C19	2D6	1A2	3A5
M14	345	9.1	3.4	5.4				
M15	329	10.0	1.5	5.3			1.3	1.4
M25	347	12.5	2.1	6.8	3.0			0.5
M18	329	13.0	2.2	6.2				0.7
M5	320	16.2	9.0	20.4				3.0
M1	299	16.8	2.4					
M2	304	17.2	5.7	6.1*				1.7*
M3	336	19.3		5.1				
CP-690,550	313	20.4	59.2	16.4	56.1	91.2	90.5	84.1
M8	329	23.8	3.4	9.6	3.9	1.7		1.4
M9	329	26.7	4.0	5.0	34.2	7.2	2.7	1.9
M22	343	30.9	2.1	2.6	1.0			
Unknown		33.6	1.7	3.7	1.8		5.5	5.2

* Mixture of M1 and M2

Study title: Identification of Human Cytochrome P450 Isoforms Responsible for In Vitro Metabolism of CP-690550

Study no.: DM2007-690550-067
Study report location: Module 4.2.2.4
Conducting laboratory and location: Department of Pharmacokinetics,
Dynamics and Metabolism
Pfizer Global Research and Development
Pfizer Inc
Groton, Connecticut 06340
Date of study initiation: Report dated Jan 11 2011, not signed
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: [¹⁴C]CP-690550-10, Lot 119931-39-
WH000A,
Specific Activity 29.83 mCi/mmol

Key Study Findings

- Incubation of CP-690550 with human liver microsomes resulted in the formation of five major metabolites. These were a demethylation (M1), formation of a cyanohydrin followed by the loss of the cyanide group (M2), a hydroxylated metabolite of M2 (M5), oxygenation of the pyrrolopyrimidine moiety (M8), and an oxygenation of the pyrrole moiety (M9).
- The results from experiments with recombinant CYP isoforms, as well as inhibition studies with isoform-selective inhibitors strongly suggest that CYP3A4 is the major contributor in the formation of these metabolites with CYP1A2 being a minor but next most important enzyme.
- The apparent Km and Vmax values for the formation of these metabolites were determined as 132µM and 517 pmol/min/mg protein, respectively.

Methods

Incubation of 10 µM [¹⁴C]CP-690550-10 with human liver microsomes (1.0 mL total) were performed in duplicate with NADPH (1.3 mM) in 15 mL glass centrifuge tubes open to air at 37°C in a shaking water bath. Samples were preincubated at 37°C for 5 minutes prior to the addition of NADPH. Each incubation contained microsomes (0, 0.1, 0.2, 0.5, 1.0, 1.5, or 2 mg of protein/mL), 0.1 M KH₂PO₄ buffer pH 7.4, MgCl₂ (3.3 µmol), and [¹⁴C]CP-690550 (10 µM). After 30 minutes, incubations were quenched with acetonitrile, centrifuged, and supernatants evaporated then reconstituted in acetonitrile for analysis by LC-MS/MS combined with a βRAM ¹⁴C detector.

Time course incubations were conducted for durations of 0, 5, 10, 15, 20, 30, and 60 minutes. Substrate saturation incubations were conducted with [¹⁴C]CP-690550 (1.0, 5.0, 10, 20, 50, 100, 150 µM) for 30 min.

Incubation with CYP inhibitors were performed with and without the CYP inhibitors present indicated in the table below. Studies with commercially available recombinant human CYP isoforms included CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4 CYP1A1, CYP2A6 +B5, CYP2C8 +OR+B5, CYP2C18 +OR, CYP2E1, and CYP2B6.

Cytochrome P450 isozymes and inhibitor	Inhibitor
1A2	Phenacetin
2A6	Coumarin
2C9	Diclofenac
2C19	Mephenyloin
3A	Midazolam
2B6	Bupropion
2D6	Bufuralol
1A2	furafylline,
2C9	sulfaphenazole
2C19	(+)N-3-benzylrivanol
2D6	quinidine
3A4	ketoconazole

The quantification of metabolites was carried out by measuring the radioactivity in the individual peaks separated in the HPLC column using a β RAM detector connected to an HPLC/MS system. Apparent K_m and V_{max} values for human liver microsomes were determined directly from the Michaelis-Menten plots.

Results

Five radiolabeled metabolites M1, M2, M5, M8, and M10 were formed by incubation of [14 C] CP-690550 with human liver microsomes. The formation of these metabolites was significantly reduced in the presence of ketoconazole indicating that CYP3A was primarily responsible.

Michaelis-Menten kinetic analysis of metabolite formation with human liver microsomes yielded a K_m of 132 μ M and a V_{max} of 517 pmol/min/mg protein,

[14 C] CP-690550 and human liver microsome with specific CYP isoform inhibitors found that ketoconazole at 1 μ M concentration significantly inhibited metabolite formation and had the greatest effect. Furafylline at 10 μ M concentration moderately inhibited metabolite formation. Inhibition of metabolism by sulfaphenazole (10 μ M) and (+)N-3-benzylrivanol (10 μ M) was minor compared to metabolism without inhibitor.

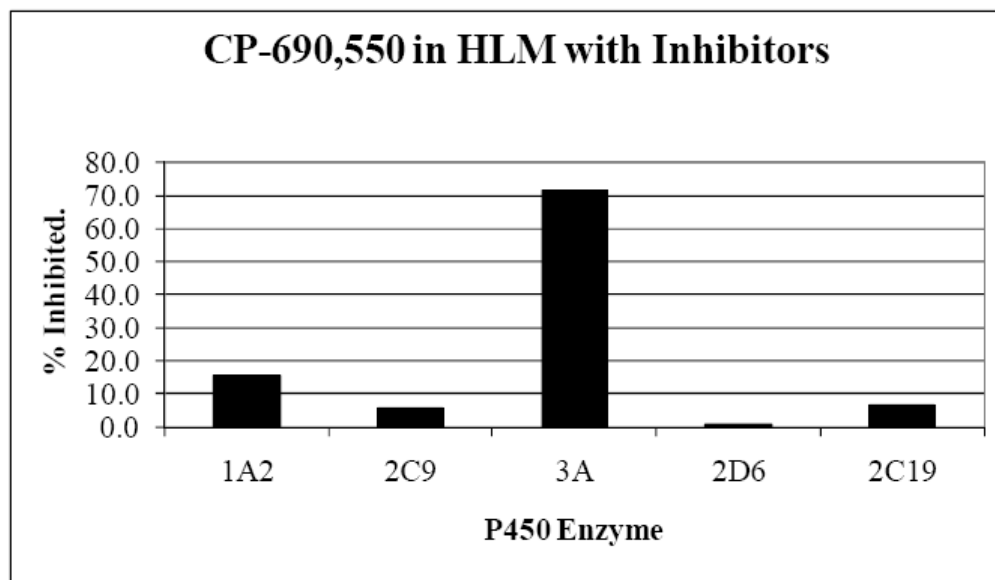


FIGURE 5. Percent inhibition of CP-690,550 (10 μ M) human liver microsomal metabolism (30 minute incubation time and 1 mg/mL protein content) using various isoform selective chemical inhibitors

When recombinant human CYP450 isoforms were incubated with 10 μ M [14 C] CP-690,550, CYP3A4 was the most active in metabolizing CP-690550 with CYP2C19 being moderately active and the activity of CYP1A2 was minor. Values for CYP2C18, CYP2E1, CYP2C8, and CYP1A1 were determined since their V_{max} data was not available for the lots of recombinant enzyme tested to correct for differences in relative activity.

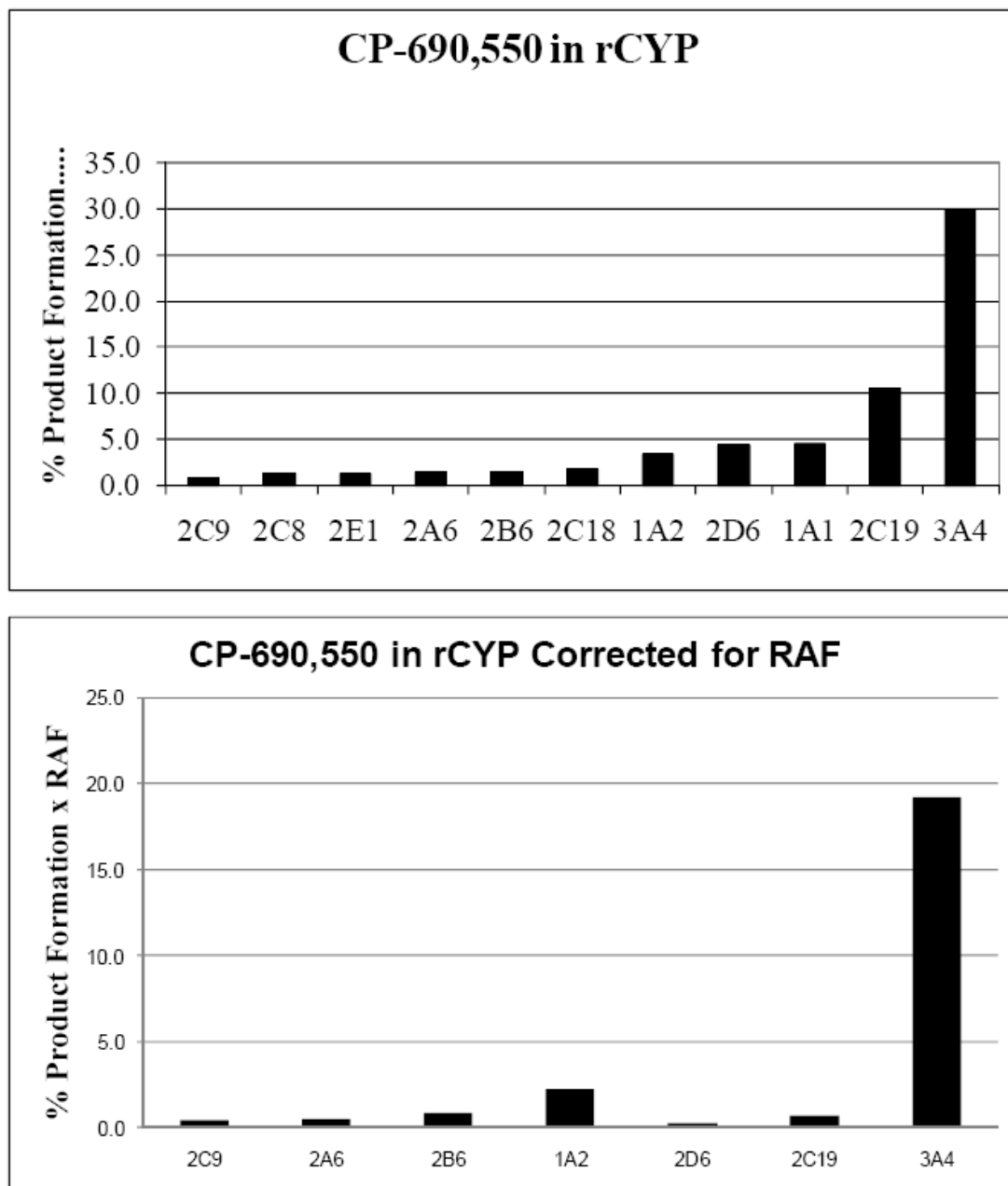


FIGURE 6. Relative metabolism of CP-690,550 to various recombinant human CYP450 enzymes (Top) and correcting for relative activity factor (Bottom).

EXCRETION

The same studies referred to in the Metabolism section provided information about the excretion CP-690550. The predominant route of elimination was through urine, with unchanged drug comprising approximately 10% in male rat, 30% in female rat, and approximately 10% in monkey. There were no major differences in the qualitative pattern of excretion among species and between genders. The primary clearance mechanism for CP-690,550 in humans appeared to be CYP450-mediated oxidation and renal clearance of parent drug, with metabolism accounting for approximately 70% of the clearance and renal approximately 30%. The parent CP-690550 and metabolite profiles for the rat, monkey, and human in plasma, urine and feces are summarized in the Table below.

Table 18: Parent and Metabolite Profiling of CP-690,550 in Human, Monkey, and Rat Plasma, Urine, and Feces

Metabolite	Human (Male)			Monkey (Male/Female)			Rat (Male/Female)		
	Plasma (% Total Radioactivity)	Urine (% Dose)	Feces (% Dose)	Plasma (% Total Radioactivity)	Urine (% Dose)	Feces (% Dose)	Plasma (% Total Radioactivity)	Urine (% Dose)	Feces (% Dose)
CP-690,550 (Parent)	69.4	28.8	0.9	30.8/48.6	6.1/10.9	1.5/1.9	60.5/58.2	11.7/33.9	5.3/15.6
M1/M2 ^a	7.4	3.6		7.6/9.0	2.5/3.3	2.7/3.7	15.5/13.8	14.3/5.2	11.0/12.0
M2			0.5						
M4	3.9	8.2		2.5/3.9			2.4/2.6	4.1/2.7	2.4/ND
M4/M18 ^a			3.4			9.4/8.4			
M6				1.8/2.0	1.8/1.8	3.6/3.2			2.2/1.1
M6/M21 ^a								2.3/2.2	
M8		1.4			0.2/0.2				
M9	1.0	19.6	1.6	0.5/0.5	3.5/3.6	2.8/2.8	0.5/0.9		2.9/3.5
M11			1.5			1.3/1.9			
M11/M29 ^a		10.6							
M11/M20/M29 ^a	6.2			35.5/21.6					
M13							1.4/ND	5.1/ND	2.8/ND
M14	3.2	3.5	1.9	2.2/2.9	1.6/2.8	2.6/2.7	1.5/1.8	3.6/3.2	5.4/1.9
M19					ND/1.1				
M20		2.2			4.0/5.6	ND/0.5			
M21							3.2/3.5		
M22			1.8			1.1/1.3			
M23				10.9/5.7	5.3/4.7				
M26				0.5/2.0	0.9/0.9				
M28				6.6/1.4	4.1/3.8				
M29					11.4/14.9		0.8/2.4	0.3/1.7	
M31		1.4			0.8/0.4	0.4/0.6			
Unknown			2.2			1.8/1.7			7.5/5.8

Data are expressed as mean percentages of CP-690,550 and metabolites.

^a Coeluting metabolites.

Table 19: Summary of Excretion of CP-690550

	Mean % of Dose Recovered				
	Mouse (31 mg/kg; 0-96 h)	Rat (10 mg/kg; 0-168 h)	Rabbit (30 mg/kg; 0-48 h)	Monkey (5 mg/kg; 0-168 h)	Human (50 mg; 0-192 h)
Urine	10.1/32.1	48.8/54.5	51.5	42.6/55.6	80.1
Feces	72.1/51.2	46.6/42.7	25.0	27.2/28.7	13.8
Total ^a	87.7/88.3	96.2/97.6	76.5	87.4/91.7	93.9

Data are represented as male/female in mouse, rat, and monkey; values are for females in rabbit and males in human.

^a Total equals the combined excretion of drug-derived radioactivity (% of dose) in urine, feces, carcass, and cage wash for nonclinical species, and urine and feces for human.

Study title: Lacteal Excretion of CP-690550 Following Administration of a Single Oral Dose to Rats

Study no.: 103847

Study report location: Module 4.2.2.5

Conducting laboratory and location:

(b) (4)

Date of study initiation: Dec 6 2010

GLP compliance: No

QA statement: No

Drug, lot #, and % purity: CP-690550-10, Lot Issue A, Purity 99.3%

Purity states 0.7% on Certificate of Analysis, but this is probably the impurity %, the purity would then be 99.3%, in line with other documented values for other Lots of CP690660-10

Composition:
62.5% CP-690550
37.5% citric acid counterion

Key Study Findings

- In lactating rats, CP-690550 is concentrated into milk at an approximately 2-fold greater level than in serum. o lactating female rats resulted in an initial concentration into milk at a level 2-fold greater than serum.
- Similar elimination rates occurred for serum and milk
- The milk:serum AUC_{0-∞} of 2.08.

Methods

Pregnant female Sprague Dawley rats were dosed by oral gavage on days 11 to 13 postpartum (body weight 265 to 326 g) at 10 mg/kg of CP-690550 in 0.5% methylcellulose vehicle. Animals were checked daily for general health, mortality, signs of pain and distress. Litters were culled to 8 pups at 4 days postpartum, then to 4 pups on the day before milk collection, which were removed 4 hours before milk collection. Milk and blood were collected from four animals/time points at 1, 3, 8, and 24 hours postdose. For each timepoint, animals received a subcutaneous injection of oxytocin before milking to stimulate lactation, and were anesthetized (type of anesthetic not mentioned) immediately before the start of milk collection, and milk (approximately 1 mL, more if possible) was collected using a specially constructed milking machine.

Serum and milk samples were analyzed by LC-MS/MS for CP-690550 concentrations. Although CP-690550 extractions and detection methodology were presented, the extraction efficacy from milk and plasma along with other assay validation information for milk samples were not provided.

Results

Table 20: Mean Serum and Milk Concentrations in Lactating Rats

Time point	Serum (ng/mL)	Milk (ng/mL)
1 hour	1180	22700
3 hour	390	734
8 hour	44	76
24 hour	BLQ	BLQ
Summary Toxicokinetics		
C_{max}	1180	2700
T_{max}	1	1
AUC_{0-t}	3240	6960
t_{1/2}	1.49	1.39

BLQ, below the limit of quantitation (<1.0 ng/mL)

DRUG INTERACTION

Study title: The in vitro study of P-glycoprotein inhibition by (b) (4) (CP-690550) in Caco-2 Cells

Study no.: 060532
Study report location: Mod 4. 2.2.6
Conducting laboratory and location: Pfizer Global Research and Development,
Department of Pharmacokinetics
Dynamics and Metabolism
Sandwich, Kent, UK
Date of study initiation: Sept 22, 2008
GLP compliance: No
QA statement: Yes
Drug, lot #, and % purity: (b) (4) (CP-690550), Lot (b) (4)
(b) (4) 10-0001, Purity >97.9%

Key Study Findings

- CP-690550 is a weak inhibitor of P-glycoprotein.

Methods

Caco-2 cells (human epithelial colorectal adenocarcinoma cell line) were cultured in transwell plates. Donor solutions contained 5µM digoxin in buffer, and acceptor solution consisted of buffer alone. Concentrations of CP-690550 up to 1000 µM were added to each donor and acceptor solution to keep the final concentration of DMSO below 1% (v/v). [³H]-digoxin (1mCi/ml, a P-glycoprotein substrate) was added to each donor solution at 1 µl/mL. Donor and acceptor solutions were added to the apical (250 µL) and basolateral (1000 µL) compartments, respectively. Following a 2 hour incubation with shaking, transport was stopped by separating the transwell insert from the base plate.

Levels of [³H]-digoxin from each compartment were determined by liquid scintillation counting for total radioactivity. The cellular monolayer integrity was checked by measuring the flux of Lucifer yellow across each monolayer. Ketoconazole was used as positive control.

Net flux, apparent permeability values (P_{app}), and IC_{50} values were calculated. P_{app} and net flux values were determined for digoxin in the presence of (b) (4) at each concentration. The determined P_{app} and net flux values were then used to calculate % activity of P-gp and efflux ratio.

Results

The apparent permeability value for digoxin in the absence of CP-690550 was 1.76×10^{-6} cm/s in the absorptive direction and 12.25×10^{-6} cm/s in the secretory direction. In the

presence of CP-690550, digoxin flux was inhibited up to 72%, but not completely (IC_{50} 311 μ M). Ketoconazole completely inhibited digoxin flux (IC_{50} 6.3 μ M). Therefore CP-690550 was a low potency inhibitor of digoxin flux in Caco-2 cells, i.e. a weak inhibitor of P-glycoprotein.

Table 21: Effect of CP-690550 on P-glycoprotein Activity

Table 1
Digoxin Papp and net secretory flux values across Caco-2 cell monolayers in the presence of increasing concentrations of (b) (4)

(b) (4) (μ M)	Mean A-B Papp ($\times 10^{-6}$ cm/s, \pm SD) (n=3)	Mean B-A Papp ($\times 10^{-6}$ cm/s, \pm SD) (n=3)	Net Secretory Flux (nmol/cm ² /h)	Degree of activity of digoxin flux (%)
0	1.76 \pm 0.05	12.25 \pm 1.16	0.19	100
1	1.69 \pm 0.04	10.58 \pm 1.67	0.16	84.70
5	1.67 \pm 0.06	11.99 \pm 0.17	0.19	98.28
10	1.61 \pm 0.13	11.47 \pm 0.23	0.18	93.90
25	2.13 \pm 0.10	12.07 \pm 0.75	0.18	94.69
50	2.12 \pm 0.16	11.42 \pm 0.67	0.17	88.56
70	2.04 \pm 0.17	10.67 \pm 0.86	0.14	72.81
100	2.16 \pm 0.04	11.98 \pm 0.46	0.18	93.64
250	2.65 \pm 0.07	10.37 \pm 0.35	0.14	73.51
500	3.75 \pm 0.23	8.69 \pm 0.33	0.09	47.02
750	3.61 \pm 0.33	6.72 \pm 0.98	0.06	29.60
1000	3.39 \pm 0.19	6.32 \pm 0.98	0.05	27.90

Study title: In Vitro Studies in MDCK-MDR1 Cells to Identify Compound (b) (4) as Substrate for P-Glycoprotein

Study no.: (b) (4)
 Study report location: Module 4.2.2.6
 Conducting laboratory and location: Pfizer Global Research and Development,
 Department of Pharmacokinetics Dynamics and Metabolism
 Sandwich, Kent, UK
 Date of study initiation: Nov 14 2008
 GLP compliance: No
 QA statement: No
 Drug, lot #, and % purity: (b) (4) (CP-690550-10)
 C- (b) (4)
 Specific Activity 0.18 mCi/ml

Key Study Findings

- CP-690550 is a substrate for P-glycoprotein

Methods

A series of studies were carried out in transfected MDCKII cells, a dog kidney epithelial cell line, stably expressing human MDR1 (*ABCB1*), the gene that encodes human P-gp. Parental (non-transfected) MDCKII cells were used as a control. Cells were cultured on a porous filters in 24-well Millipore Transwell inserts to form a tight monolayer producing two solute compartments, one above (apical side) and one below (basolateral side) the cell monolayer. Concentrations of CP-690550 were 1, 10 and 100 μM plus 0.3 $\mu\text{L/mL}$ ^{14}C -CP-690550, resulting in final CP-690550 concentrations of 3, 12 and 102 μM . The total DMSO concentration was kept below 1%.

Confluency of the monolayers was confirmed by trans-epithelial electric resistance of each well prior to treatment. Cells were incubated with CP-690550 or control compounds added to the apical or basolateral chamber. Apical to basolateral and basolateral to apical permeability of lucifer yellow and antipyrine was assessed for low and high permeability control, respectively. Digoxin was used as a positive control. CP-690550 was sampled from the donor compartments before and after incubation to determine the initial concentration and recovery. The bidirectional transport of (b) (4) on parental and MDR1 transfected MDCKII cells was determined in the presence and absence of P-glycoprotein inhibitors ketoconazole 50 μM and verapamil 100 μM applied to both compartments.

^{14}C -(b) (4) was used for sample analysis counted in a liquid scintillation counter and the apparent permeability was calculated.

Results

Post-assay apical to basolateral lucifer yellow incubations indicated the integrity of the monolayers was acceptable. The positive control, digoxin produced the expected inhibition of efflux. The efflux of CP-690550 was approximately 11, consistent with significant efflux due to human P-gp. The efflux ratio of CP-690550 at the high dose 102 μM was 20 which is interpreted as partial saturation of efflux. Positive control P-glycoprotein inhibitors, ketoconazole and verapamil, inhibited CP-690550 efflux by at least 97%.

Study title: In Vitro Inhibition of OATP 1B1 by CP-690550

Study no.: 192119
Study report location: Mod 4. 2.2.6
Conducting laboratory and location: Pharmacokinetics, Dynamics and Metabolism, PGRD, Sandwich, UK
Date of study initiation: Not provided, report signed Oct 22 2010
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: CP-690550-10, Lot E010009450, Purity not provided

Key Study Findings

CP-690550 is an inhibitor of the human hepatic uptake transporter OATP 1B1.

1.

Methods

Wild type (wt) HEK-293 cells and HEK-293 cells over-expressing recombinant human OATP 1B1 (HEK OATP 1B1 cells) cultured in plates were treated with a range of CP-690550 concentrations in combination with 10 μ M (b) (4). Rifamycin (30 μ M) in (b) (4) buffer was used as a positive control. The final concentration of DMSO was kept below 1%. After a 3 min incubation, cells were lysed and CP-690550 content was determined by LC/MS/MS. Samples were also analysed for (b) (4) (and an internal standard added to the lysing solution). Total cell protein per well was also determined.

The amount of (b) (4) uptake was calculated as analyte peak area ratio/min/mg protein. The uptake of (b) (4) observed in HEK-OATP 1B1 cells was background corrected by subtracting the uptake observed in HEK-wt cells at each inhibitor concentration. Then IC_{50} values were determined. The uptake of probe (b) (4) by OATP 1B1 was normalised using the uptake observed in wild type HEK293 cells (representing passive diffusion).

Results

CP-690550 produced a concentration-dependent inhibition of human OATP 1B1 uptake (94% at 1000 μ M) and an IC_{50} of 55.3 μ M. Rifamycin produced 100% inhibition at 30 μ M.

Table 22: Effect of CP690550 on OATP 1B1 Activity**5.1. The Effect of CP-690550 on the Uptake of (b) (4) by OATP 1B1**

Concentration of CP-690550 (μM)	(b) (4) Uptake by HEK-OATP 1B1 (peak area/mg protein/min $\times 10^5$)	Degree of Inhibition (%)
0	35.60 ± 3.25	-
0.1	29.82 ± 0.19	16.4
10	28.11 ± 1.72	21.3
31.25	19.97 ± 1.19	44.4
62.5	14.75 ± 0.80	59.2
125	11.60 ± 0.45	68.2
250	7.81 ± 0.38	79.0
375	7.79 ± 0.10	79.0
500	5.53 ± 0.17	85.4
750	4.31 ± 0.15	88.9
1000	2.50 ± 0.81	94.0
Rifamycin SV (30 μM)	0.40 ± 0.05	100.0

Data are mean \pm SD of triplicate measurements**Study title: In Vitro Inhibition of OATP 1B3 by CP-690550**

Study no.: 095440
 Study report location: Mod 4. 2.2.6
 Conducting laboratory and location: Pharmacokinetics, Dynamics and Metabolism, PGRD, Sandwich, UK
 Date of study initiation: Not provided, report signed Sept 30 2010
 GLP compliance: No
 QA statement: No
 Drug, lot #, and % purity: CP-690550, Batch E0101009450, Purity not provided

Key Study Findings

- CP-690550 is not an inhibitor of OATP 1B3 *in vitro*.

Methods

Wild type (wt) HEK-293 cells were treated with the solutions of rosuvastatin (5 μM) containing increasing concentration of CP-690550 (0.01 to 100 μM). Rifampicin (30 μM) was used as for the positive control. DMSO concentrations were kept below 1% (v/v). Cells were incubated for 2 minutes at 37°C with shaking. The experiment was stopped by removal of uptake buffer, and washes with ice cold blank uptake buffer. Intracellular rosuvastatin samples were retrieved by lysing the cells with 0.5 mL per well 100% methanol containing internal standard (b) (4) 500 ng/mL. Cell lysates analysed by mass spectrometer.

The amount of Rosuvastatin uptake was calculated after correcting for background uptake as analyte peak area: internal standard peak area ratio per mg protein per min.

Results

The uptake of rosuvastatin was not inhibited by CP-690550 up to 100 μ M. Rifampicin at 30 μ M inhibited rosuvastatin uptake by approximately 99%. Therefore CP-690550 had no activity on OATP 1B3

Table 23: Effect of CP-690550 on OATP 1B3 Activity

Table 1. The Uptake of Rosuvastatin by HEK-OATP 1B3 Cells in the Presence of CP-690550 at Increasing Concentrations

Concentration of CP-690550 (μ M)	Rosuvastatin Uptake by HEK-OATP 1B3 Corrected for Passive Uptake by HEK-wt (IS peak area ratio/mg protein/min) \pm SD
0	0.99487 \pm 0.1168
0.01	1.12413 \pm 0.0064
0.03	1.05020 \pm 0.0754
0.1	1.03094 \pm 0.0554
0.3	0.99790 \pm 0.0240
1	1.11001 \pm 0.0342
3	1.02018 \pm 0.0480
10	1.02668 \pm 0.0489
30	0.93663 \pm 0.1149
70	1.02198 \pm 0.0552
100	0.97763 \pm 0.0269
Rifampicin (30 μ M)	0.10352 \pm 0.0145

Data are mean of n=3

Study title: In Vitro Renal Transport Inhibition by CP-690550

Study no.: 135323
 Study report location: Mod 4. 2.2.6
 Conducting laboratory and location: Department of Pharmacokinetics, Dynamics and Metabolism, Pfizer Global Research and Development Groton, Connecticut 06340
 Date of study initiation: Not provided, report signed June 19 2008
 GLP compliance: No
 QA statement: No
 Drug, lot #, and % purity: CP-690550, Batch not provided, Purity not provided

Key Study Findings

- CP-690550 inhibits human mediated cellular uptake.

Methods

Human embryonic kidney (HEK 293) cells transfected with the kidney tubule organic ion transporter human OCT2 were incubated for 2 min with a solution (250 μ L) of 5 μ M [14 C]-creatinine and concentrations (1 μ M-4.1 mM) of CP-690550, cimetidine, or quinidine. The cells were lysed in 1% SDS and the accumulated radioactivity counted. Percent activity calculations were made using zero inhibitor as 100% activity and IC₅₀ determined.

Results

CP-690550 inhibited the uptake of creatinine mediated by hOCT2 in a dose-dependent manner with an IC₅₀ of 150 μ M. The positive controls had IC₅₀ of 406 μ M for cimetidine and 70 μ M for quinidine.

Figure 6: Effect of CP-690550 on OCT2 Activity

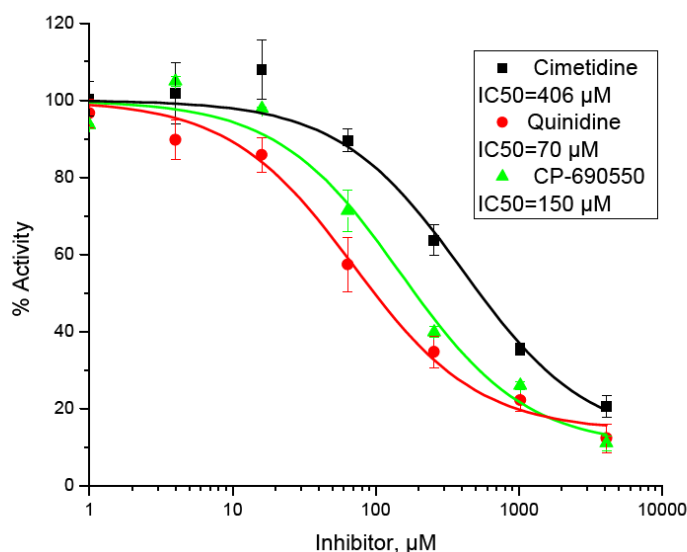


Figure 1: CP-690,550 inhibition of 5 μ M Creatinine uptake mediated by hOCT2. Cimetidine and Quinidine were used as positive controls. The estimated IC₅₀s for CP-690,550, Cimetidine and Quinidine were 150 μ M, 406 μ M and 70 μ M, respectively.

Study title: CP-690550: BCRP Substrate Evaluation

Study no.: 175813
Study report location: Mod 4. 2.2.6
Conducting laboratory and location: Pharmacokinetics, Dynamics and Metabolism, Pfizer, Inc., La Jolla, CA,
Date of study initiation: Not provided, report signed Oct 20 2010
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: CP-690550 E010009450.

Key Study Findings

CP-690550 is not a substrate for efflux by breast cancer resistance protein (BCRP).

Methods

The human transporter gene BCRP (breast cancer resistance protein) was transfected into a Madin-Darby canine kidney (MDCK) cell line that has a low native efflux activity. Apical to basolateral (AB) and basolateral to apical (BA) permeability were measured to determine the efflux ratio ($BA P_{app}/AB P_{app}$) of CP-690550 (2 and 20 μ M) by itself and in the presence of Ko143 (a BCRP inhibitor, 10 μ M). Topotecan (a BCRP substrate, 2 μ M) was the positive control. Atenolol (10 μ M) was added to each well of the plate to assess monolayer integrity. The DMSO content was 1% in the final incubation solution. Incubations lasted 120 min at 37°C and 5% CO₂. LC-MS/MS was used to quantitate the amount of parent CP-690550. These were used to calculate the P_{app} and efflux ratios of CP-690550 and topotecan.

Results

The efflux ratios for CP-690550 were less than 2.5, the cutoff values for being considered a substrate of BCRP efflux, and Ko143 had no effect on these low values. The positive control topotecan had an efflux ratio of 5.8 which was inhibited by Ko143 to a ratio of 1. The atenolol results indicated the cell monolayers were intact, necessary for a valid assay.

Table 24: Effect of CP-690550 on BCRP Activity**Table 1. Permeability and Efflux Ratio Results**

Compound(s)	P _{app} Avg. (AB)	P _{app} Avg. (BA)	Efflux Ratio
CP-690550 – 2 µM	13.5	12.7	0.94
CP-690550 – 2 µM + Ko143	12.9	13.1	1.02
CP-690550 – 20 µM	12.7	13.4	1.05
CP-690550 – 20 µM + Ko143	13.8	14.0	1.01
Topotecan – 2 µM	1.28	7.44	5.83
Topotecan – 2 µM + Ko143	3.64	3.38	0.93

Additional Information:

* P_{app} = x10⁻⁶ cm/sec

* Efflux Ratio = BA average P_{app} / AB average P_{app},
Efflux Ratio > 2.5 = substrate for BCRP efflux

* Ko143 – BCRP inhibitor marker, tested at 10 µM

* Topotecan – BCRP substrate marker

Study title: Effect of CP-690550 on Human Drug Metabolizing Enzymes In Vitro

Study no.: DM2001-690550-020
Study report location: Mod 4. 2.2.6
Conducting laboratory and location: Department of Pharmacokinetics,
Dynamics, and Metabolism
Pfizer Global Research and Development
Pfizer, Inc.
Groton, Connecticut 06340
Date of study initiation: Not provided, report signed Dec 1 2004
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: CP-690550-10, Lot 43798-2-1H, Purity
not provided

Key Study Findings

- CP-690550 had no effect on the in vitro activity of the following metabolic enzymes: CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A.

Methods

Cytochrome substrates were incubated with pooled human liver microsomes. The substrate concentrations were determined empirically to be near the K_m values. CP-690550-10 was tested at 0 (control), 0.30, 3.0, and 30 μM , for CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A, and at 0 (control), 0.0952, 0.301, 0.951, 3, 9.49, and 30 μM for CYP2B6 and CYP2C8. After incubations, an internal standard was added to the termination solution, samples filtered and quantitated from HPLC/MS/MS analysis.

Results

A summary of percentage inhibition at 30 μM of substrate are listed below. CP-690550 did not inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 or CYP3A (felodipine oxidase, midazolam 1'-hydroxylase, testosterone 6 β -hydroxylase) activities. and therefore IC_{50} values could not be calculated.

Summary of IC₅₀ Data for CP-690,550 in Human Liver Microsomes

Marker Substrate Activity	Enzyme	% of control at [I] = 30 µM		IC ₅₀ (µM)	
				Mean	± SE
Phenacetin <i>O</i> -Deethylase	CYP1A2	100		>30	
Bupropion Hydroxylase	CYP2B6	81		>30	
Amodiaquine <i>N</i> -Deethylase	CYP2C8	95		>30	
Diclofenac 4'-Hydroxylase	CYP2C9	81		>30	
S-Mephenytoin 4'-Hydroxylase	CYP2C19	96		>30	
Dextromethorphan <i>O</i> -Demethylase	CYP2D6	110		>30	
Felodipine Oxidase	CYP3A	73		>30	
Midazolam 1'-Hydroxylase	CYP3A	94		>30	
Testosterone 6β-Hydroxylase	CYP3A	84		>30	

Summary of Incubation Conditions: IC₅₀ Determination

Marker Substrate Activity	Enzyme	Substrate Concentration (µM)	Microsomal Protein Concentration (mg/mL)	Incubation Time (min)	Termination Solvent
Phenacetin <i>O</i> -Deethylase	CYP1A2	50 µM	0.03	30	5/92/3
Bupropion Hydroxylase	CYP2B6	80 µM	0.05	20	5/92/3
Amodiaquine <i>N</i> -Deethylase	CYP2C8	1.9 µM	0.025	10	5/92/3
Diclofenac 4'-Hydroxylase	CYP2C9	4 µM	0.03	10	5/92/3
S-Mephenytoin 4'-Hydroxylase	CYP2C19	60 µM	0.2	40	5/92/3
Dextromethorphan <i>O</i> -Demethylase	CYP2D6	5 µM	0.03	10	5/92/3
Felodipine Oxidase	CYP3A	5 µM	0.01	10	50/47/3
Midazolam 1'-Hydroxylase	CYP3A	2.5 µM	0.03	4	92/5/3
Testosterone 6β-Hydroxylase	CYP3A	50 µM	0.03	10	5/92/3

Termination solvent ratio = Acetonitrile/Water/Formic Acid

Study title: An Investigation of the Potential for (b) (4) to induce CYP3A4 and CYP1A2 in human hepatocytes

Study no.: DM2007 (b) (4) 001
Study report location: Module 4.2.2.6
Conducting laboratory and location: Induction CoE Lab
Pharmacokinetics, Dynamics, and
Metabolism Department
Pfizer Global Research and Development
Pfizer Inc
Groton, Connecticut 06340
Date of study initiation: Not provided, report signed Oct 9 2008
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: (b) (4) (CP-690550), Lot Lot
Number (b) (4), Purity
not provided

Key Study Findings

- In vitro studies with Fa2N-4 immortalized human hepatocytes and cryopreserved human hepatocytes found that CP-690550 induced CYP3A4, but not CYP1A2 enzymes.
- The induction of CYP3A4 was demonstrated through CP-690550 dose-dependent induction of CYP3A4 mRNA in both types of cells and the induction of testosterone 6 β -hydroxylase activity in Fa2N-4 cells. CP-690550 did not increase testosterone 6 β -hydroxylase activity in cryopreserved human hepatocytes
- There was no effect of CP-690550 on ethoxyresorufin-O-deethylation activity in either cell type.

Methods

Two types of human cells, Fa2N-4 immortalized human hepatocytes and cryopreserved human hepatocytes were used in these studies. The cells were treated for 76 hours with 8 concentrations of CP-690550 for the Fa2N-4 cells (0.78 to 100 μ M) and 5 concentrations for the cryopreserved human hepatocytes (6.25 to 100 μ M). Positive controls were rifampin (25 μ M) for CYP3A and omeprazole (50 μ M) for CYP1A2. The rate of product formation was determined by fluorescence detection, and results were normalized to percent of control using background (vehicle) as 1 fold. Measurements of CYP3A4 mRNA levels were performed using the commercial Invader assay.

As part of the demonstration of assay validity, Fa2N-4 cells and cryopreserved human hepatocytes (Lot HU4026 was tested, Lot RCP was not tested) were examined for cytotoxicity using the WST-1 assay. The cells "appeared normal throughout the experiments." Fa2N-4 cells and cryopreserved human hepatocytes of Lot HU4026 showed no evidence of cytotoxicity.

Results

Effect on CYP3A4

Positive control:

Rifampin produced a significant induction of CYP3A4 relative to the 0.1% DMSO vehicle control in both cell lines. In Fa2N-4 cells, rifampin produced a 10-fold induction of CYP3A4 mRNA transcript, and 3.1-fold induction of testosterone 6 β -hydroxylase activity. In cryopreserved human hepatocytes rifampin produced a 7.7-fold induction of CYP3A4 mRNA transcript, and 15-fold induction testosterone 6 β -hydroxylase activities. In a second lot of cryopreserved human hepatocytes, rifampin induced a 30-fold increase in CYP3A4 mRNA transcript.

CP-690550:

Treatment of the Fa2N-4 cells with CP-690550 (0.78 to 100 μ M) resulted in a 1.2 to 2.5-fold induction of CYP3A4 mRNA and testosterone 6 β -hydroxylase activity. Treatment of the cryopreserved human hepatocytes with CP-690550 (6.25-100 μ M) resulted in a 2.5 to 6.3-fold induction of CYP3A4 mRNA for one lot of hepatocytes (Lot RCP) and a 1.9 to 13-fold induction of CYP3A4 mRNA for a different lot of hepatocytes (Lot HU4026). There was no significant induction of testosterone 6 β -hydroxylase activity with cryopreserved hepatocytes of Lot RCP, and technical malfunction hindered the study with the other lot of cells.

Effect on CYP1A2

Positive control:

In the Fa2N-4 cells, omeprazole induced a 32-fold increase in CYP1A2, based on the increase in ethoxyresorufin-O-deethylation activity. In cryopreserved human hepatocytes, omeprazole induced a 36-fold (Lot RCP) and 6.8-fold (Lot HU4026) induction of CYP1A2.

CP-690550:

CP-690550 did not induce CYP1A2 in either Fa2N-4 cells (0.78 to 100 μ M CP-690550) or cryopreserved human hepatocytes (6.25 to 100 μ M CP-690550).

6 General Toxicology

6.1 Single-Dose Toxicity

Study title: Acute Oral Toxicity Study in Sprague-Dawley Rats

Study no.:	01-2063-07
Study report location:	4.2.3.1-single-dose-tox
Conducting laboratory and location:	Pfizer Global Research and Development, Groton, CT
Date of study initiation:	March 2, 2001
GLP compliance:	Yes, with the exception that a non-validated assay was used for plasma drug analysis
QA statement:	Yes
Drug, lot #, and % purity:	CP-690550-10; Lot # 43798-2-1H; Purity 96.9% Composition 60% active moiety 38.1% citric acid salt;

There was no Certificate of Analysis included with this report. .

Key Study Findings

- A maximum tolerated dose was not determined due to CP-690550-related lethality at all doses, 500, 1000 and 2000 mg/kg. Deaths occurred within 2 days after drug administration for all animals in the intermediate and high dose groups and one female dosed at 500 mg/kg. All these animals were noted as having stomach distention with fluid and gas, which was verified at necropsy and the stomach contained white pasty contents, likely unabsorbed drug substance.
- The reported toxicokinetic parameters were considered preliminary since the assay was not yet fully validated. The AUC₀₋₂₄ at 500 mg/kg was 504000 ng·h/mL. Toxicokinetic analysis for the mid and high doses groups were limited due to early deaths.
- The primary clinical signs included salivation, stained fur, eye staining, lacrimation, partially closed eyes, nasal discharge, slow respiration, labored respiration, decreased activity, lethargy, and cold to the touch.
- Histopathological changes included hepatocyte vacuolation (centrilobular, periportal, and generalized) at all doses and single cell liver necrosis at the 1000 and 2000 mg/kg dose. At all doses, lymphocytolysis with a reduction in the lymphocyte population occurred in the mesenteric lymph node and spleen.
- On day 2 in the 500 mg/kg dose group, mean white blood cell counts were reduced in males and females (69% and 72% of control values, respectively) due to decreased eosinophils, monocytes, and neutrophils. Red blood cell counts were slightly lower in males and females (92% and 95%, respectively), associated with a slight decrease in hemoglobin (93% and 96% respectively).

Additional changes included decreased fibrinogen (79% and 73% in males and females, respectively) increases ALT (288% and 152%), AST (250% and 251%), glucose (147% and 124%), and BUN (200% and 167%). All parameters recovered to control levels by day 15.

Methods

Doses: 0, 500, 1000, 2000 mg/kg
(mg/kg dose is based on the active moiety composition of the drug substance)

Frequency of dosing: single dose

Route of administration: oral by gavage

Dose volume: 20 mL/kg

Formulation/Vehicle: 0.5% methylcellulose

Species/Strain: Sprague-Dawley rats

Number/Sex/Group: 3/sex/dose

Age: Approximately 9 weeks old at study initiation

Weight: males: 299 to 327 g; females: 184 to 219 g

Satellite groups: Toxicokinetic: 5/sex/group

Study design:

Table 1. Dose groups in the main study

Group	Dose (mg/kg)	Dose Volume (mL/kg)	Drug Concentration (mg/mL)	Animal Numbers	
				Males	Females
1	0	20	0	1-3	13-15
2	500	20	25	4-6	16-18
3	1000	20	50	7-9	19-21
4	2000	20	100	10-12	22-24

Deviation from study protocol: These were not indicated in this non-GLP study.

Observations and Results

Mortality

Eleven out of 13 rats died during the study. All the rats dosed at 1000 and 2000 mg/kg and one female at 500 mg/kg died before day 3. All animals were noted as having stomach distention with fluid and gas, which was verified at necropsy.

Table 25: Summary of Mortalities

Mortality								
Dose (mg/kg/day)	0		500		1000		2000	
Sex	M	F	M	F	M	F	M	F
N	3	3	3	3	3	3	3	3
Deaths day 1: 4-10 h	0	0	0	1	1	0	3	2
Deaths on day 2	0	0	0	0	2	3	0	1
Survivors at end of day 2	3	3	3	2	0	0	0	0

Clinical signs

Monitored once daily from day -16 through the day before treatment (day -1), and then twice daily from day 1 (day of dosing) through the end of the study (day 15).

Control rats showed no clinical signs. Most of the clinical signs in CP-690550-dosed animals began 2 hours post-dose corresponding with the T_{max} from 2 to 4 hours postdose.

The female found dead 7 hours postdose in the 500 mg/kg group had signs of decreased activity, lethargy, partially closed eyes, and salivation, and there was 1 male with no clinical signs in this dose group. Other clinical signs included decreased activity and lethargy, partially closed eyes, labored respiration, and salivation. On day 2 there were no clinical signs except for the 1 male with red stained fur around the nose and muzzle there were fewer clinical signs.

At higher doses, similar clinical signs were observed almost all animals with the addition of lacrimation, cold to the touch and slowed respiration. One male dosed at 1000 mg/kg had a decrease in activity, was lethargic at 2 hours post-dose, and was dead 4 hours post-dose.

Body weights

Weighed once pre-study, 6 days before treatment (day -6), once pre-dose on day 1, then once each on days 5, 9, and 13.

There were no CP-690550-related effects on bodyweight or weight gain in the 500 mg/kg dose group. Due to mortalities, higher dose groups were not analyzed.

Food consumption

not monitored

Hematology

Blood samples collected on days 2, 7, and 14 of surviving animals. The parameters evaluated are listed here:

red blood cell count (RBC, $\times 10^6/\text{mm}^3$)
 hemoglobin concentration (HGB, g/dL)
 hematocrit (HCT, %)
 platelet count (PLT, $\times 10^3/\text{mm}^3$)
 mean corpuscular volume (MCV, fL)
 mean corpuscular hemoglobin (MCH, pg)
 mean corpuscular hemoglobin concentration (MCHC, %)
 white blood cell count (WBC, $\times 10^3/\text{mm}^3$)
 white blood cell differential count (WBC Differential, % and absolute)
 neutrophils (N, %) (NCT, $/\text{mm}^3$)
 lymphocytes (L, %) (LCT, $/\text{mm}^3$)
 monocytes (MO, %) (MOCT, $/\text{mm}^3$)
 eosinophils (EO, %) (EOCT, $/\text{mm}^3$)
 basophils (B, %) (BCT, $/\text{mm}^3$)
 large unstained cells (LUC, %) (LUCT, $/\text{mm}^3$)
 reticulocyte counts (RET, %)

Fibrinogen (FIBR, mg/dL) was measured using a manual procedure.

An adequate hematological battery was evaluated. Due to acute mortality in the higher dose groups, only the control and 500 mg/kg dose groups were analyzed.

Mean WBC were reduced on day 2 in males and females (69% and 72% of control values, respectively), due to decreased eosinophils (12% and 14% of control values, respectively), monocytes (75% and 60% of control values, respectively), and neutrophils (88% and 49% of control values, respectively). Red blood cell counts were slightly lower in males and females on days 2 (92% and 95% of control values, respectively), associated with a slight decrease in hemoglobin (93% and 96% of control values, respectively). Fibrinogen was reduced on day 2 (79% and 73% of control values, respectively). Few of these values were statistically significant due to large variations among animals within a dose group and limited (n=3) animal numbers. All values were similar to control values by day 15.

Table 26: Hematology Summary

Dose (mg/kg/day)	Day	0		500	
Sex		M	F	M	F
N		3	3	3	2
WBC ($/\text{mm}^3$)	2	12.1	13.1	8.4 (69%)	9.4 (72%)
	7	14.9	14.3	13.6	13.0
	14	13.3	13.0	13.5	12.0

Dose (mg/kg/day)	Day	0		500	
Sex		M	F	M	F
N		3	3	3	2
Monocytes (/mm³)	2	373	341	280 (75%)	203 (60%)
	7	388	356	399	530
	14	368	276	441	430
Neutrophils (/mm³)	2	1575	1415	1381 (88%)	697 (49%)
	7	1906	1517	1793	1286
	14	1336	1286	1707	1544
Eosinophils (/mm³)	2	110	69	12.7 (12%)	9.5 (14%)
	7	123	105	20	40
	14	183	175	82	112
RBC (mil/mm³)	2	7.9	8.2	7.3 (92%)	7.8 (95%)
	7				
	14	8.3 8.3	8.0 8.1	7.8 7.8	7.6 8.2
Hemoglobin (g/dL)	2	15.8	15.9	14.7 (93%)	15.2 (96%)
	7	16.3	15.8	15.2	14.9
	14	15.8	15.9	15.3	15.9
Fibrinogen	2	325	294	257 (79%)	214 (73%)
	7	355	296	318	245
	14	301	268	324	238

Clinical chemistry

blood samples collected on days 2, 7, and 14 of surviving animals, parameters evaluated are listed here:

alanine aminotransferase (ALT, U/L)	aspartate aminotransferase (AST, U/L)
γ-glutamyl transferase (GGT, U/L)	5' nucleotidase (5'NT, U/L)
total bilirubin (TB, mg/dL)	total protein (TP, g/dL)
albumin (ALBM, g/dL)	cholesterol (CHOL, mg/dL)
glucose (GLUC, mg/dL)	blood urea nitrogen (BUN, mg/dL)
creatinine (CREA, mg/dL)	sodium (NA, meq/L)
potassium (K, meq/L)	chloride (CL, meq/L)
calcium (CA, mg/dL)	

globulin (GLOB, g/dL) was calculated.

An adequate clinical chemistry battery was assessed. On day 2, at 500 mg/kg, there were increases in ALT (288% and 152% of control for males and females, respectively),

AST (250% and 251% of control for males and females, respectively), glucose (147% and 124% of control for males and females, respectively), and BUN (200% and 167% of control for males and females, respectively). The values were similar to controls by day 15.

Table 27: Serum Chemistry Summary

Dose (mg/kg/day)	Day	0		500	
Sex		M	F	M	F
N		3	3	3	2
ALT (U/L)	2	48	40	138 (288%)	61 (152%)
	7	49	44	35	36
	14	47	41	45	39
AST (U/L)	2	102	83	255 (250%)	208 (251%)
	7	96	88	80	73
	14	88	83	91	75
Glucose (mg/dL)	2	99	116	146 (147%)	144 (124%)
	7	102	106	98	112
	14	102	102	94	106
BUN (mg/dL)	2	12	12	24 (200%)	20 (167%)
	7	13	13	14	18
	14	12	14	14	14

Gross pathology

All surviving animals were necropsied on day 15, non-survivors were necropsied as soon as possible after found. All of the organs listed in the histopathology section that were collected were examined grossly as well as any lesions in tissues not collected.

There were no pathological findings in the control and 500 mg/kg dose groups.

All animals dosed at 1000 and 2000 mg/kg showed signs of stomach distention with fluid and gas. The stomach contents were described as a white pasty material. Two animals had dry contents within the cecum. Four animals had regions of jejunum which were described as dark red transmurally, and contained dark red fluid contents. The applicant believed this to be the result of autolysis, although drug-related hemorrhage is a more probable interpretation with gastrointestinal hemorrhage as a cause of death. The lungs of two animals found dead had areas of dark red discoloration consistent with microscopic findings suggesting congestion and hemorrhage which is another cause of death.

Organ weights

not conducted

Histopathology

Adequate Battery: Yes, the battery of tissues assessed was adequate, however the study design was inadequate. There was no interim assessment within 1-2 days of drug administration to examine acute drug toxicities.

Peer review: Not an outside pathologist, it was reviewed by an in-house pathologist

kidneys	parathyroid
urinary bladder	testes (left and right)
liver (left and right lateral lobes)	epididymides
thymus	prostate
spleen	seminal vesicle
mesenteric lymph node	ovaries
esophagus	uterus
stomach	vagina
duodenum	trachea
jejunum	lung (both diaphragmatic lobes)
ileum	heart
cecum	peripheral nerve
colon	brain (cerebrum, cerebellum and pons)
pituitary gland	spinal cord (cervical)
salivary gland	Harderian gland
skeletal muscle	eyes
pancreas	skin and adnexa (including mammary gland)
adrenal glands	bone (sternum, including bone marrow)
thyroid gland	

There were no findings at the low dose, 500 mg/kg, examined at day 15. There was no interim analysis for acute effects on day 2 or 3.

In animals that did not survive to termination, there were pathological findings in the liver, mesenteric lymph node, and spleen. For the males in the 500 mg/kg dose group, all histopathological finding severities were slight, for females they were also slight except for 1 animal in which lung hemorrhage and congestion was moderate. Many of the organs and tissues from the animals that died early had autolytic findings.

Liver

Vacuolation (centrilobular, periportal, and generalized) and necrosis (single cell or generalized) occurred in the liver.

Lung

Hemorrhage and congestion occurred in the 1000 and 2000 mg/kg dose groups. This was not associated with acute inflammation which was only found in the control and low dose males.

Lymphoid Tissue: Mesenteric Lymph Node and Spleen

Lymphocytolysis was present in the mesenteric lymph node and spleen. There was a decrease in the number of lymphocytes in the marginal zone of the splenic white pulp.

Heart

One high dose female had myocardial inflammation. There was no associated myonecrosis or fibrosis.

Table 28: Histopathology Summary

Dose (mg/kg/day)	0		500		1000		2000	
Sex	M	F	M	F	M	F	M	F
N	3	3	3	3	3	3	3	3
Liver								
necrosis	0	0	0	0	0	1	0	0
single cell necrosis	0	0	0	0	1	0	1	0
vacuolation, general	0	0	0	0	0	1	0	1
centrilobular	0	0	0	1	1	0	0	0
periportal	0	0	0	0	0	1	0	0
Heart								
myocardial inflammation	0	0	0	0	0	0	0	1
Lung								
acute inflammation	2	0	2	0	0	0	0	0
hemorrhage	0	0	0	1	1	1	2	1
congestion	0	0	0	1	3	3	3	3
Mesenteric Lymph Node								
lymphocytolysis	0	0	0	1	3	3	3	3
Spleen								
lymphocytolysis	0	0	0	0	2	3	1	2
lymphoid depletion	0	0	0	1	3	3	3	3

Toxicokinetics

Blood was collected at 0.5, 1, 2, 4, 8, and 24 hours post-dose. Plasma CP-690550 concentrations were determined using a non-validated LC-MS/MS method at the Drug Metabolism Division, Groton, CT.

The toxicokinetic data presented below is considered preliminary and not acceptable for regulatory decisions since the methodology for CP-690550 measurement was not validated.

For the 500 mg/kg group, t_{max} , C_{max} , and AUC_{0-24h} were determined. However, because of animal deaths in the other groups, only $AUC_{(0-8)}$ could be determined for the 1000 mg/kg group, and AUC_{0-4h} for the 2000 mg/kg group. C_{max} and AUC_{0-4h} both appeared to

increase proportionally as the dose was increased from 500 to 2000 mg/kg. There were no sex differences in the TK parameters.

Dose (mg/kg)	Gender	Mean C _{max} (µg/mL)	Mean T _{max} (h)	Mean AUC _(0-4h) (µg•hr/mL)	Mean AUC _(0-8h) (µg•hr/mL)	Mean AUC _(0-24h) (µg•hr/mL)
500	M	26.4	4.0	86	176	339
	F	39.9	8.0	130	286	683
	M+F	32.4	4.0	108	231	504
1000	M	65.2	8.0	203	441	^a
	F	77.5	2.0	247	482	
	M+F	69.0	2.0	225	461	
2000 ^b	M	125	4.0	379		
	F	125	4.0	415		
	M+F	125	4.0	398		

^a Only one male sampled. All other animals died.

^b All animals died prior to 8 and 24 hour post-dose blood sampling.

Study title: Single Dose Intravenous Toxicity Study of CP-690550 in Rats with a 14-Day Recovery

Study no.: 09GR453
 Study report location: 4.2.3.1-single-dose-tox
 Conducting laboratory and location: Drug Safety Research & Development,
 Pfizer Global Research & Development,
 Groton, CT
 Date of study initiation: Jan 8, 2010
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: CP-690550-10 (citrate salt),
 Lot GR02684 subplot of Lot E010009450,
 Purity 100.2%
 Composition: active moiety 61.8%

There was no Certificate of Analysis included with this report. Purity was indicated in Table 2.6.7.4 in Module 2.

Key Study Findings

- This study was conducted to determine the safety of an intravenous formulation of the compound that will be used in a single dose human bioavailability study involving intravenous administration. The emphasis of the submitted toxicology study was local injection site reactions.
- Administration of CP-690,550 as a single intravenous bolus at 0.5, 1, or 3 mg/kg did not result in CP-690550-related gross or microscopic findings at the injection site.

- The NOAEL was 3 mg/kg corresponding to serum CP-690,550 concentration of 1140-1870 ng/mL 10 minutes following IV injection.

Methods

Doses: 0, 0.5, 1, and 3 mg/kg
(mg/kg dose is based on the active moiety composition of the drug substance).

Frequency of dosing: Single dose

Route of administration: Intravenous

Dose volume: 3 mL/kg

Formulation/Vehicle: 10 mM lactic acid in normal saline

Species/Strain: Sprague-Dawley (CrI:CD®[SD]) rats

Number/Sex/Group: 5/sex/dose

Age: 7-8 weeks

Weight: males 241.3-311.1g; females 169.8-216.6g

Satellite groups: Recovery (day 15 termination) 5/sex/dose
Toxicokinetic (day 1, 5/sex/dose/timepoint)

Study design:

Group Number	Dose (mg/kg/day)	Dose Volume (mL/kg)	Main Study Animal Numbers		Recovery Animal Numbers	
			Males	Females	Males	Females
1	0	3	1-5	41-45		
2	0.5	0.5	6-10	46-50		
3	1	1	11-15	51-55		
4	3	3	16-20	56-60		
5	0	3			21-25	61-65
6	0.5	0.5			26-30	66-70
7	1	1			31-35	71-75
8	3	3			36-40	76-80

All dose levels were expressed as mg of active drug moiety per kg of body weight per day.

Animals were administered a single dose via intravenous injection. The dose volume for each animal (listed above) was based on Day 1 individual body weights and administered as a slow bolus dose. Animals in Groups 1-4 were necropsied on Day 2; animals in Groups 5-8 were necropsied on Day 15.

Main study animals were euthanized on day 2.

Recovery animals were euthanized on day 15.

Deviation from study protocol: There were no deviations that affected the study conclusions.

Observations and Results

Clinical Signs: On the day of dosing, all animals were observed predose, approximately 1-2 hours postdose, and near the end of the workday, and subsequently twice daily until necropsy.

Bodyweights: recorded pretreatment, prior to dosing on day 1, and on days 2, 4, 8, and 15.

Food consumption: recorded weekly (days 8 and 15) for the recovery animals (Groups 5-8).

Results

There were no mortalities. There were no CP-690550-related effects for clinical observations, body weight, food consumption, and gross necropsy findings. Assessments of ophthalmology, hematology, clinical chemistry, urinalysis, and organ weights were not conducted.

Histopathology

A gross examination was performed at necropsy, and representative samples of injection site tissues were collected from all animals. Injection site tissues that were collected from main study animals in the control and 3 mg/kg dose groups on day 2 (Groups 1 and 4) and recovery animals on day 15 (Groups 5 and 8) were evaluated microscopically.

Minimal perivascular hemorrhage and minimal perivascular and/or dermal inflammation was noted for most injection sites in control and high dose rats sacrificed on day 2. Hemorrhage and most inflammatory findings had resolved by day 15.

Table 29: Histopathology Summary

Histopathology								
Dose (mg/kg)	0		0.5		1		3	
Sex	M	F	M	F	M	F	M	F
N	5	5	5	5	5	5	5	5
Day 2								
Injection Site 1								
Number examined	5	5	0	0	0	0	5	5
Thrombosis, grade 1	1	0	-	-	-	-	0	0
Hemorrhage, grade 1	5	5	-	-	-	-	4	3
Inflammation, grade 1	3	4	-	-	-	-	4	5
Recovery, Day 15								
Injection Site 1								
Number examined	5	5	0	0	0	0	5	5
Thrombosis, grade 1	1	0	-	-	-	-	0	0
Fibrosis, grade 1	1	0	-	-	-	-	0	0
Inflammation, grade 1	0	0	-	-	-	-	1	2

Histopathology								
Dose (mg/kg)	0		0.5		1		3	
Sex	M	F	M	F	M	F	M	F
N	5	5	5	5	5	5	5	5
Skin and adnexa								
Number examined	0	0	0	0	1	0	0	0
chronic inflammation, grade 2	-	-	-	-	1	-	-	-
- no tissue sample was collected								

Toxicokinetics

Blood samples were collected from the jugular vein of 5 animals/sex (Groups 1-4) on day 1 at approximately 10 minutes postdose for measurement of CP-690,550 serum concentrations.

Samples were analyzed by the (b) (4). The results from the complete validation of CP-690550 in rat serum are given in (b) (4) Validation Report AR690A, not submitted, but summarized in the Bioanalytical report.

Systemic exposure following a single intravenous bolus increased with dose and there were no exposure differences between sexes.

Table 30: Toxicokinetics of CP-690550*

Dose (mg/kg)	0	0.5	1	3
CP-690550 (ng/mL)	-	182-323	337-569	1140-1870

* 10 min postdose sample, combined sexes with n=5/sex/dose

Drug Stability and Homogeneity

Stability was verified previously and the solutions were maintained under those previously verified conditions. Homogeneity: was not assessed. The dose concentration analysis was 100-101% of intended concentrations.

Study title: Single Day Oral Toxicity Study in Cynomolgus Monkeys

Study no.: 00-2063-04
 Study report location: 4.2.3.1-single-dose-tox
 Conducting laboratory and location: Drug Safety Evaluation Department, Pfizer Global Research and Development, Groton, CT 06340
 Date of study initiation: Oct 11, 2000
 GLP compliance: Yes, except a non-validated assay was used for plasma CP-690550 analysis
 QA statement: Yes
 Drug, lot #, and % purity: CP-690550-10; Lot # 44207-207-1; Purity 97.15%

Composition 61.9% active moiety
38.1% citric acid salt;

There was no Certificate of Analysis included with this report. Purity was indicated in Table 2.6.7.4 in Module 2.

Key Study Findings

- CP-690550-10 administered orally to cynomolgus monkeys (2/sex/dose) on a single day, as a split oral dose of 13, 67, or 333 mg/kg TID, corresponding to a total daily dose of ~40, ~200, and ~1000 mg/kg, respectively.
- At ≥ 67 mg/kg TID (≥ 200 mg/kg/day), signs of emesis and decreased activity were noted. There were no terminal studies to assess macroscopic or microscopic pathology.
- Preliminary assessment of toxicokinetic parameters with a non-validated assay indicated an increase in plasma exposure with increasing dose and no differences between sexes. For the low dose with no identified toxicity, the AUC₀₋₂₄ was 40,200 ng-h/mL.

Methods

Doses:	0, 40, 200, 1000 mg/kg/day single dose, but split as three dose administrations per day of 13, 67, and 333 mg/kg
	(mg/kg dose is based on the active moiety composition of the drug substance)
Frequency of dosing:	TID approximately 7 hours apart
Route of administration:	Orally, by gavage
Dose volume:	7 mL/kg
Formulation/Vehicle:	0.5% methylcellulose
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	2/sex/dose
Age:	Not available
Weight:	males: 3.4 kg; females: 12.9 kg
Satellite groups:	None
Unique study design:	Necropsy and histopathology were not conducted since the animals were not terminated at the study completion.

Table 1. Dose groups

Group	Dose (mg/kg)	Dose Volume (mL/kg)	Drug Concentration (mg/mL)	Animal Numbers	
				Males	Females
1	0	7	0	1-2	9-10
2	40	7	1.9	3-4	11-12
3	200	7	9.52	5-6	13-14
4	1000	7	47.62	7-8	15-16

Deviation from study
protocol:

Observations and Results

Mortality

Animals were checked at least twice daily.
There were no deaths during the course of the study

Clinical signs

Animals were checked at least twice on the day of dosing, and then at least once a day for 14 days after dosing.

Clinical signs included emesis on days 1 and 2 and decreased activity at doses of 200 and 1000 mg/kg.

Body weight

Measured once pre-study, and on days 1, 4, 8 and 15.

There was no effect of CP-690550 on body weights or weight gain.

Food consumption

Estimated daily as within the categories of <25%, ~25%, ~50%, ~75%, ~100%, or spill of the total ration.

There were no effects on food consumption in the study. With only 2 animals per group and qualitative assessments, only rather large differences would be detected, indicative of animals off-feed, not eating, possible due to toxicity. There were numerous other influences on feeding in the study design, such as stress associated with TID dosing, restraint, and multiple bleeds for toxicokinetics.

Hematology

Blood samples were collected pre-dose, Day 2, and Day 15. The parameters evaluated are presented below.

red blood cell count (RBC, $\times 10^6/\text{mm}^3$)
 hemoglobin concentration (HGB, g/dL)
 hematocrit (HCT, %)
 platelet count (PLT, $\times 10^3/\text{mm}^3$)
 mean corpuscular volume (MCV, fL)
 mean corpuscular hemoglobin (MCH, pg)
 mean corpuscular hemoglobin concentration (MCHC, %)
 white blood cell count (WBC, $\times 10^3/\text{mm}^3$)
 white blood cell differential count (WBC Differential, % and absolute)
 neutrophils (N, %) (NCT, $/\text{mm}^3$)
 lymphocytes (L, %) (LCT, $/\text{mm}^3$)
 monocytes (MO, %) (MOCT, $/\text{mm}^3$)
 eosinophils (EO, %) (EOCT, $/\text{mm}^3$)
 basophils (B, %) (BCT, $/\text{mm}^3$)
 large unstained cells (LUC, %) (LUCT, $/\text{mm}^3$)

Reticulocyte counts (RET, %) were determined on the Adiva 120.

The following coagulation tests were performed using the ACL 3000+:

prothrombin time (PT, sec)
 activated partial thromboplastin time (APTT, sec)

The hematological assessment was adequate.

CP-690550-related hematological effects were not evident due to the variability associated with only 2 animals per sex per dose group. The changes observed on day 2 were attributed by the applicant to stress associated with TID dosing, restraint, and multiple bleeds for toxicokinetics. The reviewer concurs that this is a reasonable explanation for the minor changes observed, although an effect due to CP-690550 cannot be eliminated. These changes were evident in variability of vehicle control animals, for example % neutrophils in males for the two animals was 20% and 22% at predose, 49% and 34% one day after dosing and 22% and 55% on day 15. For this reason, no confident conclusions about the effects of CP-690550 are possible, whether stress or drug induced.

Clinical chemistry

Blood samples were collected pre-dose, Day 2, and Day 15. The parameters are presented below.

alanine aminotransferase (ALT, U/L)	aspartate aminotransferase (AST, U/L)
alkaline phosphatase (ALP, U/L)	γ -glutamyl transferase (GGT, U/L)
total bilirubin (TB, mg/dL)	total protein (TP, g/dL)
bile acids (BILA, $\mu\text{M/L}$)	cholesterol (CHOL, mg/dL)

albumin (ALBM, g/dL)
triglycerides (TRIG, mg/dL)
creatinine (CREA, mg/dL)
sodium (NA, meq/L)
chloride (CL, meq/L)

glucose (GLUC, mg/dL)
blood urea nitrogen (BUN, mg/dL)
potassium (K, meq/L)
calcium (CA, mg/dL)

Globulin (GLOB, g/dL) was calculated.

The clinical chemistry battery was adequate. CP-690550-related effects on chemistry parameters were not able to be discerned for the same reasons presented in the hematology section, above.

Gross pathology

No terminal studies were performed.

Organ Weights

No terminal studies were performed.

Histopathology

No terminal studies were performed.

Toxicokinetics

For the toxicokinetic study, femoral vein blood samples were collected from each animal at approximately 0.5 h after each (split) dose, 0.5 h before the second and third split doses, and 24 h after the first dose. Plasma samples were analyzed for CP-690550 using a non-validated HPLC/MS/MS method the Drug Metabolism Division, Groton, CT.

The toxicokinetic data presented below is considered preliminary and not acceptable for regulatory decisions since the measurement of CP-690550 was not validated. A reasonable limited assessment indicated that exposure increased with dose, despite the large variability among animals at higher doses.

Table 31: Toxicokinetic Summary

Dose (mg/kg)	Monkey #	Sex	AUC _(0-24h) (ng*h/mL)	Avg AUC _(0-24h) (ng*h/mL)
40	3	M	5860	6570
	4	M	7280	
	11	F	7530	6645
	12	F	5760	
200	5	M	32900	46500
	6	M	60100	
	13	F	27400	28050
	14	F	28700	
1000	7	M	29100	53350
	8	M	77600	
	15	F	53600	75750
	16	F	97900	

**Repeat-Dose Toxicity
STUDIES IN RATS****Study title: A Two-Week Oral Exploratory Toxicity Study with CP-690550-10 in
Sprague-Dawley Rats**

Study no.: 00-2063-03
Study report location: 4.2.3.2
Conducting laboratory and location: Pfizer Global Research and Development,
Groton, CT
Date of study initiation: 9 Jan 2003
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: CP-690550-10, lot #44207-207-1, Purity
97.15%,
Composition: 61.9% active moiety

There was no Certificate of Analysis included with this report. A description of HPLC evaluation was included.

Purity/composition: Based on HPLC evaluation there were 3 impurities which were (b) (4) than CP-690,550 ranging from an area of (b) (4) and 6 impurities which were (b) (4) than CP-690,550 ranging from an area of (b) (4). Based on this, the purity of CP-690,550 was 97.15%.

Key Study Findings:

- CP-690550 was administered orally to Sprague Dawley rats (n=5/sex/dose) at doses of 0, 10 and 100 mg/kg/day. To determine tolerability another dose group of 30 mg/kg/day was escalated to 300 mg/kg/day on day 7, then to 1000 mg/kg/day on day 11. There was one death in the study, a female on the second day (study day 12) after receiving 1000 mg/kg/day.
- At 1000 mg/kg/day, clinical signs were salivation, noisy respiration, hair loss, and staining of the eyes and muzzle. There were no clinical signs at lower doses.
- Hematologic findings included decreased WBC and lymphocytes (≥ 10 mg/kg/day), erythroid parameters (RBC, Hb, and Hct) and platelets (at 1000 mg/kg/day), and reticulocytes (at all doses). In the high dose group, mean erythroid parameters were also decreased on day 14 corresponding to 3 days of the 1000 mg/kg/day dose (RBC 76-78%, Hct 73-74%, Hb 77-79%, reticulocytes 6-14% of control values). At ≥ 10 mg/kg/day reticulocytes were reduced on day 7. For white blood cells, maximal reductions in the high dose group were WBC 33%, Lymph 10% of control value) with a dose- and time-dependency. Also mean eosinophil and basophil counts were also reduced in all treated groups on day 7, 10, and 14 (maximal reductions occurred at 1000 mg/kg/day on days 10 or 14, Eos 8%, Bas 10%).

- Chemistry findings included increases in ALT, AST, and GGT in the 30/ 300/1000 mg/kg/day dose group which corresponded with liver necrosis in 1 male, and increased liver weight and centrilobular hypertrophy in other animals.
- CP-690550 produced generalized depletion in the bone marrow at ≥ 100 mg/kg/day, mostly due to erythroid depletion, and lymphoid depletion in spleen, thymus, mesenteric lymph node at ≥ 10 mg/kg/day. Substantial increases in myeloid cells and the reduction in erythroid cells in the 30/300/1000 mg/kg/day dose group resulting an M:E ratio >1300 were likely a response to severe liver (necrosis) and stomach pathology (moderate multifocal necrosis of glandular stomach) noted in some animals.
- As a non-GLP exploratory study, the study will not be used to support regulatory decisions. However, based on these data the applicant considered the 100 mg/kg/day dose, a tolerable dose, and used this dose as the high dose in the majority of the GLP toxicology studies in the rat.

Methods

Doses:	0, 10, 30, 100, 300, and 1000 mg/kg/day,
	The 300 and 1000 mg/kg/day doses were administered by an escalation of the 30 mg/kg group to 300 mg/kg on Days 7, 8, 9, and 10 and then escalated again to 1000 mg/kg on Days 11, 12, 13, and 14.
Frequency of dosing:	The mg/kg dose is based on the active moiety composition of the drug substance.
Route of administration:	once daily for 14 days
Dose volume:	oral by gavage
Formulation/Vehicle:	10 mL/kg
Species/Strain:	0.5% methylcellulose
Number/Sex/Group:	Sprague-Dawley rats
Age:	5/sex/dose
Weight:	50 days of age
	males 222.7 to 255.9 g
	females 168.6 to 201.6 g
Satellite groups:	None
Unique study design:	In order to attain a maximum tolerated dose, an escalation of the 30 mg/kg group to 300 mg/kg was performed for Days 7, 8, 9, and 10 and then escalated again to 1000 mg/kg for Days 11, 12, 13, and 14.

Group	Daily Dose* (mg/kg)	Dose Volume (ml/kg)	Drug Concentration (mg/ml)	<u>Animal Numbers</u>	
				Males	Females
Group 1 0.5% Methylcellulose	0	10	0	1-5	21-25
Group 2 (CP-690,550-10)	10	10	1	6-10	26-30
Group 3 ^a (CP-690,550-10)	30/300/1000	10	3/30/100	11-15	31-35
Group 4 (CP-690,550-10)	100	10	10	16-20	36-40

*All dose levels are expressed as mg of active moiety per kg of body weight.

^a Group 3 received 30 mg/kg on days 1-6, escalated to 300 mg/kg for days 7-10 and then was escalated to 1000 mg/kg for days 11-14.

Deviation from study protocol: There were no deviations that affected the study results and conclusions.

Results

Mortality

One 30/300/1000 mg/kg female was found dead on day 12; however, there was no macroscopic signs of a gavage error or other findings that enabled a determination of the cause of death. Microscopic examinations were limited by tissue autolysis. Based on findings of previous single dose toxicology studies in rats, and studies in monkeys this death is very likely CP-690550-related.

Clinical Observations

Animals were checked at least once daily prior to treatment initiation, then beginning on day 1 for the study period at pre-dose, ~1/2 hour post the last animal dosed and in the p.m.

Clinical signs related to CP-690550 included salivation, noisy respiration, eye staining, muzzle staining, and hair loss. These were only observed after initiation of 1000 mg/kg dosing on day 11 in the 30/300/1000 mg/kg dose group. Salivation was observed on days 12-14 at ~36-50 minutes post-dose in 1 male and 2 females. Noisy respiration was observed on days 14 and 15 in 1 females. Eye staining was observed on days 13-15 in 1 males and muzzle staining was observed on day 15 in 1 females. Hair loss was observed on days 14 and 15 in 1 males and 1 female.

Body Weight and Food Consumption

Body weights were determined pre-study, day 1 and every 2 days during the study period

Food consumption was measured every 2 days

There were no CP-690550-related effects on body weight or food consumption.

Hematology

Blood samples were collected by retro-orbital sinus puncture from all the rats on days 7 and 14 and an additional blood sample was obtained on day 10. The following parameters were evaluated and the battery was adequate:

red blood cell count (RBC, $\times 10^6/\text{mm}^3$)
 hemoglobin concentration (HGB, g/dL)
 hematocrit (HCT, %)
 platelet count (PLT, $\times 10^3/\text{mm}^3$)
 mean corpuscular volume (MCV, fL)
 mean corpuscular hemoglobin (MCH, pg)
 mean corpuscular hemoglobin concentration (MCHC, %)
 white blood cell count (WBC, $\times 10^3/\text{mm}^3$)
 white blood cell differential count (WBC Differential, % and absolute)
 neutrophils (N, %) (NCT, $/\text{mm}^3$)
 lymphocytes (L, %) (LCT, $/\text{mm}^3$)
 monocytes (MO, %) (MOCT, $/\text{mm}^3$)
 eosinophils (EO, %) (EOCT, $/\text{mm}^3$)
 basophils (B, %) (BCT, $/\text{mm}^3$)
 large unstained cells (LUC, %) (LUCT, $/\text{mm}^3$)

Reticulocyte counts (RET, %)

Fibrinogen (FIBR, mg/dL) was measured using a manual procedure.

In the high dose group, mean erythroid parameters were also decreased on day 14 corresponding to 3 days of the 1000 mg/kg/day dose (RBC 76-78%, Hct 73-74%, Hb 77-79%, reticulocytes 6-14% of control values). At ≥ 10 mg/kg/day reticulocytes were reduced on day 7.

Mean white blood cells and lymphocytes were decreased in all CP-690550 dose groups by day 14 (maximal reductions occurred at 1000 mg/kg/day, WBC 33%, Lymph 10% of control value) revealing a dose- and time-dependency. Mean eosinophil and basophil counts were also reduced in all treated groups on day 7, 10, and 14 (maximal reductions occurred at 1000 mg/kg/day on days 10 or 14, Eos 8%, Bas 10%).

Table 32: Hematology Summary[#] (values rounded by Reviewer)

Dose (mg/kg/day)	0		10		100		30/300/1000*	
Sex	M	F	M	F	M	F	M	F
N	5	5	5	5	5	5	5	4/5
RBC (10⁶/mm³)								
day 7	8.24	7.58	7.72	8.10	8.00	7.96	7.64 (93%)	7.98 (105%)
day 10	7.76	7.06	7.44	7.24	7.66	7.26	7.66 (99%)	7.30 (103%)
day 14	8.02	7.34	7.70	7.58	7.76 (97%)	7.20 (98%)	6.06 (76%)	5.75 (78%)
Hematocrit (%)								
day 7	52.0	46.4	48.9	48.3	49.7	46.8	48.7 (94%)	46.8 (101%)
day 10	47.4	42.8	46.0	42.4	46.6	42.8	47.2 (100%)	43.0 (100%)
day 14	49.0	45.2	47.2	45.2	46.9 (96%)	42.6 (94%)	36.4 (74%)	33.0 (73%)
Hemoglobin (g/dL)								
day 7	15.7	14.7	15.2	15.2	15.3	15.0	14.8 (94%)	14.9 (101%)
day 10	15.0	14.0	14.7	13.9	14.9	14.0	15.3 (102%)	13.9 (99%)
day 14	15.1	14.3	14.7	14.3	14.8 (98%)	13.7 (96%)	11.9 (79%)	11.0 (77%)
Reticulocytes (%)								
day 7	4.64	3.48	3.78 (81%)	2.04 (59%)	2.50 (54%)	1.22 (35%)	3.52 (76%)	1.78 (51%)
day 10	4.64	5.70	4.08	5.12	2.74 (59%)	3.06 (54%)	0.96 (21%)	0.40 (7%)
day 14	5.64	6.50	5.18	5.48	3.64 (64%)	4.94 (76%)	0.36 (6%)	0.93 (14%)
WBC (10³/mm³)								
day 7	12.1	11.3	8.9	7.9	6.9 (57%)	6.5 (57%)	8.8 (73%)	8.1 (72%)
day 10	12.3	12.0	8.7	8.4	6.4 (52%)	6.4 (53%)	5.0 (41%)	6.5 (54%)
day 14	12.2	11.2	7.8 (64%)	6.6 (59%)	5.7 (47%)	5.2 (46%)	4.0 (33%)	4.2 (38%)
Lymphocyte (/mm³)								
day 7	1014	9617	7522	6568	5766	5149	7211	6875

	3			(65%)	(60%)	(54%)	(71%)	(71%)
day 10	1026 8	1031 6	7235 (70%)	6235 (60%)	51885 7 (50%)	4079 (40%)	3484 (34%)	4541 (44%)
day 14	1023 1	9506	6151 (60%)	5244 (55%)	3847 (38%)	3062 (32%)	1143 (12%)	1002 (10%)
Neutrophils (/mm³)								
day 7	1421	1068	846	966	790 (56%)	962 (97%)	1163 (82%)	853 (80%)
day 10	1552	1185	1082	1768	891 (57%)	1977 (167%)	1185 (76%)	1462 (123%)
day 14	1260	908	1117	926	1301 (103%)	1626 (179%)	2326 (184%)	2780 (306%)
Eosinophils (/mm³)								
day 7	104	114	53 (51%)	57 (50%)	28 (27%)	33 (56%)	40 (38%)	43 (38%)
day 10	115	95	44 (38%)	56 (59%)	19 (16%)	27 (28%)	16 (14%)	25 (26%)
day 14	142	213	69 (48%)	67 (31%)	21 (15%)	54 (25%)	14 (10%)	17 (8%)
Basophils (/mm³)								
day 7	60	51	21 (35%)	18 (35%)	14 (23%)	13 (25%)	21 (35%)	18 (35%)
day 10	61	56	17 (28%)	18 (32%)	9 (15%)	11 (20%)	6 (10%)	8 (14%)
day 14	79	55	28 (35%)	10 (18%)	10 (13%)	7 (13%)	6 (11%)	8 (14%)
<p>* animals received 30 mg/kg on days 1-6, escalated to 300 mg/kg for days 7-10, and escalated to 1000 mg/kg for days 11-14.</p> <p># Values in bold were identified as significantly different from control (p<0.05) by the applicant.</p>								

Serum Chemistry

Blood samples were collected by retro-orbital sinus puncture from all the rats on days 7 and 14. The following parameters were evaluated and the battery of serum chemistry parameters was adequate:

alanine aminotransferase (ALT, U/L)
alkaline phosphatase (ALP, U/L)
calcium (CA, mg/dL)

aspartate aminotransferase (AST, U/L)
γ-glutamyl transferase (GGT, U/L)
5'nucleotidase (5'NT, U/L)

total bilirubin (TB, mg/dL)	sodium (NA, meq/L)
potassium (K, meq/L)	total protein (TP, g/dL)
albumin (ALBM, g/dL)	cholesterol (CHOL, mg/dL)
triglycerides (TRIG, mg/dL)	glucose (GLUC, mg/dL)
blood urea nitrogen (BUN, mg/dL)	chloride (CL, meq/L)
Globulin (GLOB, g/dL)	creatinine (CREA, mg/dL)

Increases in ALT, AST, GGT, glucose, BUN, total protein, albumin and decreases in potassium occurred at doses ≥ 100 mg/kg.

In the 1000 mg/kg/day dose group, ALT increased 240-262% of control values, AST increased 156-171% of control values, and GGT increased 167-217% of control values. These mean values were also associated with individual pathology of slight hepatocellular necrosis (animal #11) or slight centrilobular hypertrophy (animal #13).

On day 14, the high dose of 30/300/1000 mg/kg/day increased mean glucose (196-200% of control values), BUN (156-186 of control values), total protein (109-110% of control values), and albumin (118-122% of control values). In this dose group, potassium reduced (84-86% of control values).

There were essentially no effects at lower doses of CP-690550.

Table 33: Summary of Serum Chemistry[#]

Dose (mg/kg/day)	0		10		100		30/300/1000*	
Sex	M	F	M	F	M	F	M	F
N	5	5	5	5	5	5	5	5
ALT (U/L)								
day 7	35.6	32.6	33.0	32.6	37.2	31.6	35.0 (98%)	30.4 (93%)
day 14	38.4	33.8	34.8	30.6	36.4	30.0	92.4 (240%)	88.5 (262%)
AST (U/L)								
day 7	97.4	97.0	96.4	93.4	123.8	98.8	102.4 (105%)	105.6 (109%)
day 14	97.0	103.8	100.2	101.0	101.6	92.4	166.4 (171%)	161.5 (156%)
GGT (U/L)								
day 7	1.2	2.4	1.4	1.6	1.2	2.0	1.2 (100%)	2.0 (83%)
day 14	1.2	1.8	1.0	2.2	1.6	1.8	2.6 (217%)	3.0 (167%)
Glucose (mg/dL)								

Dose (mg/kg/day)	0		10		100		30/300/1000*	
Sex	M	F	M	F	M	F	M	F
N	5	5	5	5	5	5	5	5
day 7	106	115	104	111	107	112	104 (98%)	114 (99%)
day 14	112	108	95	102	107	100	219 (196%)	216 (200%)
BUN (mg/dL)								
day 7	12.4	14.8	12.4	13.2	13.8	15.2	13.4 (108%)	14.2 (96%)
day 14	13.4	15.2	13.6	14.8	14.8	14.6	25.0 (186%)	23.8 (156%)
Total Protein (g/dL)								
day 7	6.40	6.44	6.46	6.76	6.66	6.76	6.36 (99%)	6.56 (102%)
day 14	6.34	6.32	6.16	6.40	6.66	6.54	6.92 (109%)	6.98 (110%)
Albumin (g/dL)								
day 7	3.44	3.56	3.46	3.70	3.64	3.70	3.42 (99%)	3.52 (99%)
day 14	3.34	3.46	3.26	3.44	3.64	3.54	4.08 (122%)	4.10 (118%)
Potassium (meq/L)								
day 7	5.76	5.44	5.78	5.54	6.12	5.66	5.78 (100%)	5.04 (93%)
day 14	5.78	5.78	5.86	5.54	5.56	5.64	4.84 (84%)	5.00 (86%)
* animals received 30 mg/kg on day 1-6, escalated to 300 mg/kg for days 7 to 10, then escalated to 1000 mg/kg for days 11-14 # Values in bold were identified as significantly different from control (p<0.05) by the applicant.								

Organ Weights

Weights of the liver, kidneys (combined), heart, brain, adrenals (combined), and testes (combined), were obtained. Lymphoid organs were not weighed.

Increased liver weight occurred in the 30/300/1000 mg/kg/day group compared to the control (absolute weight by 120-130%; % body weight by 130-140%). This correlated microscopically with centrilobular hypertrophy in 1/5 males and 2/5 females (#13, 32, 35).

Histopathology

Adequate battery: Yes, as a non-GLP, tolerability assessment study.

Organs and tissues not examined or examined from only a few animals included eye, pancreas, urinary bladder, salivary gland, pituitary, adrenal, thyroid, parathyroid, esophagus, uterus, ovary, duodenum, jejunum, ileum, cecum, colon, Harderian gland, peripheral nerve, skin and adnexa, and spinal cord.

Peer Review: Not by an outside pathologist, only by an in-house pathologist

The following tissues were collected:

kidneys*	skeletal muscle
urinary bladder	ovaries
liver (left and right lateral lobes)*	uterus
thymus*	prostate
spleen*	epididymides*
mesenteric lymph node*	lung (both diaphragmatic lobes)*
esophagus	heart*
stomach*	testes (left and right)*
duodenum	brain (cerebrum, cerebellum and pons)*
jejunum	spinal cord (cervical)
ileum	Harderian gland
cecum	eyes
colon	skin and adnexa (including mammary gland)
pituitary gland	bone (sternum, including bone marrow)*
salivary gland	parathyroid
pancreas	bone marrow smear*
adrenal glands	peripheral nerve
thyroid gland	

CP-690550-related microscopic changes occurred at doses ≥ 10 mg/kg/day in bone marrow, spleen, thymus, mesenteric lymph nodes, liver, kidney, and stomach. Generalized cellular depletion occurred in the bone marrow at ≥ 100 mg/kg/day. At 30/300/1000 mg/kg/day an increase in liver weights correlated with centrilobular hypertrophy. In this dose group, erythroid depletion occurred in the bone marrow. Also there was multifocal slight to moderate necrosis of the glandular stomach that correlated with gastric enlargement.

A small thymus was observed in 1/5 100 and 2/5 30/300/1000 mg/kg males and in 2/5 100 and 2/4 30/300/1000 mg/kg females, which also correlated microscopically with lymphoid depletion.

Enlarged stomach was observed in 5/5 30/300/1000 mg/kg males and 2/5 30/300/1000 mg/kg females and correlated microscopically with multifocal necrosis. In addition to enlarged stomach, small ulcerations were also grossly apparent in the gastric mucosa of 1 male (#15).

Table 34: Histopathology Findings Related to Lymphoid Organs

Dose (mg/kg/day)	0		10		100		30/300/1000*	
	M	F	M	F	M	F	M	F
N (unless otherwise stated)	5	5	5	5	5	5	5	4/5[#]
Bone Marrow								
depletion, generalized	0	0	0	0	3	4	5	5
Bone Marrow Smear								
N	0	0	0	5	5	5	5	4
depletion lymphoid	-	-	-	5	5	5	5	4
depletion, erythroid	-	-	-	0	0	0	5	4
Lymph Node, Mesenteric								
lymphoid depletion	0	0	0	0	0	3	5	5
Spleen								
lymphoid depletion	0	0	0	0	4	4	5	5
Thymus								
lymphoid depletion	0	0	0	1	5	5	5	4
Stomach								
Necrosis	0	0	0	0	0	0	4	4
Kidney								
tubular dilation	0	0	0	0	0	0	1	2
Liver								
centrilobular hypertrophy	0	0	0	0	0	0	1	2
necrosis	0	0	1	0	0	0	1	0
Lung								
inflammation, subacute	4	0	0	1	0	1	0	1
congestion	0	0	0	0	0	0	0	1
Stomach								
Necrosis	0	0	0	0	0	0	4	4
* animals received 30 mg/kg on day 1-6, escalated to 300 mg/kg for days 7 to 10, then escalated to 1000 mg/kg for days 11-14 # the female (#34) that died on day 12 was included in the analysis (n=5) of the spleen, thymus, mesenteric lymph node, bone marrow and lung, but other tissues had autolysis and this animal was excluded from that dataset, (n=4). - there was no analysis of tissue from these dose groups								

Bone marrow analysis revealed an increase in the percentage of myeloid cells evident at 100 mg/kg/day and prominent after 30/300/1000 mg/kg/day dosing (176-196% of control values). There was a slight increase in the percentage of erythroid cells in the low and mid dose groups (up to 130% of control) but a marked reduction in the high dose group (11-16% of control values). The percentage of lymphocytes was reduced for all doses, with the mid dose having the greatest reduction (40-47% of control values). The increase in percent myeloid cells at the high dose, together with the

substantial erythroid cell reduction, resulted in an increase in the myeloid:erythroid ratio of 1397- 2593%. The lower doses had a slight reduction in M:E ratio, 88-91% for the 100 mg/kg/day dose. The effect at the high dose was likely due to hematological responses associated with the liver and stomach pathology noted above.

Table 35: Myeloid:Erythroid Ratio

Dose (mg/kg/day)	0		10		100		30/300/1000*	
Sex	M	F	M	F	M	F	M	F
N	5	5	5	5	5	5	5	5
% Myeloid cells	43.2	42.2	43.0 (99%)	44.7 (106%)	48.6 (112%)	50.3 (119%)	83.8 (194%)	74.3 (176%)
% Erythroid cells	31.6	29.6	32.7 (103%)	36.0 (122%)	39.6 (125%)	38.5 (130%)	3.4 (11%)	4.90 (16%)
% Lymphocytes	25.2	28.2	24.4 (97%)	19.4 (69%)	11.8 (47%)	11.2 (40%)	12.8 (51%)	20.8 (74%)
M:E Ratio (% of control)	1.40	1.46	1.34 (96%)	1.35 (92%)	1.24 (88%)	1.33 (91%)	36.3 (2593%)	20.4 (1397%)
* animals received 30 mg/kg on day 1-6, escalated to 300 mg/kg for days 7 to 10, then escalated to 1000 mg/kg for days 11-14								

Toxicokinetics

Blood samples were obtained from the retro-orbital sinus following CO₂-O₂ (70%-30%) anesthesia on days 1, 8, and 14 at 0.5, 2, and 8 hours postdose (first 3/sex/group) and 1, 4, and 24 hours postdose (second 3/sex/dose). Vehicle-control animals sampled on day 1 at 0.5 hours postdose, but the results were not included in this report. The HPLC/MS/MS assay was not validated.

In general, exposure was greater than dose proportional in males and females as dose was increased from 1 to 100 mg/kg/day, but was less than dose proportional from 100 to 1000 mg/kg/day. There was no substantial drug accumulation from day 1 to 14.

Toxicokinetic Parameters

Dose (mg/kg/day)	10		30*		100		300*		1000*	
Sex	M	F	M	F	M	F	M	F	M	F
C _{max} (ng/mL)										
day 1	1350		5070		9980		ND		ND	
day 8	1930		ND		7060		10500		ND	
day 14	2540		ND		10700		ND		48900	
AUC ₀₋₂₄ (ng-h/mL)										
day 1	3600		15000		107000		ND		ND	
day 8	3330		ND		55500		133000		ND	
day 14	4500		ND		84500		ND		342000	

* Animals were administered 30 mg/kg/day on days 1-6, escalated to 300 mg/kg/day on days 7-10, escalated once again to 1000 mg/kg/day on days 11-14.
 ND no dose and no determination of values, the dose for the column on that day was not administered, therefore there was no sample collected for that dose.

Study title: CP-690550-10: Six-Week Oral Toxicity Study with One Month Recovery in Sprague-Dawley Rats

Study no.: 01-2063-06
 Study report location: 4.2.3.2-repeat-dose-tox
 Conducting laboratory and location: Pfizer Global Research and Development, Groton, CT
 Date of study initiation: 14 March 2001
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: CP-690550-10, Lot # 43798-2-1H; Purity 0.96%
 Composition: 60% active moiety/potency, 38.1% citrate counterion,

There was no Certificate of Analysis included with this report. A description of HPLC evaluation was included.

Purity/composition:

(b) (4) CP-690,550-10 + (b) (4)
 HPLC impurities + (b) (4) impurities + (b) (4)
 (b) (4)

Key Study Findings

- CP-690550-10 was administered orally to Sprague Dawley rats for 44 days at 0, 1, 10, and 100 mg/kg/day, followed by a 1-month recovery period for animals in the 0 and 100 mg/kg/day groups.
- There were no CP-690550-related effects on mortalities, clinical signs, and body weight, food consumption, or urinalysis.
- The major treatment related hematologic findings included marked decreases in white blood cell parameters and slight time dependent decreases in red blood cell count, hemoglobin, and hematocrit. Compared to control values, for the high dose on day 43 the changes included red blood cell counts reduced red blood cell counts (M/F 93%/92%), hematocrit (M/F 93%/93%), hemoglobin (M/F 94%/94%), and reticulocytes (M/F 80%/85%), all of which returned to control levels by the end of the recovery period. For white blood cells, a similar comparison, revealed reductions in white blood cell counts (M/F 45%/50%), lymphocyte counts (M/F 36%/36%), eosinophil counts (M/F 69%/44%), basophil counts (M/F 18%/19%), large unstained cells (M/F 15%/20%), and an increase in neutrophils (M/F 122%/169%). Except for neutrophils which recovered completely the other white blood cell parameters only partially recovered.

- The thymus and spleen of animals in the 100 mg/kg dose group were noted as small examined macroscopically, were not included as weighed organs, but correlated with histopathological changes of lymphoid depletion. Lymphoid depletion was also noted in other examined lymphoid tissues, the mesenteric lymph node and bone marrow.
- Adrenal and kidney weights were reduced approximately 80% and 90% of control values, respectively, but completely recovered. There were no histopathological findings associated with the reduction.
- Exposure was greater in females than males at 1 and 10 mg/kg/day, but was similar in both sexes at 100 mg/kg/day. In general, exposure was greater than dose proportional for the dose range of to 100 mg/kg/day. There did not appear to be any significant drug accumulation from day 1 to day 44.
- A NOAEL of 1 mg/kg/day was based on the findings that the changes in hematology and bone marrow myeloid-erythroid changes were minimal and not adverse.

Methods

Doses: 0, 1, 10, and 100 mg/kg/day

The dose expressed as mg/kg is based on the active moiety composition of the drug substance.

Frequency of dosing: Once daily for 6 weeks

Route of administration: Orally, by gavage

Dose volume: 10 mL/kg

Formulation/Vehicle: 0.5% (aq) methylcellulose

Species/Strain: Sprague Dawley CD (CrI: CD (SD)IGSBR) rats

Number/Sex/Group: Control and 100 mg/kg/day: 15/sex/dose
1 and 10 mg/kg/day: 10/sex/dose

Age: Approximately 7-9 weeks old at the start of the study

Weight: males: 239.5 to 284.9 g; females: 155.0 to 206.5 g

Satellite groups: Recovery: 5/sex/dose for control and high dose only
Toxicokinetics: 5/sex/group

Unique study design:

Dose (mg/kg/day)	Animal #s	
	Males	Females
Control	1-15	51-65
1	16-25	66-75
10	26-35	76-85
100	36-50	86-100

Deviation from study protocol: There were no deviations that affected the study results or conclusions.

Mortality

Animals were checked at least twice daily.
There were no mortalities.

Clinical sign

Observations made four times a day: pre- and immediately post-dose, 1 hour after dosing, and near the end of the day. During the recovery period, observations made twice daily.

There was no CP-690550 -related clinical signs were observed. Salivation and reddened conjunctiva were observed sporadically in individual animals and were not considered by the applicant to be treatment related.

Body weights

Body weights were measured weekly

No treatment-related effects were observed in body weight or body weight gain.

Food consumption

Food consumption was measured weekly.

No treatment-related effects in food consumption were observed.

Ophthalmoscopy

Examinations were performed once prior to treatment initiation and on days 38 (dosing period) and 66 (recovery period). Mydriasis was induced by 1.0% tropicamide (Mydracyl®).

No treatment-related ophthalmoscopic changes were observed.

Hematology

Blood samples for hematology analysis were taken on Days 10, 43, and 67. The parameters examined provided an adequate assessment.

red blood cell count (RBC, $\times 10^6/\text{mm}^3$)
 hemoglobin concentration (HGB, g/dL)
 hematocrit (HCT, %)
 platelet count (PLT, $\times 10^3/\text{mm}^3$)
 mean corpuscular volume (MCV, fL)
 mean corpuscular hemoglobin (MCH, pg)
 mean corpuscular hemoglobin concentration (MCHC, %)
 white blood cell count (WBC, $\times 10^3/\text{mm}^3$)
 white blood cell differential count (WBC Differential, % and absolute)
 neutrophils (N, %) (NCT, $/\text{mm}^3$)
 lymphocytes (L, %) (LCT, $/\text{mm}^3$)
 monocytes (MO, %) (MOCT, $/\text{mm}^3$)
 eosinophils (EO, %) (EOCT, $/\text{mm}^3$)
 basophils (B, %) (BCT, $/\text{mm}^3$)
 large unstained cells (LUC, %) (LUCT, $/\text{mm}^3$)
 reticulocyte counts (RET, %)
 Fibrinogen (FIBR, mg/dL) was measured using a manual procedure.

There were decreases in both red cell and white cell parameters. A time-dependent decrease in red blood cell count, hemoglobin, and hematocrit: evident by reductions on day 43, but not day 10. The decreases in red blood cell parameters were minimal, but the time related change indicates that the reduction would be expected to be greater in a longer study. For the 100 mg/kg/day dose, mean red blood cell counts decreased to 92% of controls values, hematocrit decreased to 93% of control values, and hemoglobin decreased to 94% of control values. For reticulocytes, the reduction on day 10 was greater in females than males, and occurred in the 10 and 100 mg/kg/day dose groups (females 75% and 67% of control values, males 85% and 85% of control values, for the respective dose levels). All parameters recovered over the 24 day recovery period.

Total white blood cell count decreased by day 43 in a dose dependent manner to 59-67% of control values at 10 mg/kg/day and to 45-50% of control values at 100 mg/kg/day, and only partially recovered to 62-69% of control values during the 24 day recovery period. This decrease was primarily due to a dose dependent decrease in lymphocyte counts on days 10 and 43 (66-70% of control values on day 10, and 36 to 58% of control values on day 43. These recovered only partially (59-63% of control values) in the 100 mg/kg/day group. There were no recovery animals at the lower dose groups. Neutrophils increased at the high dose, with a slightly greater increase on day 43 (122-169% of control values) than day 10 (115-145% of control values), and a return to control levels at recovery. Eosinophil and basophil counts were substantially reduced in a dose and time dependent manner for all CP-690550 dose groups and only partially recovered. At the 100 mg/kg/day dose on day 43, eosinophils were 44-69% of control values and basophils were 18-19% of control values. Large unstained cell counts were also reduced in a dose-dependent and time-dependent manner without complete recovery reduced to 15-20% of controls values on day 43 at the high dose.

Table 36: Hematology Summary*

(values rounded by Reviewer)

Dose (mg/kg/day)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
N	15	15	10	10	10	10	15	15
N-Recovery	5	5	0	0	0	0	5	5
RBC (10⁶/μL)								
day 10	7.81	7.83	7.77	7.96	7.74	7.80	7.80 (100%)	7.75 (99%)
day 43	9.11	8.52	8.81	8.49	8.80	8.21	8.49 (93%)	7.85 (92%)
Recovery, day 67	9.18	8.78	-	-	-	-	8.84 (96%)	8.12 (92%)
Hematocrit (%)								
day 10	46.5	46.0	45.7 (98%)	46.8 (102%)	46.1 (99%)	46.2 (100%)	46.2 (99%)	45.8 (100%)
day 43	51.4	46.0	49.2 (96%)	46.2 (100%)	49.4 (96%)	44.6 (97%)	47.9 (93%)	42.7 (93%)
Recovery, day 67	48.7	47.1	-	-	-	-	48.2 (99%)	45.5 (97%)
Hemoglobin (g/dL)								
day 10	15.5	15.4	15.1	15.5	15.4	15.5	15.4 (99%)	15.3 (99%)
day 43	16.6	15.6	15.9	15.6	16.0	15.3	15.7 (94%)	14.7 (94%)
Recovery, day 67	16.1	15.7	-	-	-	-	15.9 (99%)	15.3 (97%)
Reticulocytes (%)								
day 10	3.4	2.4	3.3	2.2	2.9 (85%)	1.8 (75%)	2.9 (85%)	1.6 (67%)
day 43	2.0	2.0	1.8	2.4	1.7 (85%)	1.9 (95%)	1.5 (80%)	1.7 (85%)
Recovery, day 67	2.8	2.0	-	-	-	-	3.0 (107%)	2.4 (120%)
WBC (10³/mm³)								
day 10	15.9	11.7	14.0	11.1	11.3 (71%)	8.4 (72%)	11.6 (73%)	8.5 (73%)
day 43	15.3	11.4	13.2	9.8	9.1 (59%)	7.6 (67%)	6.9 (45%)	5.7 (50%)
Recovery, day 67	16.3	14.6	-	-	-	-	11.3 (69%)	9.0 (62%)

Lymphocyte (/mm³)								
day 10	13620	10240	11978	9270	9296 (68%)	7123 (70%)	9164 (67%)	6803 (66%)
day 43	13174	9784	11050	7931	7235 (54%)	5668 (58%)	4780 (36%)	3507 (36%)
Recovery, day 67	13822	12834	-	-	-	--	8726 (63%)	7547 (59%)
Neutrophils (/mm³)								
day 10	1713	978	1500	1347	1545	972	1970 (115%)	1421 (145%)
day 43	1458	1093	1479	1359	1400	1482	1779 (122%)	1844 (169%)
Recovery, day 67	1770	1143	-	-	-	-	1974 (112%)	1128 (99%)
Eosinophils (/mm³)								
day 10	84	124	81	99	56 (67%)	42 (34%)	72 (86%)	54 (44%)
day 43	166	124	250	105	155 (93%)	86 (69%)	114 (69%)	54 (44%)
Recovery, day 67	135	112	-	-	-	-	87 (64%)	61 (54%)
Basophils (/mm³)								
day 10	109	68	74 (68%)	58 (85%)	48 (44%)	29 (43%)	34 (31%)	22 (32%)
day 43	108	72	67 (62%)	46 (64%)	39 (36%)	19 (26%)	19 (18%)	14 (19%)
Recovery, day 67	93	89	-	-	-	-	55 (59%)	34 (38%)
Large Unstained cells (/mm³)								
day 10	115	73	77 (67%)	65 (89%)	63 (55%)	49 (67%)	51 (44%)	29 (40%)
day 43	113	80	80 (71%)	56 (70%)	38 (34%)	40 (50%)	17 (15%)	16 (20%)
Recovery, day 67	85	92	-	-	-	-	59 (69%)	24 (26%)
* Values in bold were identified as significantly different from control (p<0.05) by the applicant. - There were no low and mid dose recovery groups								

Clinical chemistry

Blood samples for serum chemistry analysis were taken on Days 10, 43, and 67. The parameters examined provided an adequate assessment.

alanine aminotransferase (ALT, U/L)	aspartate aminotransferase (AST, U/L)
total bilirubin (TB, mg/dL)	γ-glutamyl transferase (GGT, U/L)
albumin (ALBM, g/dL)	5' nucleotidase (5'NT, U/L)
cholesterol (CHOL, mg/dL)	total protein (TP, g/dL)
blood urea nitrogen (BUN, mg/dL)	glucose (GLUC, mg/dL)
potassium (K, meq/L)	creatinine (CREA, mg/dL)
calcium (CA, mg/dL)	sodium (NA, meq/L)
	chloride (CL, meq/L)

Globulin (GLOB, g/dL) was calculated.

Aspartate aminotransferase was increased (117-124% compared to control values) in the 100 mg/kg/day group on days 10 and 43 in males and on day 43 in females. Total protein and albumin were slightly increased for males in the 100 mg/kg/day on days 10 and 43 (total protein: 105% compared to control; albumin: 108-109% of control). Total protein and albumin values had not recovered by the end of the 1-month recovery period. These small changes are unlikely to be toxicologically significant. Similar changes in total protein and albumin occurred in the 2 week exploratory study (Report 00-2063-03) and the 6-month GLP study (Report 02-2063-20). In the 6-month study there was sufficient information to suggest these findings were related to salivation-induced dehydration associated with drug administration. In this 6-week study, the same 100 mg/kg/day dose induced only sporadic salivation.

Table 37: Serum Chemistry Summary* (values rounded by Reviewer)

Dose (mg/kg/day)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
N	15	15	10	10	10	10	15	15
N-Recovery	5	5	0	0	0	0	5	5
AST (U/L)								
day 10	87	94	98	90	86	95	102 (117%)	100 (106%)
day 43	80	80	79	90	83	79	99 (124%)	97 (121%)
Recovery day 67	88	116	-	-	-	-	80 (91%)	116 (100%)
Total Protein (g/dL)								
day 10	6.27	6.70	6.22	6.75	6.34	6.92	6.58 (105%)	6.81 (102%)
day 43	7.21	7.17	7.10	7.30	7.29	7.24	7.60 (105%)	7.34 (102%)

Dose (mg/kg/day)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
N	15	15	10	10	10	10	15	15
N-Recovery	5	5	0	0	0	0	5	5
Recovery day 67	7.18	7.28	-	-	-	-	7.56 (105%)	7.58 (104%)
Albumin (g/dL)								
day 10	3.27	3.57	3.28	3.62	3.35	3.58	3.55 (108%)	3.67 (103%)
day 43	3.46	3.73	3.49	3.31	3.60	3.64	3.77 (109%)	3.73 (100%)
Recovery day 67	3.36	3.62	-	-	-	-	3.56 (106%)	3.66 (101%)
* Values in bold were identified as significantly different from control (p<0.05) by the applicant. - There were no low and mid dose recovery groups								

Urinalysis

Urine was collected for about 5-6 hours each on Days 39 (dosing period) and 64 (recovery period). The parameters examined provided an adequate assessment.

volume (VOL, ml, manual)	glucose (GLU, neg to 3+*)
specific gravity (SPGR, refrac.)	urobilinogen (URO, neg to 4+)
pH (5 to 9)	bilirubin (BIL, neg to 3+*)
protein (PRO, neg to 3+*)	ketones (KET, neg to 3+)
blood (BLO, neg to 3+)	color (COLR, visual assessment)

*Protein, glucose and bilirubin could be reported up to 4+ if manually read.

Microscopic examination of sediment was performed if urine was abnormal in color and/or blood (2+) was observed.

Microscopic analysis of urine sediment includes the following parameters: WBC and RBC are rated on a scale of negative to 4+ and TNTC (too numerous to count). All other parameters are rated on a scale of negative to 3+.

casts (CAST, /lpf)	white blood cells (WBC, /lpf)
red blood cells (RBC, /lpf)	epithelia (EPH, /lpf)
calcium oxalate crystals (CAOX, /lpf)	amorphous phosphates (AMPH, /lpf)
triple phosphates (TRPH, /lpf)	amorphous urates (AMUR, /lpf)
other crystals (OTHR, /lpf)	

There were no CP-690550-related effects on urinalysis parameters.

Gross pathology

Gross pathology and organ weight determination were performed on all main study animals on day 45, or on day 73 for the recovery animals. The organs and tissues examined, besides general examination external and internal body surfaces are those collected for histopathology, (refer to the histopathology inventory table in the histopathology section) as well as any additional gross lesions. This was an adequate assesment of gross pathology.

A separate listing of gross pathological findings was no included in the report, nor were gross findings included in the individual animal histopathology listings. The applicant noted that some animals in the 100 mg/kg dose group had small thymuses and spleens. Small thymus was observed in 8/10 males and 9/10 females and small spleen was observed in 3/10 males and 4/10 females. These correlated with lymphoid depletion observed in histopathology.

Non treatment related findings consisted of fibrous adhesions of the pericardium to the epicardium and thoracic wall in a female of 1 mg/kg/day dose group which was attributed to gavage trauma, which the Reviewer concurs. An enlarged heart was observed in a female of the 100 mg/kg group, but no microscopic correlate was observed and there was no further description of this finding.

Organ weights

Organ weights were determined on the day of termination (day 45 for main study; or day 73 for recovery animals) of the liver, kidneys (combined), heart, brain, adrenals (combined), and testes (combined). Data were presented in terms of absolute weight and % body weight (% brain weight was not provided by the applicant). The organ weight presentation is an inadequate assessment, missing known target organs of CP-690550, lymphoid organs and major lymph nodes, as well as other standard organ assessments, (e.g, ovary, epididymides, spleen, prostate).

Organ weight changes that were potentially related to administration of CP-690550 were observed in kidneys and adrenals. Mean kidney weight relative to control was reduced in the 100 mg/kg/day dose in males (89% of control for absolute weight and 7% decrease for % body weight). A statistically significant decrease in mean adrenal weights was seen in females at 10 mg/kg (78% of control expressed as absolute or % of body weight) and 100 mg/kg (80-82% of control weights). There were no differences in organ weights were observed at the end of the recovery period.

Table 38: Organ Weights* (values rounded by Reviewer)

	Males				Females			
Dose (mg/kg/day)	0	1	10	100	0	1	10	100
N	10	10	10	10	10	10	10	10
N-Recovery	5	5	0	0	0	0	5	4
Kidney								
Absolute (g)	3.27	3.34	3.22	2.91 (89%)	1.92	1.88	1.87	1.87 (97%)
% body wt	0.76	0.76	0.79	0.71 (93%)	0.81	0.77	0.79	0.78 (96%)
Recovery								
Absolute (g)	3.58	-	-	3.48 (97%)	2.08	-	-	1.99 (96%)
% body wt	0.74	-	-	0.77 (104%)	0.77	-	-	0.81 (105%)
Adrenal								
Absolute (g)	0.091	0.079	0.081	0.071 (78%)	0.097	0.089 (92%)	0.076 (78%)	0.080 (82%)
% body wt	0.021	0.018	0.020	0.017 (81%)	0.041	0.036 (88%)	0.032 (78%)	0.033 (80%)
Recovery								
Absolute (g)	0.071	-	-	0.066 (93%)	0.067	-	-	0.061 (91%)
% body wt	0.015	-	-	0.015 (100%)	0.025	-	-	0.025 (100%)
- There were no low and mid dose recovery groups								
* Values in bold were identified as significantly different from control (p<0.05) by the applicant.								

Histopathology

Adequate battery: Yes, major organs and tissues were evaluated as indicated in the histopathology inventory listed below. However the evaluation and summary of the findings was inadequate. For most tissues, the low and mid dose group's tissues were not examined since there were no abnormal findings in the high dose animals. The Applicant's summary table did not separate main study findings from recovery findings for the control and high dose groups, and this was the only study in rats with recovery dose groups. Also severity was not indicated in the summary histopathology table, but was included in the individual animal listings. The Reviewer reanalyzed the data.

Peer review: Not by an outside pathologist, only by an in-house pathologist

Bone Marrow Analysis

Bone marrow smears were stained with Wright's Giemsa and 500 nucleated cell differential count was performed on a representative slide from each animal. These data were properly analyzed with main study and recovery groups separately presented. The bone marrow evaluation was adequate.

The following tissues were collected:

Histopathology inventory

Study: 01-2063-06			
Species: Sprague-Dawley Rat			
Adrenals	X, *	Nasal cavity	
Aorta		Optic nerves	
Bone Marrow smear	X	Ovaries	X
Bone (femur)		Pancreas	X
Brain	X, *	Parathyroid	X
Cecum	X	Peripheral nerve	X
Cervix		Pharynx	
Colon	X	Pituitary	X
Duodenum		Prostate	X
Epididymis	X	Rectum	
Esophagus	X	Salivary gland	X
Eye	X	Sciatic nerve	
Fallopian tube		Seminal vesicles	X
Gall bladder		Skeletal muscle	X
Gross lesions	X	Skin	X
Harderian gland	X	Spinal cord	X
Heart	X, *	Spleen	X
Ileum	X	Sternum	X
Injection site		Stomach	X
Jejunum	X	Testes	X, *
Kidneys	X, *	Thymus	X
Lachrymal gland		Thyroid	X
Larynx		Tongue	
Liver	X, *	Trachea	X
Lungs	X	Ureter	
Lymph nodes, cervical		Urinary bladder	X
Lymph nodes, mandibular		Uterus	X
Lymph nodes, mesenteric	X	Vagina	X
Mammary Gland	X	Zymbal gland	

X histopathology performed

* organ weight obtained

CP- 690550-related lymphoid depletion was prominent in lymphoid tissue of the thymus, spleen, mesenteric lymph node, and bone marrow. The severity increased with increased dose from slight at the 1 mg/kg/day dose to marked at higher doses, 10 and 100 mg/kg/day)

Cellular depletion in the bone marrow was noted in a few animals of the high dose group, and there was a dose-dependent qualitative assessment of decreased lymphocytes in blood marrow smears. Quantitative analysis of the bone marrow smear, revealed a small increase in percent myeloid cells at the 100 mg/kg/day dose (110-

117% of control), a dose-dependent increase in percent erythroid cells (up to 121-126% of control values at the 100 mg/kg/day dose which did not recover within 28 days, and a dose-dependent reduction in percent lymphocytes to 37-44% of control values at the 100 mg/kg/day dose which partly recovered. These changes resulted and in a small reduction in myeloid:erythroid ratio in high dose males (86% of control values), but no change in the ratio for females.

The histological evaluation was not deficient, since the recovery animal information was not separately analyzed from the main study animals. This study does have a signed GLP and quality control statement. The Reviewer reanalyzed the data and it is presented below.

Table 39: Histopathology Summary

Dose (mg/kg/day)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
N	10	10	10	10	10	10	10	10
Recovery N	5	5	0	0	0	0	5	5
Bone marrow								
Cellular depletion slight	0	0	0	0	0	0	2	1
recovery	0	0	-	-	-	-	0	0
Bone marrow smear								
Cellular depletion slight	0	0	0	0	0	2	4	2
mild	0	0	0	1	0	1	4	2
moderate	0	0	0	0	1	0	1	2
recovery^a								
slight	0	0	-	-	-	-	0	2
mild	0	0	-	-	-	-	0	1
Epididymis								
spermatic granuloma	1	-	-	-	-	-	4	-
recovery	0	-	-	-	-	-	0	-
Lymph Node, Mesenteric								
Lymphoid depletion slight	0	0	0	0	1	0	5	4
recovery	0	0	-	-	-	-	0	0
Spleen								
Lymphoid depletion slight	0	0	0	0	1	0	7	3
mild	0	0	0	0	0	0	2	2
recovery	0	0	-	-	-	-	0	0
Thymus								
Lymphoid depletion slight	0	0	0	0	0	0	1	1
mild	0	0	0	0	0	0	2	3

moderate	0	0	0	0	0	0	4	0
marked	0	0	0	0	0	0	0	1
recovery^a	0	0	-	-	-	-	0	0
- not applicable for female rats								
^a based on N=4 examined (missing tissue)								

Table 40: Summary of Bone Marrow Analysis

Dose (mg/kg/day)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
N	10	10	10	10	10	10	10	10
Recovery N	5	5	0	0	0	0	5	5
% Myeloid cells	47.1	40.5	44.5 (94%)	41.8 (103%)	50.2 (106%)	41.9 (103%)	52.0 (110%)	47.5 (117%)
Recovery	47.1	40.4	-	-	-	-	47.2 (100%)	41.3 (102%)
% Erythroid cells	29.9	36.3	25.2 (84%)	36.1 (99%)	34.5 (11%)	41.0 (113%)	37.7 (126%)	44.0 (121%)
Recovery	24.9	32.5	-	-	-	-	33.2 (133%)	37.0 (114%)
% Lymphocytes	23.0	23.1	30.3 (132%)	21.9 (95%)	15.3 (66%)	15.0 (65%)	10.2 (44%)	8.5 (37%)
Recovery	28.0	27.1	-	-	-	-	19.7 (70%)	21.7 (80%)
M:E Ratio (% of control)	1.64	1.25	1.82 (111%)	1.19 (95%)	1.56 (95%)	1.10 (88%)	1.42 (86%)	1.24 (99%)
Recovery	1.93	1.26	-	-	-	-	1.47 (76%)	1.14 (90%)
- not applicable or not assessed								

Toxicokinetics

Blood samples were obtained from 3/sex/dose/timepoint at 0.5, 1, 2, 4, 8, and 24 hours after CP-690550 administration on days 1 and 44. Vehicle control rats were sampled at the same time points; however, only the 0.5 h post-dose samples were analyzed. Serum drug analysis was quantitated using a validated LC/MS/MS assay at (b) (4) (b) (4)

Exposure to CP-690550, as estimated by C_{max} and AUC₀₋₈, was greater in females than males at 1 and 10 mg/kg/day, but was similar in both sexes at 100 mg/kg/day at the end of the dosing phase. The T_{max} was 0.5 h, except for the high dose on day 44 in which the T_{max} was 2 hour for males and 1 hour for females. In general, exposure was greater than dose proportional in males and females as dose was increased from 1 to

100 mg/kg/day. There was no substantial drug accumulation from Day 1 to 44. Samples from control animals were analyzed only at the 0.5 hours postdose time point; all were below the lower limit of quantification, 5 ng/mL. Mean toxicokinetic parameters are presented below:

Table 41: Toxicokinetic Parameters

Dose (mg/kg/day)	1		10		100	
Sex	M	F	M	F	M	F
C_{max} (ng/mL)						
day 1	61	152	728	2080	10400	12600
day 44	109	236	1080	2980	8130	8860
AUC_{0-last} (ng-h/mL)						
day 1	95	228	1690	4100	57200	71700
day 44	136	322	1850	4730	49400	51200

Stability and Homogeneity

Stability: The stability of CP-690550 in the vehicle was assayed using a reverse-phase high performance liquid chromatography method. The suspension was found to be stable for 3 days when stored either at room temperature, 5° C, or frozen.

Homogeneity: The dose formulation was prepared every 2 or 3 days, and was maintained by continuous magnetic stirring during the dosing period. Dosing suspensions were assayed for concentrations during weeks 1 and 7. Concentrations of the dosing were within 10% of the expected level, and this is acceptable. Data were not presented to support the results, but referenced to a study binder.

Study title: A 6-Month Oral Toxicity Study of CP-690550-10 in Rats

Study no.: 02-2063-20
 Study report location: 4.2.3.2-repeat-dose-tox
 Conducting laboratory and location: (b) (4)
 Date of study initiation: 9 Jan 2003
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: CP-690550-10, Lot 54422-88-I F, Purity 97.1%
 Composition: 60.1% active moiety/ potency 37.4% citric acid counterion

A Certificate of Analysis was included, manufactured March 7 2002, test date not indicated, signed CA May 31 2002

Key Study Findings

- CP-690550-10 was orally administered to Sprague Dawley rats, daily for 26 weeks at doses of 0, 1, 10, and 100 mg/kg/day. There was no recovery phase.
- There were 5 deaths in the CP-690550 treatment groups, at least one at each dos. Due to findings of ventral cervical and thoracic hemorrhage of skeletal muscle in these regions and near organs, and since most deaths were temporally related to jugular vein blood collections during the study, it is most likely these are unrelated to CP-690550 treatment.
- The major clinical sign was salivation at the high dose, which occurred in all animals for most of the study. This might have contributed to dehydration in this dose group evidenced by increased sodium and total protein levels during the second half of the study.
- Except for the high dose male group, there was no effect on body weight and weight gain. The mean weight of the high dose male group was 86% of the control males, and mean body weight gain was 82% of the control males.
- The major treatment-related hematologic findings included reduction in white blood cell counts (total WBC 32% of control, lymphocytes 55% of control), increases in neutrophils (398% of control) and monocytes (259% of control) and decreases in red blood cell parameters (RBC 85% of control, Hb 90% of control, Hct 88% of control) at doses ≥ 10 mg/kg/day in males and females. For doses ≥ 10 mg/kg/day, the lower dose generally required a longer treatment period to reach similar effects in the high dose group. Analysis of lymphocyte subset populations found dose-dependent decreases in all the evaluated lymphocytes subpopulations, helper T cells, cytotoxic T cells and a small subset of NK cells, total T cells, total B cells, and NK cells. These populations were ranged from 5% to 77% of controls.
- Increased in total protein, mainly due to an increase in albumin was reflected in an increase in A/G ratio. Calcium levels were also increased in association with albumin concentrations. The reported changes in glucose, triglycerides, and alkaline phosphatase were within normal variation and not toxicologically significant. Thus, the observed changes in clinical chemistry parameters were likely related to dehydration, possibly due to excessive salivation from drug administration.
- Except for control and high dose groups, not all tissues and organs were examined for pathology, therefore a NOAEL was not identified for some of the effects of CP-690550 treatment. The most prominent findings was atrophy in lymphoid tissues in the lymph nodes (inguinal, ileo-femoral and mesenteric), spleen, and thymus. This was often associated with reduced organ weights and macroscopic observations of small size. In the lungs, pulmonary histiocytosis with interstitial inflammation occurred at the high dose. There was a dose-related increase in liver weight (when expressed as % body weight) corresponding with hepatocellular hypertrophy in males and females.
- Systemic exposure was averaged 2.9, 2.2 and 1.6 -times greater in females than males at doses of 1, 10 and 100 mg/kg/day, respectively. Due to variation in the

timepoint data comprising the AUC, the sex difference in CP-690550 may not be a real finding. Most samples obtained after the 4 hour timepoint of the low and mid dose groups were below the level of CP-690550 detection, indicating that the lack of toxicity in the low and mid dose groups probably reflects their lack of continuous drug exposure (<8 hours/day).

- An overall NOAEL for the study could not be determined due to lung pathology and the lack of demonstrating recovery of anatomic and hematological findings. The animals were bleed multiple times throughout the study, which very likely contributed to some of the pathologies confounding the separation of a clear CP-690550 effect. However, for lymphoid tissues, a NOAEL of 1 mg/kg/day could be identified due to the lack of histopathological changes for tissues that were evaluated at all doses and the associated minimal hematological changes.. The lack of recovery assessment, hindered the possibility of attaining a higher dose as the NOAEL. The associated C_{max} and AUC exposure for 1 mg/kg/day at week 26 were C_{max} : male 120 mg/mL female 382 ng/mL, and AUC male 255 ng-h/mL (AUC₀₋₈), female 710 ng-h/mL (AUC₀₋₂₄).

Methods

Doses:	0 (vehicle), 1, 10, and 100 mg/kg/day and one sentinel group
Frequency of dosing:	once daily for 26 weeks
Route of administration:	orally, by gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	methylcellulose, the solvent in which the methylcellulose was dissolved and the final concentration of the vehicle was not specified.
Species/Strain:	Sprague Dawley CD (CrI: CD (SD)IGSBR) rats
Number/Sex/Group:	15/sex/dose, and 12/sex/sentinel group
Age:	Approximately 6 weeks old at the start of the study
Weight:	males: 171-209 g; females:137-182 g
Satellite groups:	toxicokinetics: 6/sex/dose group recovery
Study design:	

Group	Dose (mg/kg/day)	Animal #s			
		Main Study		Toxicokinetic Study	
		Males	Females	Males	Females
1	Control	1001-1015	1501-1515	1016-1021	1516-1521
2	1	2001-2004, 2006-2015 2022	2501-2515	2016-2021	2516-2521
3	10	3001-3015	3501-3515	3016-3021	3516-3521
4	100	4001-4015	4501-4515	4016-4021	4516-4521
5	Sentinel	5001-5012	5501-5512	-	-

Deviation from study protocol:

There were no deviations of the protocol that affected the study results or conclusions.

Observations and Results Results

Mortality

Animals were checked at least twice daily

There were 5 deaths during the study, 1 or 2 per dose group. Although there were no deaths in the control group, no death could be clearly attributed to CP-690550 treatment, rather all deaths appeared to be iatrogenic, related to blood collection or gavage administration. Three of the 5 deaths occurred during or following blood collection (via the jugular vein) for laboratory testing. Upon necropsy two animals (#2506, #3513) and had thoracic region skeletal muscle hemorrhage, hemorrhage of the thymus, dark discolored kidney probably due to accumulation of myoglobin and hemoglobin and their breakdown products, the third (#2510), had dorsal cervical skeletal muscle hemorrhage and subcutaneous axillary hemorrhage. Animal #4504 had a linear array of dark areas in the glandular region of the stomach and this was likely due to a gavage dosing error within a few days of death. Animal #3012 was found with dark fluid in the abdominal cavity, multiple fissures and multifocal hemorrhage in the liver, congested lung and dark foci in the thymus, also indicative of gavage error. There were no deaths in the 6-week rat study and no deaths at the 100 mg/kg/day dose in the 2-week rat study.

Table 42: Summary of Mortalities

Dose (mg/kg/day)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Main Study day	0	0	0	2 #2506 d 88 #2510 d 184	1 #3012 d 126	1 #3513 d 88	0	1 #4504 d 180
Toxicokinetic	0	0	0	0	0	0	0	0

Study								
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Clinical signs

Animals were checked twice daily and weekly complete physical examinations

Salivation following drug administration occurred in all animals of the 100 mg/kg/day dose group, occurring 780 times in males and 1089 times in females during the study. Findings in the high dose group only included decreased activity (M 1/15, F 1/15), abnormal breathing sounds (F 1/15), pale eyeball (M 1/15, F 1/15), red fur stain near mouth (M 4/15, F 2/15), red fur staining periorbital (M 4/15), malocclusion (M 2/15), broken teeth (M 1/15, F 2/15), swollen forelimb/axillary (M 1/15, F 1/15), skin papule on muzzle (F 1/15). The low incidence of these findings indicates they may not be CP-690550-related effects.

Body weights

Animals were weighed weekly

A reduction in body weight gain occurred in males in the 100 mg/kg/day dose group, beginning around week 6 compared to the control group. At the end of the 26-week treatment period, the high dose males had a mean body weight gain 82% of the weight of the control group. There was no effect on bodyweights and bodyweight gains for the other treatment groups. For females, there was a small increase in body weight gain compared to the control for all doses, up to 111% for the mid dose.

Table 43: Body Weight and Body Weight Gain (values rounded by Reviewer)

Sex	Males				Females			
Dose (mg/kg/day)	0	1	10	100	0	1	10	100
N	15	15	15	15	15	15	15	15
Week -1	147	145	148	146	127	128	127	127
Week 1	195	193	196	194	158	155	155	156
Week 6	410	396	399	392 (96%)	240	237	238	251 (105%)
Week 13	542	524	519	484 (89%)	289	296	296	308 (106%)
Week 26	652	637 (98%)	616 (94%)	559 (86%)	334	351 (105%)	354 (106%)	348 (104%)
Body Weight Gain (week -1 to 26)								
absolute	505	492	468	413	207	223	227	221
% gain	343%	340%	318%	283%	163%	174%	181%	173%
difference from control in		-3% (99%)	-25% (93%)	-60% (82%)		11% (107%)	18% (111%)	10% (106%)

% gain (% of control gain)								
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Food consumption

Food consumption was measured weekly

There were small significant decreases in mean food consumption in males in the 100 mg/kg/day, during weeks 10-12, 16-19, 22-23, and 25-26 (control ranged from 26 to 31 g/animal/day, 100 mg/kg/day ranged from 24 to 27 g/animal/day over these time periods).

There was no effect on food consumption for the other treatment groups.

Ophthalmoscopy

Eyes were examined before treatment and during study weeks 4, 13 and 26 using funduscopy (indirect ophthalmoscopy) and biomicroscopic (slit lamp)

There were no effects of CP-690550 treatment on ophthalmoscopic findings.

Hematology

Blood samples were collected before start of treatment and during weeks 4, 13, and 26.

Parameters examined:

+*activated partial thromboplastin time
blood cell morphology
erythrocyte indices (MCV, MCH, MCHC and RDW)

+*fibrinogen
hematocrit
hemoglobin
mean platelet volume
platelet count
+*prothrombin time
red blood cell count
reticulocyte count
white blood cell count (total, absolute and percent differential)

+ At termination only

Dose-dependent and time-dependent reductions in white and red blood cell parameters occurred in males and females at doses ≥ 10 mg/kg/day. For the lower dose group it generally took a longer treatment period to produce significant effects on hematological parameters.

Table 44: Summary of Red Cell Hematology and Fibrinogen

Dose (mg/kg/day)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
RBC (10⁶/μL)								
Predose	6.82	7.06	6.72	7.14	6.72	6.97	6.88	7.13
Week 4 (% of control)	8.59	8.59	8.94	8.55	8.71	8.32	8.80 (102)	8.11 (94)
Week 13	9.60	8.98	9.59	8.74	9.40 (98)	8.56 (95)	8.91 (93)	8.08 (90)
Week 26	9.49	8.53	9.36 (99)	8.42 (99)	8.80 (93)	8.30 (97)	8.29 (87)	7.27 (85)
Hematocrit (%)								
Predose	46.3	47.4	46.6	48.4	46.2	46.1	46.9	48.8
Week 4	50.1	50.4	53.0	50.9	51.7	48.8	52.0	47.8
Week 13	50.7	49.2	50.9	48.4	49.3 (97)	46.7 (95)	47.7 (94)	45.6 (93)
Week 26	50.8	47.8	50.9 (100)	48.1 (101)	47.2 (93)	46.7 (98)	44.6 (88)	42.1 (88)
Hemoglobin (g/dL)								
Predose	13.9	14.6	14.0	14.7	13.9	14.2	14.2	14.6
Week 4 (% of control)	16.6	16.7	17.3	16.8	16.8 (101)	16.2 (97)	17.0 (102)	15.8 (95)
Week 13	16.7	16.4	16.8	16.1	16.5 (99)	15.8 (96)	15.7 (94)	15.3 (93)
Week 26)	16.5	16.2	16.6 (101)	16.1 (99)	15.7 (95)	15.9 (98)	14.8 (90)	14.6 (90)
Fibrinogen (mg/dL)								
Week 26 (% of control)	229	164	216 (94)	173 (105)	224 (98)	181 (110)	230 (100)	202 (123)

Dose-dependent decreases in total white blood cell count occurred at doses ≥ 10 mg/kg/day in weeks 4, 13, and 26 (males and females) and at ≥ 1 mg/kg/day during weeks 13 and 26 (females only). These were primarily due to decreases in lymphocytes. Increases in neutrophils (approximately 400% above control) and monocytes (approximately 250% above control) occurred in the high dose.

Table 45: Summary of White Cell Hematology

Hematology								
Dose (mg/kg/day)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
WBC (10³/μL)								
Predose	8.24	8.15	6.97	9.40	7.81	7.85	7.20	7.88
Week 4 (% of control)	11.70	8.80	10.16	8.15	8.81	5.06 (57)	5.23 (45)	2.79 (32)
Week 13	11.48	8.36	9.86	6.89	7.68	3.90	3.84	3.03
Week 26	9.34	6.31	7.56 (81)	5.14 (81)	6.23 (67)	4.02 (64)	3.51 (38)	2.55 (40)
% Lymphocyte								
Predose	85.0	87.1	86.3	86.2	89.2	86.6	87.8	85.3
Week 4	84.9	88.7	86.2	83.1	83.7	82.6	74.2 (87)	70.0 (79)
Week 26 (% of control)	80.0	84.0	77.1 (96)	79.8 (95)	76.7 (96)	72.9 (87)	49.4 (62)	46.6 (55)
% Neutrophils								
Predose	11.0	9.7	9.9	10.3	7.7	9.7	8.3	11.0
Week 4 (% of control)	11.2	7.4	10.0	12.3	12.5	13.1	21.2 (189)	24.5 (331)
Week 13	13.8	10.6	18.7	17.5	17.1	20.5	36.7 (266)	40.9 (386)
Week 26	14.2	11.2	17.4 (122)	14.7 (131)	17.9 (126)	21.1 (188)	43.0 (303)	44.6 (398)
% Monocytes								
Predose	2.5	1.53	2.4	1.65	1.8	1.92	2.0	1.78
Week 4 (% of control)	1.83	1.63	1.76	2.50	2.05	2.43	3.05 (167)	3.86 (211)
Week 13	2.47	2.36	3.14	3.34	3.20	3.44	5.58 (226)	6.11 (259)
Week 26 (% of control)	3.57	2.61	3.05 (85)	2.93 (112)	3.18 (89)	3.63 (139)	5.59 (156)	6.12 (234)

Immunophenotyping

For weeks 6, 14 and 26, the absolute cell count and relative proportion of lymphocyte subsets were determined using the following antigen markers: anti-CD4 (helper T cells), anti-CD8a (cytotoxic T cells and a small subset of NK cells), anti-CD3 (total T cells), anti-CD45RA (total B cells) and anti-CD161a (NKR-P1A; NK cells and a small subset of T cells).

There were dose-dependent reductions (ranging from 5% to 77%) in CD3+, CD4+, CD8a+, CD45RA+, and CD161a+ lymphocyte sub-populations at weeks 14 and 26. The effect at week 14 was generally not further reduced at week 26 for doses of ≥ 10 mg/kg/day, but for the low dose, longer duration of treatment further reduced some subpopulations as indicated in the table below. These subpopulation reductions paralleled the decrease in total lymphocyte counts at weeks 14 and 26 noted above for both males and females at doses of ≥ 10 mg/kg/day. At the lowest dose, 1 mg/kg, CD8a+ and CD161a+ lymphocytes were decreased at weeks 14 and 26 in both males and females. CD45RA+ lymphocytes decreased in females at week 14 and in both males and females at week 26.

The hematology and immunophenotyping evaluations were adequate.

Table 46: Summary of the Effect of CP690550 on Lymphocyte Subpopulations**Table 61: Summary table (continued)**

Parameter	Study Day	Group 1		Group 2		Group 3		Group 4	
		Male	Female	Male	Female	Male	Female	Male	Female
Mean absolute CD4 ⁺ lymphocyte count (a)	Week 6	-	-	-	-	-	-	-	-
	Week 14	3022.42	2663.61	2725.89 (-9.8)	2465.72 (-7.4)	2114.80 D (-30.0)	1322.51 E (-50.3)	823.99 F (-72.7)	707.69 F (-73.4)
	Necropsy	2035.42	1424.10	1717.94 (-15.6)	1407.09 (-1.2)	1415.49 D (-30.5)	1089.06 A (-23.5)	562.44 F (-72.4)	482.01 C (-66.2)
Mean absolute CD8a ⁺ lymphocyte count (a)	Week 6	-	-	-	-	-	-	-	-
	Week 14	1792.35	1471.58	1395.05 (-22.2)	1024.56 (-30.4)	814.77 F (-54.5)	381.79 F (-74.1)	149.38 F (-91.7)	62.44 F (-95.8)
	Necropsy	1352.11	909.18	983.75 (-27.2)	572.36 (-37.0)	517.69 F (-61.7)	305.02 F (-66.5)	55.01 F (-95.9)	32.12 F (-96.5)
Mean absolute CD3 ⁺ lymphocyte count (a)	Week 6	-	-	-	-	-	-	-	-
	Week 14	4939.19	4493.20	4075.78 (-17.5)	3735.75 (-16.9)	2914.51 E (-41.0)	1753.64 F (-61.0)	974.07 F (-80.3)	775.06 F (-82.8)
	Necropsy	3425.76	2295.77	2805.81 (-18.1)	1953.37 (-14.9)	2030.48 E (-40.7)	1360.13 C (-40.8)	626.45 F (-81.7)	482.26 C (-79.0)
Mean absolute CD45RA ⁺ lymphocyte count (a)	Week 6	-	-	-	-	-	-	-	-
	Week 14	3885.04	2677.38	3235.67 (-16.7)	2050.50 B (-23.4)	2828.56 (-27.2)	1182.51 C (-55.8)	1230.01 F (-68.3)	778.39 C (-70.9)
	Necropsy	2840.93	1729.24	1820.57 (-35.9)	1280.03 (-26.0)	1873.42 (-34.1)	857.00 F (-50.4)	825.51 F (-70.9)	566.64 F (-67.2)
Mean absolute CD161a ⁺ lymphocyte count (a)	Week 6	-	-	-	-	-	-	-	-
	Week 14	1020.78	677.04	774.04 (-24.2)	439.29 (-35.1)	571.64 D (-44.0)	197.78 F (-70.8)	174.25 F (-82.9)	127.45 F (-81.2)
	Necropsy	461.36	309.62	308.00 (-33.2)	143.52 D (-53.6)	173.45 F (-62.4)	97.76 F (-68.4)	49.97 F (-89.2)	43.74 F (-85.9)

a: Percent differences compared to control Group 1 are shown in parenthesis

Significantly different from control group (Group 1) value: A - $P \leq 0.05$ B - $P \leq 0.01$ C - $P \leq 0.001$ (Dunnett)D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)**Clinical chemistry**

Blood samples were collected before start of treatment and during weeks 4, 13, and 26

Parameters examined:

A/G ratio (calculated)
alanine aminotransferase
albumin
alkaline phosphatase
aspartate aminotransferase
blood urea nitrogen
calcium
chloride
cholesterol
creatinine
gamma glutamyl transferase
globulin (calculated)
glucose
inorganic phosphorus
potassium
sodium
total bilirubin
total protein
triglycerides

The most notable effect was an increase in total protein at the high dose associated with an increase in albumin and increased A/G ratio. The increase in calcium at the high dose is likely related to the increase in serum albumin. The increase in sodium in association with protein may also reflect a degree of dehydration in these animals.

There was no treatment-related effect on alkaline phosphatase concentrations, glucose and triglycerides in contrast to the applicant's conclusions. Alkaline phosphatase concentrations decreased during the study in all dose groups including the controls by approximately the same amount. There was no toxicologically significant effect of CP-690550 on triglyceride levels. The lower values in the mid and high dose female groups were due to an increase in levels in the control group during the later half of the study. The high dose groups had values similar to their predose levels. The changes in alkaline phosphatase, glucose and triglycerides were within the range of normal fluctuations and not toxicologically significant.

The clinical chemistry evaluation was adequate.

Clinical Chemistry								
Dose (mg/kg/day)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Alkaline Phosphatase (U/L)								
Predose	297	236	264	242	292	255	311	216
Week 4 (% of control)	204	128	182	143	203	160	221 (108)	150 (117)
Week 13	93	52	87	60	96	74	108 (116)	76 (146)
Week 26	77	33	72	34	79	54	96 (124)	60 (182)
Glucose (mg/dL)								
Predose	58	70	64	80	54	78	62	60
Week 4 (% of control)	90	87	92	92	94	97	113 (125)	107 (123)
Week 13	102	95	105	96	109	102	113	107
Week 26	96	83	86	87	95 (99)	99 (119)	111 (116)	104 (125)
Na (mEq/L)								
Predose	150	150	150	149	147	148	148	150
Week 4	148	148	150	150	150	150	148 (100)	151 (102)
Week 13	146	147	149	149	151 (103)	151 (103)	152 (103)	153 (104)
Week 26	149	147	150	149	150 (101)	150 (102)	152 (102)	151 (103)
Calcium (mg/dL)								
Predose	11.0	11.1	11.01	11.1	11.0	11.2	11.2	11.3
Week 4	11.0	10.9	10.9	11.2	10.9	10.9	10.8	11.3
Week 13 (% of control)	11.5	11.5	11.4	11.6	11.7	11.5	11.6 (101)	12.3 (107)
Week 26	11.3	11.5	11.2	11.7	11.3	11.5	11.6 (103)	11.9 (103)
Triglycerides (mg/dL)								
Predose	86	74	62	82	124	84	79	74
Week 4 (% of control)	68	50	60	66	75	50	97 (143)	62 (124)
Week 13	135	84	116	98	125	68	98 (72)	68 (81)

Week 26	228	148	195	153	207 (91)	98 (66)	174 (76)	89 (60)
Total Protein (g/dL)								
Predose	5.93	6.26	5.97	6.28	5.72	6.10	5.77	6.35
Week 4 (% of control)	6.77	6.96	6.87	7.07	6.85	6.85	7.02 (104)	7.33 (105)
Week 13	7.45	7.57	7.42	7.65	7.53	7.54	8.10 (109)	7.97 (105)
Week 26	7.93	8.29	7.99	8.31	7.97 (100)	7.94 (96)	8.77 (110)	8.59 (104)
Albumin (g/dL)								
Predose	4.35	4.65	4.42	4.64	4.20	4.52	4.29	4.66
Week 4 (% of control)	4.35	4.85	4.45	4.95	4.47	4.74	4.69 (108)	4.97 (102)
Week 13	4.83	5.41	4.85	5.42	4.98	5.24	5.47 (113)	5.37 (99)
Week 26	4.85	5.78	5.07	5.84	5.10 (105)	5.36 (93)	5.73 (118)	5.60 (97)
Globulin (g/dL)								
Predose	1.58	1.61	1.55	1.64	1.52	1.58	1.49	1.72
Week 4 (% of control)	2.42	2.11	2.42	2.13	2.37	2.11	2.33 (96)	2.35 (2.12)
Week 13	2.61	2.17	2.57	2.23	2.55	2.30	2.63 (101)	2.61 (120)
Week 26	3.03	2.51	2.93	2.48	2.87	2.59	3.03 (100)	2.99 (119)
A/G ratio								
Predose	2.77	2.91	2.87	2.86	2.78	2.87	2.91	2.73
Week 4 (% of control)	1.81	2.32	1.85	2.34	1.89	2.28	2.03 (2.51)	2.12 (91)
Week 13	1.87	2.52	1.90	2.45	1.97	2.29	2.11 (113%)	2.07 (82)
Week 26	1.62	2.32	1.74	2.38	1.80	2.08	1.91 (118)	1.88 (81)

Urinalysis

Urine samples were collected before the start of treatment and during Weeks 4, 13, and 26

Parameters examined:

bilirubin	nitrite
blood	pH
color and appearance	protein
glucose	specific gravity
ketones	urobilinogen
microscopy of centrifuged deposit	volume

There were no effects of CP-690-550 on urinalysis parameters.

The urinalysis evaluation was adequate.

Gross pathology

Necropsy occurred at the end of week 26. Each animal was examined externally and internally for lesions, and tissues and organs were examined before processing for histopathology (refer to the Histopathology inventory table in the histopathology section of this study review. The gross pathology examination was adequate.

Dose-related macroscopic changes were noted in the lungs, liver, and lymphoid organs (ileo-femoral and inguinal lymph nodes, spleen, and thymus). In the lungs of 1 male and 1 female at 100 mg/kg/day, pale foci (attributed to alveolar histiocytosis) were observed. Lymph nodes, spleen, and thymus were visually decreased in size, with a higher incidence of this effect in rats of the high dose group. There was a dose-related increased incidence for liver enlargement.

Table 47: Gross Pathological Findings

Dose (mg/kg/day)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
N examined (unless indicated otherwise)	15	15	15	15	15	15	15	15
Kidney , area pale	0	0	0	0	0	0	1	1
area raised	0	0	0	0	0	0	0	1
dilatation pelvis	0	0	0	0	1	0	0	1
Lung , pale foci	0	0	0	0	0	0	1	1
dark foci	0	0	0	0	0	0	1	1
Liver , enlarged	1	0	2	0	3	0	4	1

enlargement	1	0	2	0	3	0	4	1
Lymph node, mesenteric, dark foci	0	0	0	0	0	1	0	0
Lymph node, inguinal, small foci dark	0 0	0 0	1 2	0 0	5 2	3 0	11 2	11 0
Lymph node, iliofemoral, small discoloration dark foci dark	0 0 0	1 0 0	0 0 0	0 0 1	0 0 0	2 1 0	2 0 0	6 1 1
Spleen, small	0	0	0	0	0	0	2	1
Stomach, dark foci or area	0	0	0	0	2	0	2	1
Thymus, small	0	0	0	1	1	2	9	12

Organ weights

The weighed organs were adrenal glands, brain, epididymides, heart, kidneys, liver, ovaries, prostate, spleen, testes, and thymus. Relative organ weight was only calculated as % body weight, % brain weight was not calculated in the applicant's analysis.

CP-690550-related organ weight changes occurred in spleen, liver, and thymus. A decrease in spleen weight relative to control was seen at all doses in males and females. Thymus weight was decreased in high dose males and females. A small increase in relative liver weight occurred in the high dose.

Table 48: Organ Weights (values rounded by Reviewer)

	Males				Female			
Dose (mg/kg/day)	0	1	10	100	0	1	10	100
Spleen g	0.92	0.78	0.63	0.44	0.55	0.47	0.45	0.35
% body weight	0.15	0.13	0.11	0.08	0.18	0.15	0.14	0.11
Thymus g	0.16	0.16	0.14	0.08	0.13	0.14	0.13	0.06
% body weight	0.02	0.03	0.02	0.01	0.04	0.05	0.04	0.02

Liver g	16.8	16.5	16.1	16.3	7.87	7.87	7.97	9.18
% body weight	2.71	2.73	2.78	3.05	2.56	2.47	2.48	2.87

Histopathology

Adequate battery: Yes

Histopathologic examination was performed on tissues from all animals from Groups 1 and 4, and any tissues showing abnormalities during gross examination from animals from Groups 2 and 3. Since pathologies identified at the high dose may not have been examined at lower doses, a NOAEL could not be identified. The battery of tissues was adequate, the evaluation was not optimal.

Peer review: Not by an outside pathologist, the peer review pathologist was employed by the applicant. The peer review pathologist's signed statement indicated that the study pathologist and review pathologist discussed differences in opinion of study findings and resolved these differences. These resolved differences of opinion between the two pathologists were presumably incorporated into the study pathologist's final report.

Table 49: Histopathology Inventory**Histopathology inventory**

Study: 77435			
Species: Sprague Dawley Rat			
Adrenals	X, *	Nasal cavity	
Aorta	X	Optic nerves	X
Bone Marrow smear	X	Ovaries	X, *
Bone (femur)	X	Pancreas	X
Brain	X, *	Parathyroid	X
Cecum	X	Peripheral nerve	
Cervix	X	Pharynx	
Colon	X	Pituitary	X
Duodenum	X	Prostate	X, *
Epididymis	X, *	Rectum	X
Esophagus	X	Salivary gland	X
Eye	X	Sciatic nerve	X
Fallopian tube		Seminal vesicles	X
Gall bladder		Skeletal muscle	X
Gross lesions	X	Skin	X
Harderian gland	X	Spinal cord	X
Heart	X, *	Spleen	X, *
Ileum	X	Sternum	
Injection site	NA	Stomach	X
Jejunum	X	Testes	X, *
Kidneys	X, *	Thymus	X, *
Lachrymal gland		Thyroid	X
Larynx		Tongue	X
Liver	X, *	Trachea	X
Lungs	X	Ureter	X
Lymph nodes, cervical		Urinary bladder	X
Lymph nodes, mandibular		Uterus	X
Lymph nodes, mesenteric	X	Vagina	X
Mammary Gland	X	Zymbal gland	

X histopathology performed

* organ weight obtained

CP- 690550-related effects occurred in lymphoid tissues, lungs, liver, pancreas, and adrenals.

Lymphoid Tissue (lymph nodes, spleen, thymus)

Atrophy of lymphoid tissue in the lymph nodes, spleen, and thymus occurred at doses ≥ 10 mg/kg/day. These generally correlated with the small size of these organs upon macroscopic observation and the decreases in the organ weights. Atrophy of the GALT

was only noted in males and females of the 100 mg/kg/day dose group, however there were many tissues in which it was not present in the examined sections. Still, there were sufficient tissues to confirm CP690550 associated atrophy in GALT. The lymphoid atrophy was not restricted to specific subregions of the lymphoid organs, but affected the cortex and paracortex in the lymph nodes, PALS and mantle zone in the spleen and the cortex and medulla in the thymus. In the spleen, lymphoid atrophy was accompanied by a relative diminution in number of hematopoietic cells in the red pulp, primarily in rats given 100 mg/kg/day. The change was described as minimal to slight decreased extramedullary hematopoiesis

Lungs

In the lungs, there was an increase in incidence and severity of alveolar histiocytosis and interstitial inflammation was noted in males at doses ≥ 10 mg/kg/day and in 100 mg/kg/day females. These changes were characterized by variable sized focal/multifocal aggregates of macrophages within the alveolar spaces accompanied by multifocal infiltration of mononuclear cells in the alveolar wall and/or perivascular/peribronchiolar spaces. Of particular concern were the findings of hemorrhage at all doses including controls. It was not obvious from the locations of hemorrhage that these were associated with jugular vein blood collection throughout the study, although that is a possibility since the technique can be difficult, particularly after a few collections. The lung findings contributed to the lack of an identifiable NOAEL.

Liver

Minimal to slight hepatocellular hypertrophy occurred in the liver of the high dose groups. This was not consistently correlated with individual liver weights but did correlate with macroscopic observations of liver enlargement.

Pancreas

Minimal degeneration of the pancreatic islets was observed only in high dose, but was also present in male control and treatment groups. Not all animals were evaluated at the low and middle dose, so no dose-related incidence could be determined. The finding generally affected one or more islets, and characterized by fragmentation of the islets into smaller nests by fibrous tissues admixed with rare mononuclear cells.

Adrenal

In the high dose males group and 1 high dose female, there was an increase in adrenal gland vacuolation, described as a diffuse vacuolation of the zona fasciculata.

The overall histological evaluation was not adequate.

Table 50: Histopathology Summary

Revised Table

Group		1	2	3	4
Dose (mg/kg/day)		0	1	10	100

		M	F	M	F	M	F	M	F
N		15	15	15	15	15	15	15	15
AUC₀₋₂₄ (ng-h/mL) at week 26 * (AUC_{0-8h})		-	-	(255)*	742 2.9X	3440	7680 2.2X	43200	68800 1.6X
Adrenal									
N		15	15	15	3	15	1	15	15
cortical hypertrophy	Grade 1	2	1	0	0	1	0	2	0
	Grade 2	2	1	0	0	0	0	0	0
cortical vacuolation	Grade 1	0	0	2	0	3	0	6	1
	Grade 2	0	0	0	0	4	0	3	0
	Grade 3	0	1	0	0	0	0	1	0
Bone marrow									
N		15	15	15	2	15	1	15	15
hypocellularity, hematopoietic	Grade 1	0	0	0	0	0	0	0	1
hypercellularity, myeloid	Grade 2	0	0	0	0	0	0	0	1
Duodenum									
N		15	15	0	2	1	1	15	15
hyperplasia, villous/mucosal	Grade 1	0	0	0	0	0	0	0	2
Jejunum									
N		15	15	15	15	15	15	15	15
lymphoid atrophy GALT	Grade 1	0	0	0	0	0	0	2	4
	Grade 2	0	0	0	0	0	0	3	1
Kidney									
N		15	15	1	2	2	1	15	15
urothithiasis	Grade 1	0	0	0	0	0	0	0	1
dilatation, pelvis	Grade 1	0	0	0	0	1	0	0	0
	Grade 2	0	0	0	0	1	0	0	2
Liver									
N		15	15	15	15	15	15	15	15
necrosis, (incl. single cell multifocal)	Grade 1	0	1	0	0	0	0	1	0
	Grade 2	0	0	0	0	0	0	0	0
	Grade 3	0	0	0	0	1	0	1	0
	Grade 4	0	0	0	0	1	0	0	0
inflammation	Grade 1	3	7	0	3	0	2	1	1
	Grade 2	0	0	0	0	0	0	0	0
	Grade 3	0	0	0	0	1	0	0	0
hepatocellular	Grade 1	0	0	0	0	0	0	4	3

hypertrophy	Grade 2	0	0	0	0	0	0	3	1
Lung									
N		15	15	15	15	15	15	15	15
histiocytosis	Grade 1	1	1	1	0	2	2	5	3
	Grade 2	0	0	0	0	1	0	6	8
	Grade 3	0	0	0	0	0	0	3	0
	Grade 4	0	0	0	0	0	0	0	1
interstitial inflammation	Grade 1	3	1	2	1	1	3	6	1
	Grade 2	0	0	0	0	1	0	2	3
	Grade 3	0	0	0	0	0	1	1	0
	Grade 4	0	0	0	0	0	0	0	1
hemorrhage, (with/without inflammation)	Grade 1	2	1	4	2	8	0	3	1
	Grade 2	0	1	0	0	0	0	0	6
	Grade 3	0	0	0	0	0	0	0	1
Lymph Node, Mandibular (selected from gross lesion obs)									
N		5	0	2	1	5	0	1	1
atrophy, lymphoid	Grade 1	0	0	0	0	0	0	1	0
erythrocytosis/hemorrhage, sinusal	Grade 1	5	0	2	1	5	0	0	0
	Grade 2	0	0	0	0	0	0	1	1
Iliofemoral LN									
N		12	15	14	15	12	12	11	14
atrophy/necrosis, lymphoid	Grade 1	0	0	0	0	0	1	5	4
	Grade 2	0	0	0	0	0	1	4	6
	Grade 3	0	0	0	0	0	0	1	2
erythrocytosis/hemorrhage, sinusal	Grade 1	0	1	0	0	0	0	2	1
	Grade 2	0	0	0	0	0	0	1	0
Inguinal LN									
N		14	13	15	15	15	15	11	12
atrophy/necrosis, lymphoid	Grade 1	0	0	0	0	1	4	3	2
	Grade 2	0	0	0	0	0	1	7	8
	Grade 3	0	0	0	0	0	0	0	1
Mesenteric LN									
N		15	15	15	15	15	15	15	13
atrophy/necrosis, lymphoid	Grade 1	0	0	0	0	0	8	3	4
	Grade 2	0	0	0	0	0	1	8	7
	Grade 3	0	0	0	0	0	0	0	2
Pancreas									
N		15	15	0	15	1	15	15	15
degeneration, islet	Grade 1	5	0	0	0	0	0	5	6
	Grade 2	3	0	0	0	0	0	5	0
acinar cell	Grade 1	4	1	0	0	0	0	2	1

atrophy, multifocal	Grade 2	0	0	0	0	1	0	1	0
	Grade 3	0	0	0	0	0	0	0	0
Spleen									
N		15	15	15	15	15	15	15	15
atrophy lymphoid	Grade 1	0	0	0	0	0	5	6	3
	Grade 2	0	0	0	0	0	0	7	9
	Grade 3	0	0	0	0	0	0	0	3
decreased extramedullary hematopoiesis	Grade 1	1	0	0	0	0	0	5	7
	Grade 2	0	0	0	0	0	0	4	6
Stomach									
N		15	15	2	2	1	3	15	15
erosion, glandular	Grade 1	0	0	0	0	0	0	2	1
mucosa inflammation	Grade 1	0	0	0	0	0	0	1	0
Thymus									
N		15	15	15	15	15	15	15	15
atrophy, lymphoid	Grade 1	0	0	0	0	0	5	4	2
	Grade 2	0	0	0	0	0	0	8	6
	Grade 3	0	0	0	0	0	0	2	6
	Grade 4	0	0	0	0	0	0	0	1
hemorrhage, focal of multifocal	Grade 1	4	5	5	2	5	2	1	0
	Grade 2	2	0	3	2	3	1	0	0
	Grade 3	0	0	0	0	0	0	0	0
Grade Description									
1 = Minimal / very few / very small									
2 = Slight / few / small									
3 = Moderate / moderate number / moderate size									
4 = Marked / many / large									
5 = Severe / extensive number / extensive size									

Toxicokinetics

Blood samples were obtained from three animals per sex in each of the four toxicokinetic groups at each timepoint, 0.5, 1, 2, 4, 8, and 24 hours post dosing on day 1 and during weeks 4, 13, and 26. The lower limit of quantification was 5 ng/mL. Samples were analyzed by (b) (4) (b) (4)

(b) (4) using a validated S/MS assay for rat serum,
(b) (4) Study No. PFICT01H-24.

Serum concentrations of CP-690550 were below the detection limits for most samples after 4 hours in the 1 mg/kg/day and 10 mg/kg/day dose groups (refer to graphical presentations below). Exposure to CP-690550 was not continuous, but intermittent comprising up to approximately 8 hours for the low and mid dose groups as indicated in

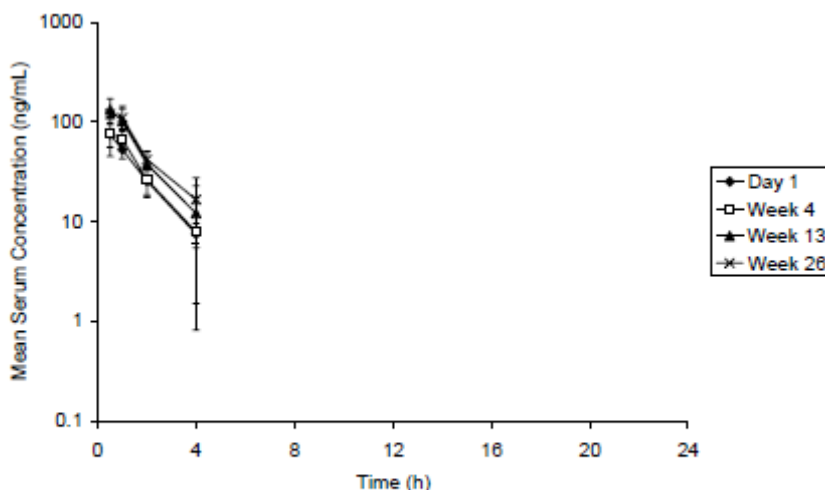
the graphs below. Based on mean C_{max} and AUC_{0-8h} values, throughout the study females consistently had greater exposure to CP-690550 (approximately 2-3.5-fold higher) than males for the 1 and 10 mg/kg/day dose groups, but not at 100 mg/kg/day due to variation in the data. The C_{max} and AUC at week 26 at summarized in the table below.

Table 51: Toxicokinetics at Week 26 in Rats

Dose (mg/kg/day)	1		10		100	
	M	F	M	F	M	F
C_{max} (ng/mL)	120	382	1640	3040	9670	10600
AUC_{0-24} (ng-h/mL)	255	742	3440	7680	43200	68800

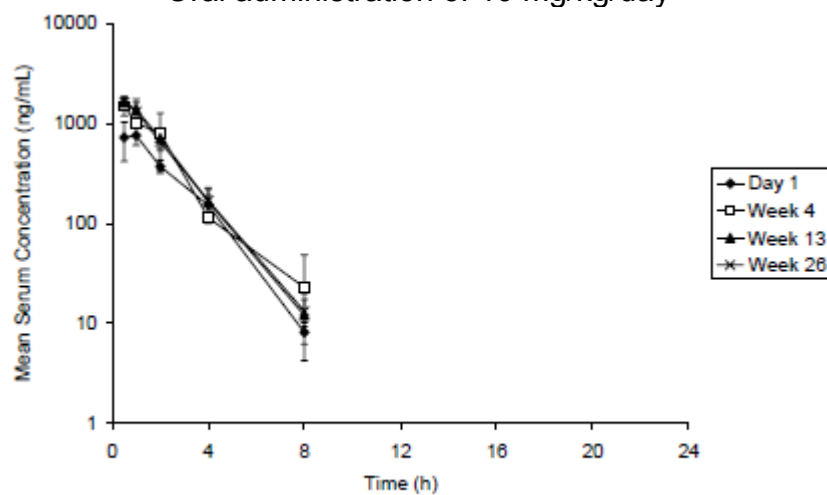
Figure 7: Toxicokinetic Profiles for the 3 doses of CP-690550

Oral administration of 1 mg/kg/day



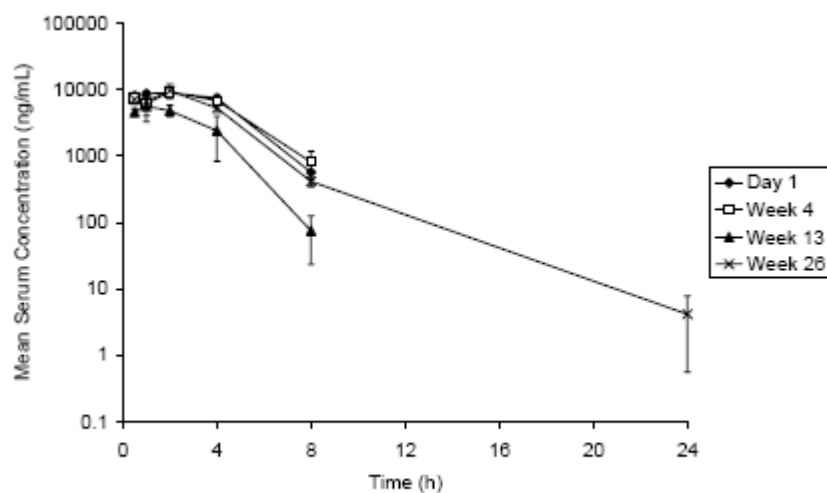
Data are expressed as mean \pm SD, n = 3/sex/dose group/time point

Oral administration of 10 mg/kg/day



Data are expressed as mean \pm SD, n = 3/sex/dose group/time point

Oral administration of 100 mg/kg/day



Data are expressed as mean \pm SD, n = 3/sex/dose group/time point

Table 52: Toxicokinetic Summary

Table 4. Mean Toxicokinetic Parameters

	Day 1, males			Day 1, females		
	1	10	100	1	10	100
t_{max} (h)	0.5	1.0	2.0	1.0	0.5	2.0
C_{max} (ng/mL)	75	781	9000	179	2480	10900
$AUC_{(0-8h)}$ (ng ² h/mL)	138	1970	47700	485	5410	46100
$AUC_{(0-24h)}$ (ng ² h/mL)	NR	2030	52300	513	5850	NR ^a

	Week 4, males			Week 4, females		
	1	10	100	1	10	100
t_{max} (h)	0.5	0.5	2.0	0.5	0.5	1.0
C_{max} (ng/mL)	76	1500	9270	227	2900	9020
$AUC_{(0-8h)}$ (ng ² h/mL)	151	3100	44400	460	6380	46900
$AUC_{(0-24h)}$ (ng ² h/mL)	NR	3280	51000	NR	6820	66200

	Week 13, males			Week 13, females		
	1	10	100	1	10	100
t_{max} (h)	0.5	0.5	1.0	0.5	0.5	1.0
C_{max} (ng/mL)	132	1630	5630	343	3390	7640
$AUC_{(0-8h)}$ (ng ² h/mL)	234	3460	21200	681	7520	24400
$AUC_{(0-24h)}$ (ng ² h/mL)	NR	3550	21800	725	8000	30900

	Week 26, males			Week 26, females		
	1	10	100	1	10	100
t_{max} (h)	0.5	0.5	2.0	0.5	0.5	1.0
C_{max} (ng/mL)	120	1640	9670	382	3040	10600
$AUC_{(0-8h)}$ (ng ² h/mL)	255	3330	39800	710	6860	49100
$AUC_{(0-24h)}$ (ng ² h/mL)	NR	3440	43200	742	7680	68800

NR Not reportable due to insufficient concentration data

NR^a Not reportable because $AUC_{(8-24h)}$ was greater than 30% of the $AUC_{(0-24h)}$

Sentinel Monitoring (Serology Report)

Blood from three rats per gender were examined for presence of viral antibodies after arrival and during weeks 4, 13, and at the completion of the study. These assays were conducted by (b) (4)

Table 53: List of Infectious Disease Organism Monitoring

Name	Family/Genus	Report Abbrev.
Sendai virus	Parainfluenza	SEND
Pneumonia virus of mice	Paramyxo	PVM
Rat Sialodacryoadenitis	Corona	SDAV
Kilham Rat Virus	Parvo	KRV
Toolan's H-1 Virus	Parvo	H-1
Mycoplasma Pulmonis	Mycoplasma	MPUL
Encephalitozoon cuniculi	Pleistophoridae	ECUN
Cilia-Associated Respiratory Bacillus	NA	CARB
Rat parvovirus	Parvo	NS-1
Mouse poliovirus	Picorna	GDVII
Reovirus Type 3	Reo	REO
Mouse adenovirus	Adeno	MAV (1 & 2)
Lymphocytic choriomeningitis virus	Arena	LCMV
Hantaan virus	Hanta	HANT

No abnormal results were found for the 14 serological tests.

Stability and Homogeneity

Stability: The applicant mentioned that the stability of the formulations was previously demonstrated, but no study report or protocol was referenced.

Homogeneity: Samples collected from weeks 1, 12, and 24 were analyzed for homogeneity of the dosing solutions. Samples were homogeneous, within $\pm 10\%$ of nominal values.

Concentration Verification: Samples from the middle of the container of each dose formulation on the day of preparation from weeks 4, 8, 16 and 20 were analyzed for concentration. Samples were within $\pm 10\%$ of nominal values except for for week 1 in which the 1 mg/kg/day group dosing solution was 130.5-132.4% of the nominal values. Except for slightly greater toxicokinetic values on day 1, this would not affect the study results or conclusions.

STUDIES IN JUVENILE RATS

Study title: Oral Dose Range-Finding Study of CP-690550 in Juvenile Rats

Study no.:	09GR249
Study report location:	Mod. 4.2.3.5.4
Conducting laboratory and location:	Pfizer Global Research & Development Drug Safety Research & Development Eastern Point Road, Groton, CT
Date of study initiation:	Sept 29 2009
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	CP-690550-10,;Lot GR02684, a sub-lot of Lot E010009450), Purity: 102% (by HPLC) Composition: active moiety 61.8% citrate counterion 38.1%

Key Study Findings

- CP-690550-10 was administered orally to rats pups from postnatal day 12 through day 35 at doses of 1, 10 and 100 mg/kg/day.
- There were no mortalities and no effects on clinical signs, body weight, or body weight gain. Clinical and anatomic pathology were not conducted.
- CP-690550 plasma concentration, determined from a single blood sample obtained 0.5 hours after administration, increased with increasing dose. Exposures of females at PND 35 were greater than males (2-fold at the low and mid doses, 1.3-fold at the high dose), but there was no evidence for CP-690550 accumulation between PND 12 and 35.

Methods	
Doses:	0, 1.0, 10, and 100 mg/kg Dose levels expressed as mg/kg refer to mg active moiety of compound.
Frequency of dosing:	Once daily from PND 21-35
Route of administration:	Orally by gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% methylcellulose
Species/Strain:	Sprague-Dawley rat (CrI:CD®[SD])
Number/Sex/Group:	10/sex/dose
Age:	PND 21 at the start of dosing
Weight:	Males 42.8 g to 66.9 g Females: 44.3 g to 67.8 g
Satellite groups:	Toxicokinetic: 3/sex/dose obtained from the main study animals

Unique study design:	<p>8 litters of lactating Sprague-Dawley rats [CrI:CD®(SD)] were obtained with each litter consisting of 6 F₁ males and 6 F₁ females. None of the F₀ females were treated. By PND 14, litters were culled to 5 males and 5 females. These pups were assigned to 4 groups of 10/sex/group (3 dose groups and 1 control group).</p> <p>After weaning on PND 21, pups were housed by sex within study groups, up to 5 animals/cage. On PND 28, pups were housed individually.</p>																																
<table><tr><th rowspan="2">Group Number</th><th rowspan="2">Daily Dose^a (mg/kg)</th><th rowspan="2">Drug Concentration (mg/mL)</th><th rowspan="2">Dose Volume (mL/kg)</th><th colspan="2">Animal Numbers</th></tr><tr><th>Males</th><th>Females</th></tr><tr><td>1</td><td>0</td><td>0</td><td>10</td><td>1-10</td><td>41-50</td></tr><tr><td>2</td><td>1</td><td>0.1</td><td>10</td><td>11-20</td><td>51-60</td></tr><tr><td>3</td><td>10</td><td>1.0</td><td>10</td><td>21-30</td><td>61-70</td></tr><tr><td>4</td><td>100</td><td>10</td><td>10</td><td>31-40</td><td>71-80</td></tr></table> <p>^aAll dose levels are expressed as mg of active moiety per kg of body weight.</p>		Group Number	Daily Dose ^a (mg/kg)	Drug Concentration (mg/mL)	Dose Volume (mL/kg)	Animal Numbers		Males	Females	1	0	0	10	1-10	41-50	2	1	0.1	10	11-20	51-60	3	10	1.0	10	21-30	61-70	4	100	10	10	31-40	71-80
Group Number	Daily Dose ^a (mg/kg)					Drug Concentration (mg/mL)	Dose Volume (mL/kg)	Animal Numbers																									
		Males	Females																														
1	0	0	10	1-10	41-50																												
2	1	0.1	10	11-20	51-60																												
3	10	1.0	10	21-30	61-70																												
4	100	10	10	31-40	71-80																												
Deviation from study protocol:	There were no deviations mentioned in the study.																																

Observations and Results

Mortality

There were no mortalities.

Clinical Signs

Clinical signs on the F₀ dams and F₁ preweaning pups were recorded at least twice daily throughout the study. During the treatment period, surviving F₁ animals were observed at least 3 times daily, generally predose, ~30 minutes post the last animal dosed, and near the end of the workday.

There were no CP-690550-related effects on clinical observations.

Body Weights

F₀ females were weighed on the day of arrival and on LD 10, 14, 17, and 20. For F₁ pups, preweaning body weights were collected on PND 14, 17, and 20, after weaning body weights were collected on PND 21, 23, 25, 28, 30, 32, and 35.

There were no CP-690550-related effects on body weights or body weight gain.

Feed Consumption

Food consumption was not monitored in this study.

Hematology, Clinical Chemistry and Urinalysis

Clinical pathology evaluations were not performed.

Gross Pathology

No gross pathology, organ weights, or histopathology was performed.

Toxicokinetics

Jugular vein blood samples were obtained under CO₂-O₂ anesthesia at 30 min after the first dose on PND 21 and after the last dose on PND 35.

Serum CP-690550 at 0.5 h following the first and last dose (PND 21 and PND 35, respectively) increased with increasing dose. Exposures between males and females were generally similar on PND 21, but on PND 35 females had a 2-fold higher exposure at the low and mid doses, but only a 1.3-fold higher exposure at the high dose.

Table 54: Toxicokinetic Parameters

Dose (mg/kg)	1		10		100	
Sex	M	F	M	F	M	F
Conc (ng/mL)						
day 1 (PND 21)	129	113	1440	1563	8830	10727
day 14 (PND 35)	89	180	1073	2153	5603	7530

Stability and Homogeneity

Stability: CP-690550 was previously demonstrated to be stable in 0.5% methylcellulose over a concentration range of 0.05 mg to 200 mg CP-690550/mL for up to 8 days when stored at room temperature or refrigerated.

Dose formulations were not sampled for analysis.

Study title: 1-Month Oral Toxicity Study of Tasocitinib (CP-690550) in Juvenile Rats with a 2-Month Recovery

Study no.:	10GR307
Study report location:	Mod. 4.2.3.5.4
Conducting laboratory and location:	Pfizer Worldwide Research & Development Drug Safety Research & Development Eastern Point Road, Groton, CT
Date of study initiation:	Oct 21, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CP-690550-10, Lot GR02684 (sub-lot of Lot E010009450), Purity 100.2% (HPLC), however total impurities are 0.3%, listed on the same Certif. of Analysis Composition: active moiety 61.8% citrate counterion 38.1%

Key Study Findings

- This study focused on the effects of CP-690550 on immune system development and function in juvenile rats.
- CP-690550 was administered orally once daily to juvenile rats from post natal day (PND) 21-49 at dose levels of 0, 1, 10 and 100 mg/kg/day. A 2-month recovery period followed with animals sacrificed on PND 111 (study day 91).
- Body weight was slightly decreased during the dosing phase at 10 mg/kg and 100 mg/kg/day doses, and was still lower than controls at the end of the recovery phase for the 100 mg/kg/day dose group (M/F 97%/92% of control).
- CP-690550 administration produced dose dependent reductions and time dependent reductions in hematological parameters although these were not large changes, a slight reduction in red blood cell counts by day 30 (PND 50, M/F 93%/95%) and in reticulocytes on day 15 (M/F, 85%/83%).
- The mean white blood cell count was reduced in a dose-dependent manner by CP-690550 administration and this was attributed to dose-dependent reductions in lymphocytes (maximal reduction at the high dose: M/F 34%/24% of control on day 30), eosinophils (high dose: M/F 29%/19% of control on day 30), and basophils (high dose: M/F 25%/17% of control on day 15). These effects were generally reversible by the end of the recovery period.
- CP-690550 decreased the mean percentage of lymphocyte subpopulations. These effects were sometimes dose- or time-dependent with further reduction on day 30 (PND 50) compared to day 15 (PND 35). The maximal reductions at the high dose for males and females compared to control values were total T cells (M/F 90%/88% on day 15), cytotoxic T cells (M/F 48%/44% on day 30), natural killer cells (M/F 28%/41% on day 15) and NKT cells (M/F 31%/23% on day 30). There were no effects on B lymphocytes.

- Thymus and spleen weights were reduced in a dose dependent manner compared to control values in males and females, with maximal reductions at the high dose: spleen (M/F 50%/45% of control) and thymus (M/F 63%/49% of control).
- The reduction in organ weights were reflected in histopathological findings that all lymphoid organs examined (thymus, spleen, mesenteric lymph node, inguino-femoral lymph node and mandibular lymph node) had decreased cellularity. This was reversible by the end of the 2-month recovery period.
- The effects on hematology and lymphoid organ weights were similar to those observed in adult rats (see Reports 01-2063-06 and 02-2063-20). Since similar doses were administered in the adult and juvenile studies and generally similar magnitude of effects were observed, the juvenile and adult rat appear to have similar sensitivity to CP-690550.
- The LOAEL for this juvenile rat study is 100 mg/kg/day, based on the findings of reversibility of CP-90550-related effects and lack of clinical signs of adverse effects or mortality. Since the study focused on hematological aspects of CP-690550 and lacked analysis of serum chemistry, urinalysis, complete anatomical pathology analysis, toxicokinetic, this study would not support clinical studies in pediatric populations.

Methods	
Doses:	0, 1, 10, and 100 mg/kg/day
Frequency of dosing:	once daily from PND 21 to PND 49
Route of administration:	orally by gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% methylcellulose
Species/Strain:	Sprague-Dawley rats [CrI:CD® (SD)]
Number/Sex/Group:	16/sex/group
Age:	postnatal day 21 at the start of dosing
Weight:	Males 47.7 g - 68.2 g Females 44.7 g - 66.6 g
Satellite groups:	None
Unique study design:	Lactating Sprague-Dawley rats [CrI:CD®(SD)] were obtained on lactation days 8 and 9 (PND 8 and 9) and each dam's litter comprised 6 F ₁ males and 6 F ₁ females. By PND 17, litters were culled to 5 males and 5 females. No F ₀ females were assigned to the study. The first 8 animals/sex/group were sacrificed on PND 50. All remaining surviving animals in each group underwent a 2-month recovery period and were sacrificed on PND 111.

Group Number	Daily Dose ^a (mg/kg)	Drug Concentration (mg/mL)	Dose Volume (mL/kg)	Animal Numbers	
				Males	Females
1	0	0	10	1-16	65-80
2	1	0.1	10	17-32	81-96
3	10	1.0	10	33-48	97-112
4	100	10	10	49-64	113-128
^a All dose levels are expressed as mg of active moiety per kg of body weight.					
Deviation from study protocol:		There were no protocol deviations that affected the results or conclusions.			

Observations and Results

Mortality

checked daily

There was no mortality.

Clinical Signs

Clinical signs on the F0 dams and F1 preweaning pups were recorded twice daily until euthanasia.

There were no clinical signs related to CP-690550 treatment.

Body Weights

F₀ females were weighed on the day of arrival and on LD 16 and LD 20. F₁ pup preweaning body weights were collected on PND 16 and PND 20, and postweaning weights on PND 21, 23, 25, 28, 31, 35, 38, 41, 44, 47, 50, 55, 60, 66, 75, 84, 93, 102, and 111, corresponding to study days 1, 3, 5, 8, 11, 15, 18, 21, 24, 27, 30, 35, 40, 46, 55, 64, 73, 82, and 91, respectively.

Males in the 100 mg/kg/day dose group, had reduced mean body weights (93.5% of control) and mean body weight gain (89.1% of control) at PND 50, after 1-month of daily dosing.

Females in the 10 and 100 mg/kg/day dose groups had reduced mean body weights (91.9% and 95.3%, respectively) and body weight gains at PND 50 (88.4% and 94.2% of controls, respectively) which was not dose-dependent. The greater reduction mean body weight of females in the 10 mg/kg/day group partially recovered over the following 2 months. For females in the 100 mg/kg/day group, during the first month of the recovery period (PND 50 to PND 84) there was a further decrease in mean body weight gain (79.7% of control gain). During the second recovery month, (PND 84-111) body weight gain was 97% of control. This resulted in lower body weights for the high dose

group females throughout the recovery period, ending at body weights 8.3% lower than controls, but this was not statistically significant.

Table 55: Summary of Body Weight and Body Weight Gain

(g and % of control values; selected days, values rounded by Reviewer)

	Males				Females			
Dose (mg/kg/day)	0	1	10	100	0	1	10	100
N	16	16	16	16	16	16	16	16
Body Weight (g)								
day 1 (PND 21)	58	58	58	58	58	56	57	57
day 8	101	102	101	102	96	94	92	94
day 15	165	165	162	162	143	140	135	140
day 21	223	225	218	213	177	170	162	168
day 30 (PND 51)	306	307 (100%)	301 (98%)	286 (93%)	214	206 (96%)	195 (91%)	204 (95%)
Body Weight Gain (days 1-30)	232	249 (107%)	243 (105%)	228 (98%)	156	150 (96%)	138 (88%)	147 (94%)
Recovery, N	8	8	8	8	8	8	8	8
day 35	355	345	345	322	235	234	216	217
day 64	515	512	526	488	305	309	284	277
day 91	594	592 (100%)	618 (104%)	578 (97%)	345	357 (103%)	326 (94%)	316 (92%)
Body Weight Gain (days 30-91)	288	285 (99%)	317 (110%)	292 (101%)	131	151 (115%)	131 (100%)	112 (85%)

Feed Consumption

Food consumption was not monitored in this study.

Hematology

Blood was collected by jugular venipuncture from all rats on PND 35, PND 50, PND 84 (recovery animals), and PND 111 (recovery animals). PND 35, 50, 84 and 111 correspond to study days 15, 30, 64R and 91R reported in the study tables respectively. At the terminal blood collections on PND 50 and PND 111, additional blood was collected for isolation of peripheral blood mononuclear cells (PBMCs) The following parameters were assessed:

Red Blood Cells	Red Cell Distribution Width
Hemoglobin	Reticulocyte, Absolute
Hematocrit	Platelets
Mean Cell Volume	Mean Platelet Volume
Mean Cell Hemoglobin	White Blood Cells
Mean Cell Hemoglobin Conc	White Cell Differential

In addition, blood cell morphology was evaluated microscopically from blood smears from at least 5 vehicle control and 5 high dose animals of each sex.

CP-690550 administration produced a slight reduction in red blood cell counts by day 30 (PND 50, M/F 93%/95%) and in reticulocytes on day 15 (M/F, 85%/83%) but this was not a correlated relationship based on mean counts between dose group. Red blood cell counts were similar to controls at the end of the 2-month recovery period, although for males the mean counts on day 64 and 91 (PND 84 and PND 111) at the high dose and mid dose on day 91 were statistically reduced. There were no effects on hemoglobin concentrations or hematocrit in contrast to effects noted in adult toxicology studies, but dosing was longer in those studies.

The mean white blood cell count was reduced in a dose-dependent manner by CP-690550 administration and this was attributed to dose-dependent reductions in lymphocytes, eosinophils, and basophils. The decrease in lymphocytes was the main contributor to the reduction in WBC due to its substantially larger cellular population. The reductions were also time-dependent with further reduction on day 30 compared to day 15, except for basophils. The maximal reductions at the high dose for males and females were in white blood cell counts (M/F 47%/34% of control on day 30) lymphocytes (M/F 34%/24% of control on day 30), eosinophils (M/F 29%/19% of control on day 30), and basophils (M/F 25%/17% of control on day 15). Significant reductions in white blood cells tended to occur at the low dose in females, without a corresponding significant effect in males, as if females were more sensitive to the hematological effect of CP690550. However, toxicokinetic data are lacking in this study to eliminate the possibility of exposure to a larger amount of CP-690550 in females that was demonstrated in the juvenile rat dose-range-finding study (Report 09GR249). All CP-690550 treatment-related WBC parameters were not statistically different from control values by 1-month into the recovery period, although mean values were still reduced for some parameters.

Table 56: Summary of Hematology (values rounded by Reviewer)

Dose (mg/kg/day)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
RBC (10 ⁶ /μL)								

Day 15	5.51	5.63	5.46	5.73	5.52	5.66	5.20 (94%)	5.53 (98%)
Day 30	6.46	6.66	6.36	6.80	6.33	6.67	6.03 (93%)	6.36 (95%)
Recovery Day 64	8.40	6.01	8.58	8.32	8.19 (98%)	7.75 (129%)	8.07 (96%)	7.67 (128%)
Day 91	8.52	7.79	8.33	7.95	8.12 (95%)	7.62 (98%)	8.23 (96%)	7.77 (100%)
Reticulocytes(10³/μL)								
Day 15	707	605	670 (95%)	588 (97%)	647 (92%)	524 (87%)	602 (85%)	504 (83%)
Day 30	355	217	336	219	321 (90%)	192 (88%)	338 (95%)	206 (95%)
Recovery Day 64	178	220	190	174	204 (115%)	186 (219%)	240 (134%)	188 (85%)
Day 91	217	192	197	164	206	145	272 (125%)	194 (101%)
WBC (10³/μL)								
Day 15	11.27	11.38	10.28 (91%)	9.21 (81%)	7.33 (65%)	7.32 (64%)	6.06 (54%)	5.36 (47%)
Day 30	12.00	10.94	10.33 (86%)	7.31 (67%)	7.37 (61%)	5.28 (48%)	5.63 (47%)	3.67 (34%)
Recovery Day 64	12.63	13.0	12.50	10.83	12.20	12.24	11.79 (86%)	9.37 (72%)
Day 91	11.25	9.50	9.31	6.38	10.16	6.03	11.96 (106%)	7.08 (74%)
Lymphocyte (10³/μL)								
Day 15	9.61	9.76	8.76 (91%)	7.87 (81%)	5.99 (62%)	6.13 (63%)	3.94 (41%)	3.75 (38%)
Day 30	10.40	9.70	8.93 (86%)	6.18 (64%)	6.05 (58%)	4.30 (44%)	3.50 (34%)	2.30 (24%)
Recovery Day 64	10.82	11.34	10.68	9.41	10.28	10.70	9.65 (89%)	7.94 (70%)
Day 91	9.58	8.15	7.97	5.54	8.76	5.32	9.89 (103%)	5.91 (72%)
Eosinophils (10³/μL)								
Day 15	0.061	0.064	0.049	0.058	0.051 (84%)	0.031 (48%)	0.023 (38%)	0.020 (31%)
Day 30	0.103	0.099	0.084 (82%)	0.067 (68%)	0.067 (65%)	0.071 (72%)	0.030 (29%)	0.019 (19%)
Recovery Day 64	0.124	0.171	0.129	0.143	0.147	0.129	0.117 (94%)	0.107 (62%)
Day 91	0.150	0.089	0.099	0.079	0.114	0.049	0.120 (80%)	0.066 (74%)
Basophils (10³/μL)								

Day 15	0.024	0.023	0.023	0.019	0.011 (154%)	0.011 (48%)	0.006 (25%)	0.004 (17%)
Day 30	0.023	0.023	0.024	0.016	0.011 (48%)	0.012 (52%)	0.008 (35%)	0.005 (22%)
Recovery Day 64	0.039	0.049	0.051	0.038	0.040 (102%)	0.053 (108%)	0.036 (92%)	0.023 (47%)
Day 91	0.029	0.026	0.021	0.013	0.026	0.011	0.024 (83%)	0.019 (73%)

Peripheral Blood Immunophenotyping

Blood obtained at times described above were immunostained for CD3, CD4, CD8, CD161a and CD45RA using mouse anti-rat lymphocyte monoclonal antibodies with different fluorescent labels. The populations of the following lymphocyte subtypes were determined by flow cytometry:

Lymphocyte subsets:

CD3+ Total T cells
 CD3+CD4+ Helper T cells
 CD3+CD8+ Cytotoxic T cells
 CD3-CD45RA+ B cells
 CD3-CD161a+ Natural Killer cells
 CD3+CD161a+ NKT cells

Lymphocyte subsets were calculated as the percent of total lymphocytes. Subsequently these percentages were used to determine the absolute counts based on the absolute lymphocyte counts determined from the hematology data. Absolute counts for each lymphocyte subset (reported as cells/ μ L) were calculated from the total lymphocyte counts. The mean percent changes in absolute counts of each subset, relative to vehicle control group, were used to determine treatment-related changes

There were a number of animals (#13, 57, 77, 78, 106, 122 or 126) for which data were unable to be obtained due to clotting during processing or equipment malfunctions. However, as they were spread across treatment groups, there was unlikely to be a significant effect on results and conclusions of the study.

CP-690550 decreased the mean percentage of lymphocyte subpopulations. These effects were sometimes dose- or time-dependent with further reduction on day 30 (PND 50) compared to day 15 (PND 35). The maximal reductions at the high dose for males and females compared to control values were total T cells (M/F 90%/88% on day 15), cytotoxic T cells (M/F 48%/44% on day 30), natural killer cells (M/F 28%/41% on day 15) and NKT cells (M/F 31%/23% on day 30). There were no effects on B lymphocytes. There was a small increase in T helper cells in females at the mid and high dose (114% and 118% of control, respectively) on day 30 but no effect for males. All lymphocyte subsets recovered levels not statistically different from control values during the 2-

month recovery period, despite the appearance of substantially different mean percent values.

Table 57: Summary of Effects on Lymphocyte Subsets

(values rounded by Reviewer)

Dose (mg/kg/day)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
% T cell								
Day 15	54.5	60.8	56.9	60.4	55.5	58.8	48.9 (90%)	53.4 (88%)
Day 30	57.5	60.0	59.2	61.6	58.0	62.0	50.5 (88%)	57.3 (96%)
Recovery Day 64	63.7	63.6	68.0	63.0	64.2	64.5	62.3 (98%)	66.7 (105%)
Day 91	57.7	61.6	60.4	64.1	61.3	67.7	52.5 (91%)	66.4 (108%)
% T Cytotoxic cells								
Day 15	18.7	19.8	17.8	18.1	15.7 (84%)	16.3 (82%)	11.3 (60%)	12.3 (62%)
Day 30	20.4	19.6	18.7	18.1	16.2 (79%)	15.2 (77%)	9.9 (48%)	8.6 (44%)
Recovery Day 64	24.0	20.4	22.8	20.4	21.0 (88%)	18.3 (90%)	19.1 (80%)	19.2 (94%)
Day 91	22.0	20.9	20.7	20.8	20.7	19.3	17.2 (78%)	18.5 (88%)
T Helper cells								
Day 15	37.0	42.2	40.2	43.3	40.7 (110%)	43.1 (116%)	38.0 (90%)	41.3 (103%)
Day 30	37.9	41.2	41.2	44.2	42.4 (112%)	47.1 (114%)	40.7 (107%)	48.8 (118%)
Recovery Day 64	40.6	43.8	46.2	43.4	44.2 (109%)	46.9 (115%)	44.0 (108%)	48.8 (111%)
Day 91	36.4	41.3	40.5	44.0	41.3	46.1	38.6	48.4
% NK cells								
Day 1538.6	3.41	2.94	2.56	2.47	1.91 (56%)	0.68 (23%)	0.97 (28%)	1.21 (41%)
Day 30	4.44	3.07	3.41 (77%)	2.90 (94%)	3.04 (68%)	0.91 (30%)	1.33 (30%)	1.43 (46%)
Recovery Day 64	4.86	3.93	3.63	6.36	6.04 (124%)	3.95 (100%)	6.53 (134%)	5.73 (146%)
Day 91	6.06	2.63	4.88	4.60	5.45 (90%)	1.27 (48%)	5.15 (85%)	3.79 (144%)

% NKT cells								
Day 15	0.34	0.34	0.28	0.28	0.22 (65%)	0.19 (56%)	0.14 (41%)	0.13 (38%)
Day 30	0.29	0.26	0.20 (69%)	0.21 (81%)	0.17 (59%)	0.16 (62%)	0.09 (31%)	0.06 (23%)
Recovery Day 64	0.29	0.25	0.20	0.31	0.31	0.29	0.34 (117%)	0.27 (108%)
Day 91	0.26	0.40	0.28	0.38	0.25	0.27	0.29	0.24

Clinical Chemistry

There was no evaluation of clinical chemistry parameters.

Gross Pathology

Animals were terminated on PND 50 and PND 111 by isoflurane anesthesia.

There were no gross pathology findings related to CP-690550 treatment.

Organ Weights

Organ weights were obtained for the brain, spleen, thymus, and interscapular brown fat weights.

Thymus and spleen mean absolute weights, organ-to-body weight and organ-to-brain weight ratios were reduced in a dose dependent manner compared to control values in males and females. Expressed in terms of body weight maximal reduction in spleen weight was 50% for males and 45% for females, and the maximal reduction of thymus weight was 63% for males and 49% for females. These weight changes correlated with the microscopic observation of decreased lymphocyte cellularity. After the 2-month recovery period, there were no differences between control and CP-690550 treatment groups in spleen and thymus weights.

There was a dose-related trend for increased weight in males (113%, 135%, and 170% of control for 1, 10 and 100 mg/kg/day doses, respectively, expressed as % body weight) and females (108%, 120%, and 125% of control, respectively) although statistical significance occurred only for the high dose males compared to control. This trend was absent at the end of the 2-month recovery period.

Table 58: Organ Weights (values rounded by Reviewer)

	Males				Female			
52.5Dose (mg/kg/day)	0	1	10	100	0	1	10	100
Spleen								

g	0.704	0.725	0.537 (76%)	0.339 (48%)	0.530	0.424 (80%)	0.306 (58%)	0.224 (42%)
% body weight	0.232	0.232	0.177 (76%)	0.115 (50%)	0.243	0.212 (87%)	0.160 (66%)	0.110 (45%)
% brain wt	0.379	0.365	0.277 (73%)	0.174 (46%)	0.285	0.231 (81%)	0.168 (59%)	0.125 (44%)
Recovery (day 91)								
g	0.957	0.877	0.971	0.932 (97%)	0.646	0.589	0.567	0.612 (95%)
% body weight	0.163	0.149	0.159	0.163 (100%)	0.191	0.169	0.176	0.196 (103%)
% brain wt	0.422	0.407	0.462	0.435 (103%)	0.326	0.291	0.280	0.304 (93%)
Thymus								
g	0.769	0.702	0.630 (82%)	0.474 (62%)	0.656	0.561	0.482 (70%)	0.304 (46%)
% body weight	0.253	0.226	0.208 (82%)	0.160 (63%)	0.300	0.281	0.250 (83%)	0.148 (49%)
% brain wt	0.411	0.354	0.325 (79%)	0.243 (59%)	0.352	0.306	0.265 (75%)	0.170 (48%)
Recovery (day 91)								
g	0.507	0.570	0.613	0.545 (107%)	0.453	0.501	0.465	0.467 (103%)
% body weight	0.088	0.096	0.099	0.095 (108%)	0.134	0.143	0.143	0.150 (112%)
% brain wt	0.224	0.262	0.290	0.254 (113%)	0.226	0.248	0.232	0.232 (103%)
Brown adipose tissue								
g	0.346	0.435 (126%)	0.463 (134%)	0.577 (166%)	0.274	0.268 (98%)	0.284 (104%)	0.320 (117%)
% body weight	0.113	0.138 (113%)	0.153 (135%)	0.192 (170%)	0.125	0.135 (108%)	0.150 (120%)	0.156 (125%)
% brain wt	0.186	0.217	0.238	0.296 (159%)	0.147	0.147	0.156	0.179 (122%)
Recovery (day 91)								
g	0.381	0.336	0.370	0.372 (98%)	0.387	0.452	0.416	0.413 (107%)
% body weight	0.065	0.060	0.060	0.065 (100%)	0.112	0.128	0.129	0.133 (119%)
% brain wt	0.177	0.156	0.175	0.174 (98%)	0.120	0.222	0.206	0.206 (172%)

Values in bold were determined to be statistically significant , $p < 0.05$, by the applicant

Macroscopic Findings

There were no CP-690550-related macroscopic findings. There was a single incidence of wound/scar/crust was noted in a 1 mg/kg/day male and a single incidence of small left testis in a 100 mg/kg/day male. These tissues were not examined microscopically. One female in the recovery 10 mg/kg/day dose group had an enlarged inguino-femoral lymph node which was also not examined microscopically.

Histopathology

Adequate Battery: No

.Peer Review: Not by an outside pathologist, only by an in-house pathologist

The following tissues were collected but only a few tissues were examined histologically. There were liver kidney, spleen, thymus, lymph nodes (mesenteric, inguino-femoral, mandibular) and interscapular brown fat. Except for liver and kidney, tissues from all main study animals were examined, but only high dose and control recovery groups were examined. For the liver and kidney, only the main study control and high dose groups were examined.

Table 59: Histopathology Inventory

Adrenal	Optic Nerve
Aorta	Ovary
Bone Marrow	Oviduct
Brain	Pancreas
Cecum	Parathyroid
Cervix	Peripheral Nerve
Colon	Pituitary
Duodenum	Prostate
Epididymis	Salivary Gland
Esophagus	Seminal Vesicle
Eye	Skeletal Muscle
GALT	Skin and Adnexa
Harderian Gland	Spinal Cord
Heart	Spleen*
Ileum	Sternum
Inguinofemoral Lymph Node *	Stomach
Interscapular brown fat	Testis
Jejunum	Thymus*
Joint, Stifle	Thyroid
Kidney	Tongue
Larynx	Trachea
Liver	Ureter
Lung	Urinary Bladder
Mammary Gland	Uterus
Mandibular Lymph Node*	Vagina
Mesenteric Lymph Node*	

* See below for further processing specifications

Histological Findings

CP-690550-related findings included decreased cellularity in all the examined lymphoid organs(thymus, spleen, mesenteric lymph node, inguinofemoral lymph node and mandibular lymph node) and increased brown adipose tissue vacuolation. There were no findings in the kidney or liver related to CP-690550 administration.

In the thymus, a minimal to mild, diffuse decrease in lymphoid cellularity occurred in the high dose of males and females, and in the mid dose of females. A similar finding occurred in the spleen at the mid and high dose groups in both sexes. In the mesenteric lymph node a minimal to mild, diffuse decrease in lymphoid cellularity occurred in the high dose of males and females, and in the mid dose of females, whereas in the inguino-femoral lymph node, this effect was noted only in the high dose females, and in

the mandibular lymph node the effects occurred only in the high dose of both male and females.

The increased vacuolation in brown adipose tissue was minimal in severity and present in males at a low incidence (≤ 2) at all doses but was not present in females. There was no evidence of associated inflammation or cellular degeneration or cell death in the brown adipose tissue.

There were no CP-690550-related microscopic findings in the vehicle and 100 mg/kg/day recovery phase animals (not presented in the table below). Due to the lack of pathology in the 100 mg/kg/day recovery group, lower doses were not examined.

Table 60: Summary of Histopathology Findings

Dose (mg/kg/day)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Brown Adipose Tissue N	8	8	8	8	8	8	8	8
Increased vacuolation Grade 1	0	0	1	0	1	0	2	0
Lymph Node N Mandibular	8	8	8	8	8	8	8	8
decreased cellularity, lymphoid Grade 1	0	0	0	0	0	0	1	2
Lymph Node, N Inguinal/femoral	8	7	8	8	6	7	8	5
decreased cellularity, lymphoid Grade 1	0	0	0	0	0	0	0	1
Lymph Node, N Mesenteric	8	8	8	8	7	8	8	8
decreased cellularity, lymphoid Grade 1	0	0	0	0	0	2	0	6
Grade 2	0	0	0	0	0	0	1	2
Spleen N	8	8	8	8	8	8	8	8
decreased cellularity, lymphoid Grade 1	0	0	0	0	2	4	2	0
Grade 2	0	0	0	0	0	0	5	8
Thymus N	8	8	8	8	8	8	8	8
decreased cellularity, lymphoid Grade 1	0	0	0	0	0	3	6	5
Grade 2	0	0	0	0	0	0	0	3

Special Evaluation

Lymphocyte Proliferation (ex-vivo ConA stimulation)

This study was a non-GLP compliant study, to examine if the lymphocytes remaining after reduction in numbers by CP690550 treatment were still capable of responding to a nonspecific mitogen, concanavalin A.

Method: Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-PaqueTMPLUS density gradients on the day of collection from blood obtained at necropsy from main study animals on PND 50 recovery animals on PND 111. Cell counts and viability was determined. Then the isolated PBMCs (1×10^5 cells/well) were added to triplicate wells of 96 well plates, and cultured with medium in the presence or absence of 1.0 µg/mL of ConA in a humidified incubator at 37°C, 5% CO₂. Following incubation for 48 hours, BrdU was added and incubated for an additional 24 hours. The incorporation of BrdU into the cells was used as an indicator of cell proliferation. Detection used a colorimetric BrdU cell proliferation ELISA assay kit.

Analysis: For each animal on each day, the average of three replicates was calculated, and then the difference between the ConA-treated average and Non-ConA-treated average per animal was calculated. All statistical analyses were done on the difference, separately for each sex and with both sexes combined.

Result: There was a slight dose-dependent increase in the ConA induced proliferative response in males at the end of the dosing period, but no effect was observed for females. For the recovery phase evaluation, PBMCs from most animals (including controls) failed to respond to ConA stimulation, rendering the recovery data uninterpretable.

Table 61: Effect on PBMC of ConA Stimulation

Dose (mg/kg/day)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Dosing Phase (PND 50)	0.31	0.65	0.72 (232%)	0.49	0.77 (248%)	0.51	0.89 (287%)	0.50
Recovery Day (PND 111)	0.06	0.03	0.11	0.09	0.10	0.07	0.05	0.09

Although the applicant explains the response for males in terms of the population difference in lymphocyte subtypes, it is premature as there are losses in the preparation

for the stimulation test, and the need to immunophenotype the population of cells in the assay. There is not a good explanation for the difference between males and females as cell viability were similar.

Toxicokinetics

Toxicokinetics were not evaluated in this study.

Stability and Homogeneity

Stability: CP-690550 is stable in 0.5% methylcellulose over a concentration range of 0.05 to 200 mg CP-690550/mL for up to 8 days when stored at room temperature or refrigerated (b) (4) project number 894-003-01 and 1100-003). CP-690550 is stable in 0.5% methylcellulose at 1.0 mg/mL and 50 mg/mL for 15 days at room temperature and refrigerated conditions (Study 6348-463). CP-690550 is stable in 0.5% methylcellulose at 0.1 mg/mL for 10 days at room temperature (Study AF-STAB-10-CP-690550-10-01).

Homogeneity: Formulations were subdivided into daily bottles, stored at room temperature and stirred for at least 30 minutes prior to dosing. During dosing, the mixture was continuously stirred with a magnetic stir bar and plate to ensure homogeneity. Predose samples of CP-690550 and vehicle control formulations were analyzed from week 1. Homogeneity of the formulation was assessed by analyzing the top, middle, and bottom of each dose concentration from the first analytical submission. Homogeneity analyses met acceptance criteria of $\leq 10\%$.

Dose Concentration Analysis: Predose samples of CP-690550 and vehicle control formulations were analyzed from weeks 1 and 4 preparations. Concentrations ranged from 97% to 104%, meeting the acceptance criteria ($\pm 10\%$ of target).

STUDIES IN CYNOMOLGUS MONKEYS**Study title: A Two-Week Oral Exploratory Toxicity Study in Cynomolgus Monkeys with CP-690550-10**

Study no.:	00-2063-05
Study report location:	4.2.3.2-repeat-dose-tox
Conducting laboratory and location:	Pfizer Global Research and Development, Groton, CT
Date of study initiation:	Nov 2, 2000
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	CP-690550-10, lot #44207-207-1 (Purity 97.15%, Composition: active moiety 61.9%)

There was no Certificate of Analysis included with this report. A description of HPLC evaluation was included.

Purity/composition: Based on HPLC evaluation there were 3 impurities which were (b) (4) than CP-690,550 ranging from an area of (b) (4) and 6 impurities which were (b) (4) than CP-690,550 ranging from an area of (b) (4). Based on this, the purity of CP-690,550 was 97.15%.

Key Study Findings

- CP-690550-10 was administered orally to cynomolgus monkeys (n=1/sex/dose) for up to 14 days at 0 (0.5% methylcellulose vehicle), 20, 50, 200, and 500 mg/kg/day as split doses TID. On day 8, the 20 mg/kg/day dose group was escalated to 500 mg/kg/day to determine a MTD. Both animals in the 500 mg/kg/day dose group and the 200 mg/kg/day female were euthanized on days 11 and 12 due to morbidity.
- No NOAEL was identified. The highest tolerated dose was 50 mg/kg/day.
- Clinical signs related to drug treatment included emesis at ≥ 50 mg/kg/day; post-dose salivation, loose stool, and hunched posture at ≥ 200 mg/kg/day; and decreased activity, ataxia, pale skin, and dehydration at ≥ 500 mg/kg/day.
- Hematological effects were leukocyte and erythroid parameters in the ≥ 50 mg/kg dose groups. Although there was only n=1/sex/dose, there were reductions in WBC, lymphocytes, red blood cell counts, hematocrit, hemoglobin, and reticulocytes.
- There were no CP-690550-related changes in serum chemistry parameters.
- Lymphoid depletion occurred in the thymus, spleen, mesenteric lymph node, and bone marrow. Viral nuclear inclusions were observed in pathology tissues in some euthanized animals with infections and inflammation (stomach, spleen).

Methods

Doses: 0, 20/500, 50, and 200 mg/kg/day as split doses administered TID (= 0.67/166.67, 16.67, and 66.67 mg/kg TID, respectively)

On day 8, the 20 mg/kg/day dose group was escalated to 500 mg/kg/day to determine a MTD (20 mg/kg/day was administered for 7 days and 500 mg/kg/day was administered for 5 days)

The dose expressed as mg/kg dose is based on mg of the active moiety of the drug substance.

Frequency of dosing: TID for 14 days, doses about 7 hours apart

Route of administration: Orally, by gavage

Dose volume: 7 mL/kg

Formulation/Vehicle: 0.5% methylcellulose

Species/Strain: Cynomolgus monkeys

Number/Sex/Group: 1/sex/dose

Age: Not provided

Weight: Males 3.3 kg, females 3.0 kg,

Satellite groups: None

Unique study design: On day 8, the dose of 20 mg/kg/day administered to 1 male and 1 female was increased so that each animal now received 500 mg/kg/day.

Dose Selection:

The 50 mg/kg/day dose was selected to provide trough coverage of approximately 0.156 mg/ml which is considered to be the efficacious human plasma level based on the mixed lymphocyte reaction (MLR) IC₉₀. The 20 and 200 mg/kg/day doses were 2.5- and 4-fold multiples below and above the 50 mg/kg/day dose, respectively.

Group	Daily Dose ¹ (mg/kg)	Dose Volume (ml/kg)	Drug Concentration (mg/ml)	<u>Animal Numbers</u>	
				Male	Female
Group 1 (0.5% Methylcellulose)	0 (0 t.i.d.)	7	0	1	5
Group 2 (CP-690,550-10)	20 (6.67 t.i.d.)	7	0.95	2	6
Group 2 (escalated) ² (CP-690,550-10)	500 (166.67 t.i.d.)	7	23.81	2	6
Group 3 (CP-690,550-10)	50 (16.67 t.i.d.)	7	2.38	3	7
Group 4 (CP-690,550-10)	200 (66.67 t.i.d.)	7	9.52	4	8

¹ All dose levels are expressed as mg active moiety per kg of body weight.

² Escalated dose began on day 8.

The monkeys were dosed for up to 14 days. For convenience, in this report study intervals are reported in units of study days.

Deviation from study protocol:

On day 9 the male in 50 mg/kg/day dose group received a second daily dose that was meant for the 500 mg/kg/day dose group. This male was not administered the third daily dose that day. This was unlikely to affect the study results that were based on samples collected on days 12 or 15.

Observations and Results

Mortality

Observations were made daily

There were 3 deaths in the study and these were considered CP-690550-related. Deaths occurred for both animals in the 20/500 mg/kg/day group after dose escalation to 500 mg/kg/day. The male monkey #2 was euthanized on day 12 and the female #6 died on day 11. Female #8 in the 200 mg/kg/day dose was euthanized on day 12. The clinical signs associated with these deaths are described below.

Clinical signs

Clinical observations were made daily.

Physical examinations (once pre-study to assess health status), and vital signs were measured twice pre-study and then on day 12.

CP-690550-related clinical signs included emesis at ≥ 50 mg/kg/day; Emesis was observed in the 50 mg/kg/day male following a dosing error on day 9, when this animal inadvertently received a single dose at 500 mg/kg/day. Emesis also occurred in the first dose of day 1 and day 3 for the female in the 50 mg/kg/day dose.

Post-dose salivation, loose stool, and hunched posture were observed at ≥ 200 mg/kg/day; and decreased activity, ataxia, pale skin, and dehydration in the 500 mg/kg/day dosing phase of the 20/500 mg/kg/day dose group.

Physical examination

Physical examinations were conducted once during pre-study to assess health status. Vital signs were obtained twice during pre-study and on day 12. Only vital signs were provided in the study report.

There were no effects on CP-690550 on physical exam findings or vital signs.

Body weights

Body weights were obtained at 3 day intervals.

The female (#6) that received 500 mg/kg/day on day 8 in the 20/500 mg/kg dose had a 12% absolute weight loss between days 7 and 10 (no weight was obtained on day 8; day 7 weight was 3.1 kg and on day 10 the weight was 2.8 kg). For comparison, the male (#2) at this dose had a 6% absolute weight loss (day 7 weight was 3.2 kg and on day 10 weighed 3.0 kg). There were no treatment-related effects for the other doses since weights fluctuated ± 0.1 kg during the study. Since there was only $n=1$ /sex/dose, a comparison with the control animals was not conducted by the Reviewer.

Food consumption

Food consumption was qualitatively noted daily

A decrease in food intake was observed in the 500 mg/kg/day male on days 10 and 11, and sporadic decreases in food intake were noted in both animals (1 male and 1 female) of the 200 mg/kg dose level. The corresponded with the reduction in body weight noted above.

EKG

EKG and indirect systolic blood pressure recordings were obtained on all monkeys twice prior to treatment initiation, on day 12 prior to the first daily dose and then ~ 0.5 hour after the first daily dose for animal #2 and #8 and ~ 0.5 hour after the third daily dose for all other surviving study animals. Heart rates from RR-I measurements were calculated for all study animals. The data table has a footnote for QTc calculations as "QT-I(c) = QT interval corrected for heart rate = $QT-I - (RR-I - 300) \times 0.347$ (linear correction derived from historical database)" without further explanation.

There were no CP-690550-related effects on electrocardiograms or blood pressure.

Insufficient information was provided concerning the QTc calculation to determine if this were an appropriate correction method.

Hematology

Blood samples were obtained once pre-study and then on days 5, 11, and 15 in addition to samples from 2 animals on day 12.

Differential counts were performed on bone marrow smears from males and females in control, 50, and 200 mg/kg/day dose groups.

Comparisons were made for each animal between the pre-dose and treatment periods, since there was only n=1/sex/dose.

red blood cell count (RBC, $\times 10^6/\text{mm}^3$)	
hemoglobin concentration (HGB, g/dL)	
hematocrit (HCT, %)	
platelet count (PLT, $\times 10^3/\text{mm}^3$)	
reticulocyte counts (RET, %)	
mean corpuscular volume (MCV, fL)	
mean corpuscular hemoglobin (MCH, pg)	
mean corpuscular hemoglobin concentration (MCHC, %)	
white blood cell count (WBC, $\times 10^3/\text{mm}^3$)	
white blood cell differential count (WBC Differential, % and absolute)	
neutrophils (N, %)	(NCT, $/\text{mm}^3$)
lymphocytes (L, %)	(LCT, $/\text{mm}^3$)
monocytes (MO, %)	(MOCT, $/\text{mm}^3$)
eosinophils (EO, %)	(EOCT, $/\text{mm}^3$)
basophils (B, %)	(BCT, $/\text{mm}^3$)
large unstained cells (LUC, %)	(LUCT, $/\text{mm}^3$)

The following coagulation tests were performed using the ACL 3000+:

prothrombin time (PT, sec)
activated partial thromboplastin time (APTT, sec)

Although there was only 1 animal per sex per dose, changes in red blood cell and white blood cell parameters were noted. In the 50 and 200 mg/kg/day these included decreased mean red blood cell counts (81% of predose values), hematocrit (78% of predose values), and hemoglobin (82% of predose values), and reticulocytes (9% of predose values). For white blood cells there were decreased white cell counts (88% of predose value), although in two animals that were euthanized on day 12 (#2 male in the 20/500 mg/kg/day dose group, and #8 female in the 200 mg/kg/day dose group) WBC counts were 153 and 199% of predose values. Percent lymphocytes were decreased (8% of predose values) except in one female with no substantial change from the predose value. The neutrophil response was variable, reduced to 28% of predose level

in male #4, but increased in two other animals 174% (female #8) and 296% (male #3) of predose values.

The bone marrow myeloid/erythroid ratio in the 200 mg/kg/day group increased (male: 866%, female 480%) compared to the controls. This was due to an increase in the myeloid cells and a reduction in erythroid cells. The cellular composition of both series had normal morphology and maturation sequence.

Table 62: Hematology Summary

Group	1		2		3		4	
Dose (mg/kg/day)	0		20/500*		50		200	
Sex	M	F	M	F	M	F	M	F
animal # (n = 1/sex/dose)	1	5	2	6	3	7	4	8
RBC ($10^6/\text{mm}^3$)								
Predose	7.0	6.2	5.3	5.4	6.3	5.2	5.7	5.1
day 5	6.4	5.8	4.7	5.3 (98%)	6.0	5.3	5.3	5.1
day 11	6.5	5.9	5.3	-	5.6	4.9	4.8	4.2
day 12	-	-	5.3 (100%)	-	-	-	-	4.3 (84%)
day 15	6.5	5.9	-	-	5.7 (90%)	4.2 (81%)	4.6 (81%)	-
Hematocrit (%)								
Predose	44.0	42.1	35.9	36.8	43.5	34.4	37.6	37.5-
day 5	39.3	38.9	31.6	35.5 (96%)	41.0	34.6	33.9	36.2-
day 11	39.4	39.7	35.8	-	38.1	31.5	31.3	29.4
day 12	-	-	35.4 (97%)	-	-	-	-	29.9 (80%)
day 15	40.5	40.2	-	-	39.2 (90%)	26.9 (78%)	29.5 (78%)	-

Hemoglobin (g/dL)								
Predose	12.0	11.8	11.2	11.2	12.4	10.3	10.7	10.6
day 5	11.4	11.3	10.4	11.3 (101%)	11.9	10.9	10.1	10.9
day 11	11.3	11.3	11.3	-	11.0	9.7	9.2	8.9
day 12	-	-	11.5 (97%)	-	-	-	-	9.1 (86%)
day 15	11.5	11.5	-	-	11.2 (90%)	8.5 (82%)	8.8 (82%)	-
Reticulocytes (%)								
Predose	7.0	6.2	5.3	5.4	6.3	5.2	5.7	5.1
day 5	6.4	5.8	4.7	5.3 (98%)	6.0	5.3	5.3	5.1
day 11	6.5	5.9	5.3	-	5.6	4.9	4.8	4.2
day 12	-	-	5.3 (100%)	-	-	-	-	4.3 (84%)
day 15	6.5	5.9	-	-	5.7 (90%)	4.2 (81%)	4.6 (81%)	-
WBC ($10^3/\text{mm}^3$)								
Predose	9.0	13.2	6.8	8.9	9.8	7.9	7.8	10.2
day 5	9.7	17.3	7.0	7.9 (89%)	9.8	6.7	5.7	11.4
day 11	7.1	10.5	16.8	-	9.3	5.7	5.4	13.3-
day 12	-	-	10.4 (153%)	-	-	-	-	20.3 (199%)
day 15	6.7	11.2	-	-	11.5 (117%)	6.9 (87%)	6.9 (88%)	-
Lymphocytes (%)								
Predose	71	43	71	50	70	48	64	40
day 5	66	44	55	58 (116%)	38	57	88	32
day 11	52	41	12	-	31	54	45	22
day 12	-	-	23 (32%)	-	-	-	-	-
day 15	67	35	-	-	19 (27%)	52 (108%)	35 (55%)	3 (8%)
Neutrophils (%)								
Predose	25	51	22	44	25	45	29	54
day 5	26	48	41	35 (80%)	57	37	8	63

day 11	40	52	84	-	63	39	48	73
day 12	-	-	72 (327%)	-	-	-	-	-
day 15	25	57	-	-	74 (296%)	43 (96%)	58 (28%)	94 (174%)
# % values are calculated as percent of predose value, * animals received 20 mg/kg on days 1-7 and were escalated to 500 mg/kg for days 8-12 - not determined, animal mortality or no sample obtained								

Table 63: Bone Marrow Analysis

(expressed as percent myeloid, erythroid, and lymphoid cells; values in parenthesis are the difference from control values expressed as a percentage of control)

Myeloid:Erythroid Ratio

Day 15

Group	1		2		3		4	
Dose	0		20/500*		50		200	
(mg/kg/day)								
Sex	M	F	M	F	M	F	M	F
N	1	1	1	1	1	1	1	1
% Myeloid cells	43	56	-	-	33 (77%)	35.4 (60%)	81.4 (189%)	69.6 (124%)
% Erythroid cells	45	34.2	-	-	52.2 (116%)	51.6 (151%)	9.8 (22%)	8.8 (26%)
% Lymphocytes	12	9.8	-	-	14.8 (123%)	13 (133%)	8.8 (73%)	21.6 (220%)
M:E Ratio	0.96	1.64	-	-	0.63 (66%)	0.69 (42%)	8.31 (866%)	7.91 (480%)

- not determined, animal mortality or no sample obtained

* animals received 20 mg/kg on days 1-7 and were escalated to 500 mg/kg for days 8-12

Serum Chemistry

Blood samples were obtained once prestudy and then on days 5 and 15 in addition to samples from 2 animals on day 12.

alanine aminotransferase (ALT, U/L)

aspartate aminotransferase (AST, U/L)

alkaline phosphatase (ALP, U/L)

γ-glutamyl transferase (GGT, U/L)

total bilirubin (TB, mg/dL)	bile acids (BILA, μ M/L)
total protein (TP, g/dL)	albumin (ALBM, g/dL)
cholesterol (CHOL, mg/dL)	triglycerides (TRIG, mg/dL)
glucose (GLUC, mg/dL)	blood urea nitrogen (BUN, mg/dL)
creatinine (CREA, mg/dL)	sodium (NA, meq/L)
potassium (K, meq/L)	chloride (CL, meq/L)
calcium (CA, mg/dL)	

Globulin (GLOB, g/dL) was calculated.

There were no treatment-related changes in serum chemistry parameters.

Urinalysis

Not conducted, no samples were collected.

Necropsy

A selected set of tissues (kidney, liver, lung, stomach, duodenum, cecum, colon, spleen, heart, mesenteric node, thymus, bone marrow, bone, testis, epididymis, ovary, uterus, cervix, and brain) was processed for microscopic examination from all animals that were euthanized at the end of the study. A complete set of tissues was examined from animals that died or were euthanized prior to the end of the study.

Gross pathology findings in the 200 mg/kg/day male included red foci in the pyloric mucosa that correlated with foci of acute inflammation in the mucosa and sub mucosa. In addition animals of the 200 mg/kg/day and 20/500 mg/kg/day dose groups exhibited dilation of the stomach and intestine with lumen content of fluid or mucosal material. There were no obvious histopathological correlates. A small thymus was observed in the 20/500 mg/kg male and 200 mg/kg female.

Organ weight

Weighed tissues were kidneys (combined), liver, testes (combined), brain and heart. Lymphoid organs were not assessed.

There were no treatment-related effects on organ weights for either males or females.

Histopathology

Adequate Battery: Yes for this non-GLP study

A selected set of tissues (kidney, liver, lung, stomach, duodenum, cecum, colon, spleen, heart, mesenteric node, thymus, bone marrow, bone, testis, epididymis, ovary, uterus, cervix, and brain) was processed for microscopic examination from all animals that were euthanized at the end of the study. A complete set of tissues was examined from animals that died or were euthanized prior to the end of the study.

Peer Review: No

kidneys	seminal vesicle
urinary bladder	ovaries
aorta	uterus
liver (left and right lateral lobes)	cervix
thymus	trachea
spleen	lung (both diaphragmatic lobes)
mesenteric lymph node	heart
esophagus	peripheral nerve
stomach	brain (cerebrum, cerebellum and pons)
duodenum	spinal cord (cervical)
jejunum	eyes
ileum	skin and adnexa
cecum	mammary gland
colon	bone (sternum, including marrow)
pituitary gland	skeletal muscle
gall bladder	salivary gland
pancreas	adrenal glands
thyroid gland	parathyroid
testes (left and right)	epididymides
prostate	bone marrow smears

The major findings were lymphoid depletion of the thymus, spleen, and mesenteric lymph node, and depletion bone marrow cells. Although there was only 2 animals per dose (1 /sex/dose), it was apparent from the pathology written description that there was a increase in serverity with dose, which was most clearly apparent in the bone marrow, evident by a reduction in the number of hematopoietic cells and a relative increase in the amount of adipose tissue. The spleen was characterized by a reduction in the cellularity and diameter of the periarteriolar lymphoid sheath. In the mesenteric lymph node lymphoid depletion was characterized by an overall decreased thickness of the cortex and a decreased cellularity in the paracortical areas. Conclusions about quantitiative results from myeloid:erythroid ratios of the bone marrow analysis, that the erythroid component was the primarily affected in the 200 mg/kg/day animals and there was no effect in the 50 mg/kg/day dose groups are premature considering so few animals per group.

There were sings of infectious disease in the stomach and spleen in the 200 mg/kg/day male monkey with inflammatory cell foci in the submucosa and mucosa, necrosis of pyloric glandular epithelium, and intranuclear inclusion bodies in epithelial cells in the areas. In the spleen at sites of inflammation, intranuclear inclusion bodies were also present. While these appear to be viral inclusion, the applicant considers these inflammatory sties and associated mononuclear infiltrates incidental, but there presence does fit the characterized pharmacodynamic aspect of CP-690550, of immunosuppression, evident even in the hematological effects in this study. Therefore, the inflammatory foci and subsequent necrosis are CP-690550-related.

Table 64: Histopathology Summary

Dose (mg/kg/day)	0		50		200		20/500*	
	M	F	M	F	M	F	M	F
N	1	1	1	1	1	1	1	1
Bone Marrow depletion, generalized	0	0	sl	sl	mod	mild	mild	mild
Bone Marrow Smear depletion, erythroid.	0	0	0	0	pres	pres	-	-
Jejunum dilatation, lacteal	-	-	-	-	-	sl	sl	0
Esophagus erosion/ulcer	-	-	-	-	-	sl	0	0
Spleen lymphoid depletion	0	0	0	0	0	0	sl	sl
acute inflammation, focal	0	0	0	0	mild	0	0	0
fibrosis, focal	0	0	0	0	0	0	0	mild
Thymus cyst	pres	pres	pres	0	0	pres	0	0
lymphoid depletion	0	0	mild	mild	mod	mild	mod	mild
Mesenteric node lymphoid depletion	0	0		0	mild	sl		0
hemosiderosis	0	0	sl	0	0	0	mild	0
Stomach acute inflammation	0	0	0	0	mod	0	0	0
<p>* dose was 20 mg/kg/day for days 1-7, then 500 mg/kg/day for days 8-12. The female died on day 11, the male was euthanized on day 12.</p> <p>- tissue not examined at this dose</p> <p>Abbreviations:</p> <p>pres = finding was present</p> <p>severity in indicated by the following:</p> <p>sl = slight</p> <p>mild = mild</p> <p>mod = moderate</p>								

Toxicokinetics

Plasma CP-690550 concentrations were measured on days 1, 8 and 14. On days 1 and 14, animals were sampled at ~0.5 hour post each dose, ~0.5 hour prior to the second and third dose and at ~24 hours post the first dose. On day 8, all animals were bled at ~0.5 hour post the first dose and ~0.5 hour prior to the second dose only. On day 12, animals were sampled at ~0.5 hour post the first dose and animal # 8 was sampled at ~0.5 hour prior to the second dose time (Animals #2 and #8 did not receive the second or third dose on day 12 as they were euthanized on this day). Vehicle control animals were sampled at each timepoint as indicated above, however, only the 0.5 hour post the first dose timepoint on day 1 was analyzed.

Systemic exposure increased with increasing dose. The concentrations obtained at 0.5 h post dose may not have been at C_{max}, and the timing of samples for AUC₀₋₂₄ calculation were unlikely to provide an accurate profile. Variability in the data (only means are presented below) and with only 1 animal per dose/sex, additional conclusions about the results need further substantiation.

Table 65: Toxicokinetic Summary

Day	Dose (mg/kg)	Monkey	Sex	AUC ₀₋₂₄ (ng-hr/ml)
1	20 ^a	2	M	4430
1	20 ^a	6	F	7570
1	50	3	M	16300
1	50	7	F	7810
1	200	4	M	14300
1	200	8	F	24200
14	50	3	M	15000
14	50	7	F	19100
14	200	4	M	73100

Study title: CP-690550-10: One Month Oral Toxicity Study with a One Month Recovery Period in Cynomolgus Monkeys

Study no.: 01-2063-09
 Study report location: 4.2.3.2-repeat-dose-tox
 Conducting laboratory and location: Pfizer Global Research and Development, Groton, CT
 Date of study initiation: March 30, 2001
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: CP-690550-10, Lot # 43798-2-1H; Purity: 96.9%
 Composition 60% active moiety/potency, 38.1% citrate counterion

There was no Certificate of Analysis included with this report. A description of HPLC evaluation was included.

Purity/composition: (b) (4) CP-690,550 + (b) (4) impurities + (b) (4) known impurities + (b) (4) + (b) (4)

Key Study Findings

- CP-690550-10 was administered orally at doses of 0 (0.5% carboxymethylcellulose vehicle), 10, 50, and 100 mg/kg/day as split doses TID (0, 3.33, 16.67, and 33.33 mg/kg) for 4 weeks followed by a 4 week recovery period.
- The doses of 50 and 100 mg/kg/day resulted in secondary infections of open wounds, or gastrointestinal erosions or ulcers resulting in all animals in the 100 mg/kg/day group being euthanized by day 12, and 3 animals in the 50 mg/kg/day group being euthanized throughout the dosing phase.
- Lymphoid depletion occurred in the spleen at 50 and 100 mg/kg/day and in the mesenteric lymph node at 100 mg/kg/day. At 10 and 50 mg/kg/day there was a reduction in the myeloid:erythroid ratio in the bone marrow of 54 to 69%, which recovered in the 50 mg/kg/day group (there was no recovery group for the 10 mg/kg/day dose). This was due to a reduction in % myeloid cells (78-83% of control levels) and an increase in %erythroid cells (108-151% of control levels), both of which recovered to control levels at the end of the recovery period.
- There was an increase in mononuclear cell infiltration in a number of organs that exceeded control levels.
- The 10 mg/kg/day dose was a tolerated dose; effects included slight to mild reductions in red blood cell counts, hematocrit, and hemoglobin, lymphocytes, and lymphocyte subsets (T-helper, cytotoxic/suppressor T-lymphocytes and natural killer cells).
- Parameters that did not recover (50 mg/kg/day dose group) within 1 month included the following (values are end of recovery as a % of predose):
 - lymphocytes (153-159% of control) reticulocytes (58% of control in females), ALT (150-263% of control levels), and
 - decreases in RBC (88% of control in males), natural killer cells (1-28% of predose levels in 2 of 4 animals), T helper-lymphocytes, cytotoxic/suppressor T-lymphocytes (210-330% of predose levels)
- A NOAEL was not identified. The low dose, 10 mg/kg/day, was the MTD in this study. The systemic exposure, AUC₀₋₂₄, at 10 mg/kg/day was 3440 and 2090 ng·h/mL for males and females, respectively.

Methods

Doses:	0, 10, 50, and 100 mg/kg/day (as TID dosing 3.33, 16.67, and 33.33 mg/kg)
	(Doses are expressed as mg of active moiety per kg of body weight per day)
Frequency of dosing:	as a split dose, three times a day, approximately 7 hours apart, for 4 weeks
Route of administration:	orally, by gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% methylcellulose
Species/Strain:	Cynomolgus monkeys

Number/Sex/Group: 3/sex/dose for vehicle control and 50 mg/kg doses with additional 2/sex/group used for recovery
 3/sex/dose for 10 and 100 mg/kg dose groups
 Age: not provided
 Weight: males 5.7 kg, females 2.6 kg
 Satellite groups: Recovery: n=2/sex/control and 50 mg/kg dose groups
 (the 100 mg/kg/day dose animals were euthanized prior to completion of the dosing phase)

Study design:

Group	Daily Dose* (mg/kg)	Dose Volume (ml/kg)	Drug Concentration (mg/ml)	Animal Numbers Males	Females
Group 1-Control (0.5% methylcellulose)	0	5	0	1-5	17-21
Group 2 (CP-690,550-10)	10	5	0.67	6-8	22-24
Group 3 (CP-690,550-10)	50	5	3.33	9-13	25-29
Group 4 (CP-690,550-10)	100	5	6.67	14-16	30-32

*All dose levels are expressed as mg active moiety per kg of body weight.

Deviation from study protocol: There were no deviations that affected the study results and conclusions.

Observations and Results

Mortality

Observations were made daily

Two 100 mg/kg animals were euthanized moribund on study days 8 and 9 (female and male, respectively) and the remaining animals were euthanized on day 11 or 12 days (females and males, respectively).

Three males in the 50 mg/kg dose group were moribund due to poor clinical condition from bacterial infection secondary from open wounds around the restraint collar. They were euthanized on days 19, 24 and 26.

All mortalities were attributed to CP-690550-related immunosuppression resulting in infections.

Table 66: Mortalities During the Study

Mortality								
Dose (mg/kg/day)	0		10		50		100	
Sex	M	F	M	F	M	F	M	F
N	5	5	3	3	5	5	3	3
Main Study	0	0	0	0	euthanized 1 on day 19, 24, and 26	0	euthanized 1 on day 9 and 2 on day 12	euthanized 1 on day 8 and 2 on day 11

Clinical signs:

Signs were recorded at least once daily before treatment initiation. During the dosing period, animals were observed pre- and immediately post-each split dose and 1 hour after the 1st and 2nd doses. During the recovery period, animals were observed twice daily. Physical examinations were performed by a clinical veterinarian once prior to treatment initiation and during dose week 4 and recovery week 3. Vital signs, including heart rate, respiration rate, and body temperature, were recorded on all monkeys twice prior to treatment initiation, during dose weeks 1 and 5 at approximately 0.5 hours post the 2nd dose, and during recovery week 3.

Due to episodes of emesis, loose stool and possible dehydration seen in the 50 and 100 mg/kg dose groups, an approximate 60 ml flush of Prang (not described) was administered as needed to the animals in these dose groups instead of a reverse osmosis water flush.

Control and 10 mg/kg/day: Clinical signs observed during the dosing period in the control and 10 mg/kg/day dose group consisted of sporadic episodes of loose stool, and one 10 mg/kg/day male (#8) had loose, mucoid stool with blood-like substance on day 18.

50 and 100 mg/kg/day: In the 50 and 100 mg/kg/day dose groups, loose stool, mucoid stool with blood-like substance, decreased activity, and emesis were observed intermittently throughout the dosing period. Swollen abdomen was observed in two 50 mg/kg/day females on dosing days 22 and 23 and was later observed in one 50 mg/kg/day animal during dosing day 31 and recovery days 1-13 and 23-28.

Onset of scabs in the 50 mg/kg/day dosed males (2/5) developed on day 6; by day 8, scabs were observed in all males. These scabs lasted throughout the dosing period and were more severe than in the control animals. The males in the 100 mg/kg/day dose group also developed scabs beginning day 8, which persisted until the animals were euthanized (days 9 and 12). These scabs frequently resulted in open wounds and were evidence of bacterial infection (which included *Streptococcus dysgalactiae* and

Staphylococcus aureus) secondary to immunosuppression. Scabs in the neck region were attributed to rubbing against the neck collars used for restraint of the animals. In the 50 and 100 mg/kg/day dose groups, these scabs were present in all animals by Day 8 of dosing. One female (#31) in the 100 mg/kg/day dose group experienced scabbing on her perioral region on study days 9, 10 and 11 and was euthanized on day 11. Due to treatment-related morbidity (decreased body weight and activity, loose stools with blood-like substance, and scabs and/or open wounds around the restraint collar), in the 100 mg/kg/day dose group, treatment was discontinued after 11/12 days (females/males, respectively) and the surviving animals of this dose group euthanized .

In the 50 mg/kg/day dose group, swollen abdomen was noted in 2 females on days 22 and 23 and was observed in one animal during dosing day 31 and recovery days 1-13 and 23-28. On day 30 in the 50 mg/kg/day dose group, salivation was noted in 1 male and in 2 females. These females also had swelling of the jaw and neck area. This was also present on day 31 in female (#28).

There were no CP-690550-related findings in the vital signs.

Body weights

Body weights were measured at least once before treatment initiation, on Day 1, then weekly during the dosing and recovery periods.

Absolute body weight was reduced in the males of the 50 mg/kg/day group (91% of control). Females at this dose gained weight similar to control group females. There was no effect in females or males at the 10 mg/kg/day dose groups.

Food consumption

Food consumption was assessed visually as either <25%, ~25%, ~50%, ~75%, ~100%, or spill of the total ration. This was recorded daily through the study period, beginning one week prior to treatment initiation.

The applicant reported decreases in food intake in individual animals of the 50 and 100 mg/kg dose groups, although the data collected were not included in the report.

Ophthalmoscopy

Examinations were performed by a clinical veterinarian once prior to treatment initiation and during dose week 4 and recovery week 3. All monkeys were anesthetized with Ketamine HCl and mydriasis was induced by 1.0% tropicamide (Mydracil®).

There were no CP-690550-related ophthalmoscopic changes.

EKG and vital signs:

EKGs and indirect systolic blood pressure recordings were obtained on all monkeys twice prior to treatment initiation, during dose weeks 1 and 5 at approximately 0.5 hours post the 2nd dose, and during recovery week 3.

QT-interval (QT-I, mSec) - The time period from the beginning of the Q-wave to the end of the T-wave.

Corrected QT-I (QT-I(c), mSec) -The QT interval corrected for heart rate. The correction method uses a linear correction formula based on the slope of the relationship of QT-I to RR-I (RR-interval) taken from in-house historical data:

$$QT-I(c) = QT-I - [(RR-I - 300)(slope)]$$

QT-I = QT interval, The time period from the beginning of the Q wave to the end of the T-wave.

There were no treatment-related changes in electrocardiograms or blood pressure.

There was insufficient information about the validation of the QTc correction method. No background data and no references were provided.

Hematology:

Blood samples for hematology analysis were taken once pre-study, and during dose weeks 2, 3, 4, and 5, and during recovery week 4.

Bone marrow smears were examined using light microscopy and a manual 500 nucleated cell differential count was performed on a representative slide from each animal. Cells were classified as myeloid, erythroid, or lymphoid and the percentage of each cell group was determined. Following completion of the study, the results were peer reviewed by a pathologist. This analysis was appropriate.

red blood cell count (RBC, $\times 10^6/\text{mm}^3$)

hemoglobin concentration (HGB, g/dL)

hematocrit (HCT, %)

platelet count (PLT, $\times 10^3/\text{mm}^3$)

mean corpuscular volume (MCV, fL)

mean corpuscular hemoglobin (MCH, pg)

mean corpuscular hemoglobin concentration (MCHC, %)

white blood cell count (WBC, $\times 10^3/\text{mm}^3$)

white blood cell differential count (WBC Differential, % and absolute)

neutrophils (N, %) (NCT, $/\text{mm}^3$)

lymphocytes (L, %) (LCT, $/\text{mm}^3$)

monocytes (MO, %) (MOCT, $/\text{mm}^3$)

eosinophils (EO, %) (EOCT, $/\text{mm}^3$)

basophils (B, %) (BCT, $/\text{mm}^3$)

large unstained cells (LUC, %) (LUCT, $/\text{mm}^3$)

reticulocyte counts (RET, %)

prothrombin time (PT, sec)

activated partial thromboplastin time (APTT, sec)

Due to the marked variation in the white blood cell parameters prior to dosing, statistical comparisons by the applicant were made with the pre-dose values of each group. However, the reviewer noted that the differences in mean values between pre-dose and control group means over time were fairly similar for most parameters.

CP-690550 treatment resulted in increased WBC (50 mg/kg/day) and neutrophils (50 mg/kg/day), and decreased lymphocytes (10 and 50 mg/kg/day) and red blood cell parameters (red blood cell counts, hematocrit and hemoglobin). The effects recovered in the only dose group studied, 50 mg/kg/day, with lymphocyte counts were elevated above the pre-dose means at the end of the recovery period. The mean WBC and neutrophil counts were mildly elevated in the 50 mg/kg dose group on Day 15 (132-211% of the pre-dose mean).

Neutrophils: Elevated neutrophil counts of several animals in the 50 and 100 mg/kg/day males were combined with immature (band) neutrophils indicating a left shift, and consistent with bacterial infection. In some animals, elevated neutrophil counts were sometimes followed by moderate drops in counts on subsequent samples, consistent with neutrophil consumption. One 100 mg/kg/day male had a severe neutropenia (4% of individual pre-dose mean) with the presence of a marked number of band neutrophils (degenerative left shift). WBC and neutrophils had returned to values comparable with pre-treatment means by day 27 of the recovery period.

Lymphocytes: Decreased lymphocyte counts occurred in the 10 and 50 mg/kg/day animals on days 15 and 27 (66 to 92% and 50-74% of pre-dose group mean, respectively). Decreases were also seen in the three 50 mg/kg/day animals euthanized moribund on Days 26, 19, and 24 (28%, 27% and 56% of individual pre-dose means) and in the 100 mg/kg/day animals prior to euthanasia (19 to 68% times individual pre-dose means). In the 50 mg/kg/day animals, the lymphocyte counts were elevated above the pre-dose means on day 27 of the recovery period (156 to 143% of the predose group means for males and females, respectively).

RBC Parameters: Decreases in RBC, hematocrit, and hemoglobin concentration (71 to 88% of control mean) were observed on days 15 and 27 in the 50 mg/kg/day animals, and decreases in hemoglobin were also observed in the 10 mg/kg females on day 15 (93% of the control mean). Decreases in the RBC parameters were also present in samples taken just prior to necropsy (from days 8 to 12) in the 100 mg/kg/day animals (62 to 80% of the individual pre-dose values). For the 50 mg/kg/day dose, partial to complete recovery was present for these three parameters on day 27 of the recovery period (81 to 105% of the control means).

The percent reticulocytes was decreased in the 50 mg/kg/day females on day 15 (59% of control mean). Decreases in the absolute reticulocytes were also observed in the 50 mg/kg/day animals on days 15 and 27 (30 to 84% of control means). On day 27 of the recovery period, the absolute reticulocytes were increased relative to the mean of the

control recovery animals (170 to 188% of control means). The reduction in absolute reticulocytes indicated the lack of a regenerative response to the reduction in RBC.

The analysis of cellular components of the RBC and WBC was inadequately summarized and inconsistently presented. Absolute cell numbers or % of RBC or WBC were presented but not both, making interpretation sometimes problematic.

Due to the limited number of animals and presence of recurring skin infections, the hematology results are not useful since obviously ill animals were combined with apparently healthy animals. Furthermore, any veterinary treatments to these animals were not documented.

Table 67: Hematology Summary
(and % of control group values)

Dose (mg/kg/day)	0		10		50		100	
Sex	M	F	M	F	M	F	M	F
N	5	5	3	3	5	5	3	3
RBC (10⁶/μL)								
Predose	6.46	6.00	6.63	6.30	6.52	6.26	6.73	6.23
day 12	-	-	-	-	-	-	4.85 (72%)	-
day 15	6.28	6.32	5.90 (89%)	5.80 (92%)	5.30 (81%)	5.58 (89%)	-	-
day 27	6.42	6.12	5.77 (87%)	5.83 (92%)	4.60 (70%)	5.16 (82%)	-	-
Recovery day 27	7.10 (110%)	6.15	-	-	5.75 (88%)	6.05 (97%)	-	-
Hematocrit (%)								
Predose	45.7	39.9	45.6	41.9	46.6	41.8	45.1	40.5
day 12	-	-	-	-	-	-	29.6 (66%)	-
day 15	42.6	41.3	39.4 (86%)	38.2 (91%)	36.3 (79%)	36.1 (86%)	-	-
day 27	43.9	40.8	39.1 (87%)	39.2 (94%)	31.4 (67%)	33.6 (80%)	-	-
Recovery day 27	47.9	39.6	-	-	43.2 (93%)	41.5 (99%)	-	-
Reticulocytes (%)								
Predose	0.40	1.14	0.80	0.87	0.62	0.86	0.70	0.57
day 12	-	-	-	-	-	-	0.10 (14%)	-
day 15	0.66	0.92	0.70	0.83	0.24	0.54	-	-

			(88%)	(95%)	(39%)	(63%)		
day 27	0.60 (150%)	0.90 (79%)	1.13 (141%)	0.90 (103%)	0.65 (105%)	0.92 (107%)	-	-
Recovery day 27	0.25 (62%)	0.25 (22%)	-	-	0.60 (97%)	0.50 (58%)	-	-
- not determined, for the 100 mg/kg/day group, dosing was discontinued on day 11 (females) and day 12 (males) and animals were euthanized, also there were no recovery period animals for the 10 mg/kg/day dose								
WBC (10³/μL)								
Predose	11.0	10.9	20.6	14.9	14.9	10.2	12.5	11.6.
day 12	-	-	-	-	-	-	7.70 (62%)	-
day 15	10.7	10.5	17.3 (84%)	9.57 (64%)	19.9 (90%)	17.8 (174%)	-	-
day 27	9.58 (87%)	9.72 (89%)	15.6 (76%)	12.5 (84%)	15.0 (101%)	11.5 (113%)	-	-
Recovery day 27	13.05 (119%)	11.20 (103%)	-	-	14.85 (100%)	13.70 (134%)	-	-
Lymphocytes (μL)								
Predose	5390	5564	7724	6913	6038	5289	7159	4938
day 12	-	-	-	-	-	-	2694 (38%)	-
day 15	4667 (86%)	4417 (79%)	4972 (64%)	4735 (68%)	5631 (93%)	4836 (91%)	-	-
day 27	4721	4748	5251 (68%)	3802 (55%)	3052 (50%)	4226 (80%)	-	-
Recovery day 27	5139	4981	-	-	9588 (159%)	8123 (153%)	-	-
Neutrophils (μL)								
Predose	4452	4566	11514	7289	7541	4337	4235	6068
day 12	-	-	-	-	-	-	4532 (107%)	-
day 15	4874	5281	11281 (98%)	4184 (57%)	13137 (174%)	11757 (271%)	-	-
day 27	3584 (80%)	4243 (93%)	8868 (77%)	7929 (109%)	10932 (145%)	6244 (144%)	-	-
Recovery day 27	6141 (138%)	5113 (112%)	-	-	4031 (53%)	4402 (101%)	-	-
- not determined, for the 100 mg/kg/day group, dosing was discontinued on day 11 (females) and day 12 (males) and animals were euthanized, also there were no recovery period animals for the 10 mg/kg/day dose								

Immunophenotype

Analysis of immunophenotypes was conducted using flow cytometry of monoclonal antibody labeled cells. The following phenotypic markers were used:

CD3+ (total T-lymphocytes),
CD4+CD3+ (T helper-lymphocytes),
CD8+CD3+ (cytotoxic/suppressor T-lymphocytes),
CD20+CD3- (B-lymphocytes)
CD16+CD3- (natural killer cells).

Evaluation of the lymphocyte subsets was based upon comparison of individuals with their pre-dose value (blood sampling closest to the start of the study) since there was large variability in between animals during the pre-study phase such that comparison with the control mean was not considered a valid comparison.

There was no effect of CP-690550 treatments on B-lymphocyte (CD20+ cells) levels. T-helper lymphocytes (CD4+,CD3+) (43%-62% of pre-dose level) were decreased in three 10 mg/kg and two 50 mg/kg animals. Cytotoxic/suppressor T-lymphocytes (CD8+,CD3+) were decreased (20% -56% of pre-dose level) in three 10 mg/kg and five 50 mg/kg animals. Natural killer cells (CD16+,CD3-) were also reduced (5%-21% of pre-dose levels) in three 10 mg/kg and seven 50 mg/kg animals.

By the end of the 1 month recovery period, on day 27 of the recovery period, T-helper lymphocytes and cytotoxic/suppressor T-lymphocytes increased 210% -330% of predose levels. Natural killer cells were still very low in 2 of 4 animals at 50 mg/kg/day (1% to 18% of predose levels), but did recover in 2 other animals.

The evaluation of immunophenotype was adequate.

Clinical chemistry

Blood samples for clinical chemistry analysis were taken once pre-study, and during dose weeks 2, 3, 4, and 5, and during recovery week 4.

alanine aminotransferase (ALT, U/L)	aspartate aminotransferase (AST, U/L)
potassium (K, meq/L)	γ -glutamyl transferase (GGT, U/L)
cholesterol (CHOL, mg/dL)	blood urea nitrogen (BUN, mg/dL)
total bilirubin (TB, mg/dL)	glucose (GLUC, mg/dL)
bile acids (BILA, μ M/L)	total protein (TP, g/dL)
albumin (ALBM, g/dL)	sodium (NA, meq/L)
creatinine (CREA, mg/dL)	calcium (CA, mg/dL)
chloride (CL, meq/L)	

Globulin (GLOB, g/dL) was calculated.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were elevated and calcium was reduced at CP-690550 doses of 50 and 100 mg/kg/day. Serum calcium returned to normal levels, and ALT and AST values partially returned to normal levels by day 27 of the recovery period.

The evaluation of serum chemistry was adequate.

Table 68: Serum Chemistry Summary

Dose (mg/kg/day)	0		10		50		100	
	M	F	M	F	M	F	M	F
ALT (U/L)								
pre-study (% of control)	43	32	42	56	99 (230%)	41	32	68
day 11/12	-	-	-	-	-	-	110 (343%)*	132 (194%)*
day 27 (% of control)	44	42	49	81	66 (150%)	81 (193%)	-	-
Recovery (% of control)	38	46	-	-	100 (263%)	69 (150%)	-	-
AST (U/L)								
pre-study	37	28	29	29	41	37	32	44
day 11/12	-	-	-	-	-	-	76 (238%)*	76 (173%)*
day 27 (% of control)	36	31	36	41	38	53 (171%)	-	-
Recovery (% of control)	30	30	-	-	38 (93%)	40 (133%)	-	-

Ca (mg/dL)								
pre-study	10.06	10.30	11.07	10.03	10.68	10.50	10.17	10.23
day 11/12 (% of control)	-	-	-	-	-	-	7.8 (77%)*	9.65 (94%)*
day 27 (% of control)	9.96	9.34	9.77	9.57	8.55 (88%)	9.04 (96%)	-	-
Recovery (% of control)	10.20		-	-	9.90 (97%)		-	-
- not determined, for the 100 mg/kg/day group, dosing was discontinued on day 11 (females) and day 12 (males) and animals were euthanized, also there were no recovery period animals for the 10 mg/kg/day dose * % of control was calculated from the mean pre-study level within dose group								

Urinalysis

Urine was collected once prior to treatment initiation, during dose week 5, and during recovery week 3.

volume (VOL, ml, manual)	glucose (GLU, neg to 3+*)
specific gravity (SPGR, refrac.)	urobilinogen (URO, neg to 4+)
pH (5 to 9)	bilirubin (BIL, neg to 3+*)
protein (PRO, neg to 3+*)	ketones (KET, neg to 3+)
blood (BLO, neg to 3+)	Color (COLR, visual assessment)

*Protein, glucose and bilirubin could be reported up to 4+ if manually read.

Microscopic analysis of urine sediment includes the following parameters: WBC and RBC are rated on a scale of negative to 4+ and TNTC (too numerous to count). All other parameters are rated on a scale of negative to 3+.

casts (CAST, /lpf)	white blood cells (WBC, /lpf)
red blood cells (RBC, /lpf)	epithelia (EPH, /lpf)
calcium oxalate crystals (CAOX, /lpf)	amorphous phosphates (AMPH, /lpf)
triple phosphates (TRPH, /lpf)	amorphous urates (AMUR, /lpf)
other crystals (OTHR, /lpf)	

There was no CP-690550-related effect on urinalysis parameters.

The evaluation of urinalysis was adequate.

Gross pathology

Termination (Day 32, main study; or recovery Day 33, recovery animals)

Macroscopic changes observed in moribund animals included abnormal surface of the skin of the neck, with extension along the face, shoulder, ventrum, limbs, and/or tail (3 males at 50 and 100 mg/kg/day each and 1 female at 100 mg/kg); a similar finding was observed in a control male. These were attributed to bacterial dermatitis secondary to superficial trauma associated with collar restraint.

Also observed were abnormal surfaces or discoloration in the heart (2 males at 50 mg/kg/day), and abnormal surfaces or abnormal contents in the stomach and/or colon (2 males at 50 mg/kg/day and 1 male and all females at 100 mg/kg/day).

One 50 mg/kg/day female had dark brown-green tissue originating in the upper left lip with dark red gelatinous tissue extending along the submucosal tissue of the left cheek into the submandibular region. Bacterial colonies and viral inclusions were also present. Opaque, white plaques were also present along the surface of the tongue, corresponding to areas of hyperkeratosis. The applicant suggested this is likely due to alterations in eating behavior of the animal due to the inflammation. This appears to be an infection with hemorrhage, whether it is due to a biting the cheek or chewing food abnormality cannot be determined from the information provided, but the presence of CP-690550 appears to prevent healing and it was not indicated in the clinical observations or physical exam.

Organ weights

Weighed organs included the kidneys (combined), liver, testes (combined), adrenals (combined), pituitary, brain and heart.

There were no CP-690550-related effects on organ weights. Lymphoid organs, the expected pharmacologic target of CP-690550 were not weighed.

The evaluation of organ weights was not adequate due to the lack of lymphoid organ weights, an expected pharmacodynamic target, for which recovery from potential effects of CP-690550 could provide additional drug safety. This was the only repeated-dose study in cynomolgus monkeys with a recovery period. A later toxicology study of 9-months duration (Report 2003-0301) found that organs weights (expressed as absolute, percent of body weight or percent of brain weight) of the thymus, spleen and adrenal were reduced by the high dose of 10 mg/kg/day in both males and females. There was no recovery phase for this study.

Histopathology

Adequate battery: yes

Peer review: Not by an outside pathologist, only by an in-house pathologist

Tissues from all animals were examined including the recovery groups for the 50 mg/kg/day dose and the control groups. The high dose, 100 mg/kg/day animals were all sacrificed on days 11 and 12, following a few early deaths in that group,

Histopathology inventory

Study: 01-2063-09			
Species: Cynomolgus monkey			
Adrenals	X, *	Nasal cavity	
Aorta	X	Optic nerves	
Bone Marrow smear	X	Ovaries	X
Bone (femur)		Pancreas	X
Brain	X, *	Parathyroid	X
Cecum	X	Peripheral nerve	X
Cervix	X	Pharynx	
Colon	X	Pituitary	X, *
Duodenum	X	Prostate	X
Epididymis	X	Rectum	
Esophagus	X	Salivary gland	X
Eye	X	Sciatic nerve	
Fallopian tube		Seminal vesicles	X
Gall bladder	X	Skeletal muscle	X
Gross lesions		Skin	X
Harderian gland	NA	Spinal cord	X
Heart	X, *	Spleen	X
Ileum	X	Sternum	X
Injection site		Stomach	X
Jejunum	X	Testes	X, *
Kidneys	X, *	Thymus	X
Lachrymal gland		Thyroid	X
Larynx		Tongue	
Liver	X, *	Trachea	X
Lungs	X	Ureter	
Lymph nodes, cervical		Urinary bladder	X
Lymph nodes, mandibular		Uterus	X
Lymph nodes, mesenteric	X	Vagina	
Mammary Gland	X	Zymbal gland	NA

X histopathology performed

* organ weight obtained

Lymphoid Organs

CP- 690,550-related main histopathology findings were thymic atrophy, generalized depletion of bone marrow cells, and increased mononuclear cell infiltration in a number of organs. The pathologist noted that the finding of thymic atrophy throughout the study was indistinguishable from normal involution. Generalized depletion of bone marrow cells was observed as a slight effect in one 10 mg/kg/day male and was mild to marked in all of the 50 mg/kg/day main study animals and in the 2 animals in the 100 mg/kg that were euthanized early, on days 8 and 9 in moribund condition. Atrophy of the thymus was also noted in recovery animals; the degree of atrophy was severe in 1 of 2 males and slight in both females. Lymphoid depletion was observed in the spleen of two 50 mg/kg/day males, in the spleen and mesenteric lymph node of one 100 mg/kg/day male, and in the mesenteric lymph node of one 100 mg/kg/day female.

Table 69: Histopathology Findings Related to Lymphoid Organs

Dose (mg/kg/day)	0		10		50*		100[#]	
	M	F	M	F	M	F	M	F
N	3	3	3	3	3	3	3	3
Bone Marrow								
depletion, generalized	0	0	1	0	3	3	2	2
Recovery (n=2) depletion, generalized	0	0	-	-	0	0	-	-
Bone Marrow Smear								
depletion, erythroid	0	0	0	0	0	0	3	3
hyperplasia, erythroid	0	0	0	1	0	0	0	0
depletion, granulocytic	0	0	0	0	0	1	0	0
Recovery (n=2) depletion, erythroid	0	0	-	-	0	0	-	-
hyperplasia, erythroid	0	0	-	-	0	0	-	-
depletion, granulocytic	0	0	-	-	0	0	-	-
Lymph Node, Mesenteric								
lymphoid depletion	0	0	0	0	0	0	1	1
Recovery (n=2) lymphoid depletion	0	0	-	-	0	0	-	-
Spleen								
lymphoid depletion	0	0	0	0	2	0	1	0
Recovery (n=2) lymphoid depletion	0	0	-	-	0	0	-	-
<p>- indicates there were no tissues to examine, there were no recovery animals for these dose groups.</p> <p>* The 3 males in the 50 mg/kg/day group were moribund due to poor clinical condition from bacterial infection secondary from open wounds around the restraint collar. They were euthanized on days 19, 24 and 26.</p> <p>[#] There were 2 animals that were moribund on day 8 and 9 and sacrificed then, the rest of the animals in this dose group were euthanized on day 11 and 12.</p>								

Bone Marrow Smears

No changes were present in the 10 mg/kg/day males, or in 2 of the 10 mg/kg/day females. Slight erythroid hyperplasia was present in one 10 mg/kg/day female. CP-690550-related changes were most prominent in the erythroid lineage producing erythroid depletion (100 mg/kg/day, males, moderate severity, and females, slight to marked severity). Other effects were decreases in the neutrophil storage pool (100 mg/kg/day, 2 males and 2 females; one 50 mg/kg/day male), an increase in immature myeloid cells (100 mg/kg/day, 2 males and 2 females), slight granulocytic depletion (one 50 mg/kg/day female), and slight erythroid hyperplasia (one 10 mg/kg/day female). Bone marrow smears were within normal limits at the end of the recovery period. The changes in the myeloid series are consistent with the increased consumption of neutrophils occurring as a result of the bacterial infections.

Table 70: Myeloid:Erythroid Ratio

Dose (mg/kg/day)	0		10		50*		100[#]	
Sex	M	F	M	F	M	F	M	F
N	3	3	3	3	3	3	3	3
% Myeloid cells	55.5	50.3	46.3 (83%)	39.4 (78%)	43.2 (78%)	39.9 (79%)	74.8 (135%)	63.2 (126%)
Recovery (N=2)	61.9	58.6	-	-	58.8 (95%)	54.4 (93%)	-	-
% Erythroid cells	38.5	35.6	49.1 (127%)	53.9 (151%)	41.6 (108%)	52.1 (146%)	14.7 (38%)	14.6 (41%)
Recovery (N=2)	29.2	32.2	-	-	32.2 (110%)	31.8 (99%)	-	-
% Lymphocytes	6.1	14.1	4.7 (77%)	6.7 (47%)	15.2 (249%)	8.1 (57%)	10.1 (16%)	22.2 (157%)
Recovery (N=2)	8.9	9.2	-	-	9.0 (101%)	13.8 (150%)	-	-
M:E Ratio (% of control)	1.49	1.44	0.95 (64%)	0.75 (69%)	1.04 (67%)	0.78 (54%)	5.15 (356%)	29.4 (2041%)
Recovery (N=2)	2.13	1.85	-	-	1.86 (87%)	1.70 (92%)	-	-
<p>* The 3 males in the 50 mg/kg/day group were moribund due to poor clinical condition from bacterial infection secondary from open wounds around the restraint collar. They were euthanized on days 19, 24 and 26.</p> <p>[#] There were 2 animals that were moribund on day 8 and 9 and sacrificed then, the rest of the animals in this dose group were euthanized on day 11 and 12.</p>								

Mononuclear Infiltrates

An increase in the incidence and severity of mononuclear cell infiltrates within the heart was observed in the 50 mg/kg/day animals. At the end of the 1-month recovery period, slight mononuclear cell infiltration of the kidney, liver, heart, lung, prostate, pancreas (1 of 2 males), salivary gland (1 of 2 males), and thyroid (1 of 2 males) was still observed in the males of the recovery group.

Mononuclear cell infiltrates were also observed at the low dose, 10 mg/kg/day, but because the incidence and severity were similar to those seen in control monkeys, these findings were not attributed to CP-690550. Although, it is somewhat difficult to attribute to CP-690550 treatment, a higher incidence of mononuclear infiltrates would be expected during immunosuppression by CP-690550. This is reflected here by the increase in the diversity of tissues with infiltrates, rather than solely in increase incidence within one type of tissue.

Histopathology Findings Related to Mononuclear Cell Infiltration and Inflammation

Dose (mg/kg/day)	0		10		50*		100[#]	
	M	F	M	F	M	F	M	F
N	3	3	3	3	3	3	3	3
Adrenal								
mononuclear cell infiltration	0	0	0	0	1	2	0	0
Recovery (n=2) mononuclear cell infiltration	0	0	-	-	0	1	-	-
Eye								
mononuclear cell infiltration	0	1	0	1	1	2	0	0
Recovery (n=2) mononuclear cell infiltration	0	0	-	-	0	1	-	-
Heart								
mononuclear cell infiltration	0	0	3	3	3	3	0	1
inflammation	0	0	0	0	1	1	1	1
inflammation, vascular	0	0	0	0	0	1	0	0
Recovery (n=2) mononuclear cell infiltration	0	0	-	-	2	2	-	-
inflammation	0	0	-	-	0	0	-	-
inflammation, vascular	0	0	-	-	0	0	-	-
Kidney								
mononuclear cell infiltration	1	1	3	3	3	2	0	1
tubular degeneration	0	0	0	0	0	1	0	0
Recovery (n=20) mononuclear cell infiltration	0	1	-	-	2	2	-	-
tubular degeneration	0	0	-	-	0	0	-	-
Liver								
mononuclear cell infiltration	0	0	1	0	1	2	0	1
Recovery (n=2) mononuclear cell infiltration	0	0	-	-	2	2	-	-
Lung								
inflammation, subacute	1	1	1	3	1	3	0	1
inflammation, vascular	0	0	0	0	0	1	0	0
Recovery (n=2) inflammation, subacute	0	0	-	-	2	2	-	-
inflammation, vascular	0	0	-	-	0	0	-	-
Pancreas								
mononuclear cell infiltration	0	0	0	0	1	2	0	0
Recovery (n=2) mononuclear cell infiltration	0	0	-	-	1	1	-	-
Prostate								
Inflammation	0	-	0	-	2	-	0	-
Recovery (n=2) Inflammation	2	-	-	-	0	-	-	-
* The 3 males in the 50 mg/kg/day group were moribund due to poor clinical condition from bacterial infection secondary from open wounds around the								

restraint collar. They were euthanized on days 19, 24 and 26.
There were 2 animals that were moribund on day 8 and 9 and sacrificed then, the rest of the animals in this dose group were euthanized on day 11 and 12.

Reproductive Organs

There were a number of sexually immature males in the main phase of the study and in the recovery phase. Ovarian tissue had no pathological findings, but this does not mean they were sexually mature.

Infections and Euthanized Animals

Severe dermal inflammation with bacterial infection in the neck/restraint collar region, inflammation of the colon (with potentially a bacterial etiology), and viral inclusions in the renal medulla of the kidney, mouth, gastrointestinal tract, heart, and salivary gland, some of which were noted in macroscopic changes, were attributed to CP-690550-related immunosuppression.

Bacterial Infections: Three 50 mg/kg/day male animals (9, 10, and 13), and all 6 monkeys (male and female) of the 100 mg/kg/day animals, were euthanized in moribund condition prior to the scheduled necropsy day. The moribund condition in several animals (#9, 10, 13, 14, 15, and 16) was at least partially due to a moderate to severe dermal inflammation of the cervical region (region of collar placement), that had spread to other areas of skin. While scabs were present on the necks of many animals due to trauma associated with the restraining collars, and in the 50 and 100 mg/kg/day males, treatment-related immunosuppression exacerbated these scabs and infection sites. In 1 animal (#10), the inflammation was particularly severe, extending into the underlying muscle and connective tissue. At the end of recovery period, there were no indication of active bacterial or viral infections and no microscopic changes in lymphoid tissues and bone marrow.

Viral Infections: (As described by the pathologist, since specific data was not provided for these additional investigative ultrastructural examinations). One 50 mg/kg/day female (#29) had viral inclusion bodies in multiple tissues, and ultrastructural evidence for infection with at least 2 distinct types of viruses (cynomolgus polyoma virus in the renal medulla of the kidney, and herpes virus in the esophagus). This animal also had necrotizing inflammation involving the mouth, and region around the mouth. One 50 mg/kg/day female (#25) had basophilic, intranuclear inclusion bodies within the epithelium of the collecting ducts of the renal medulla, similar to the polyoma virus inclusions seen in the kidney of animal 29. Three moribund 100 mg/kg/day animals had changes in the gastrointestinal tract. Two males at 100 mg/kg/day (#15 and 16) had degeneration of the villus epithelium within the ileum, and intranuclear inclusion bodies, ultrastructurally consistent with adenovirus, within the degenerate epithelium, with villus blunting in animal #16. One 100 mg/kg/day female (#31) had foci of mild inflammation within the heart that were associated with the presence of viral intranuclear inclusion bodies. These foci, and the inclusion bodies, were similar morphologically to some of the inclusions observed in animal 29. One 50 mg/kg/day male (#13) had viral intranuclear inclusion bodies with inflammation in the salivary gland.

Dermal bacterial infections were identified as *Streptococcus dysgalactiae* and *Staphylococcus aureus*. In this study they did not identify the viral infectious agents. However, in the 6 month study that follows, immunosuppression is associated with the occurrence of animals positive for antigenic exposure to lymphocryptovirus. The pathologist presented numerous cases from the literature in which opportunistic infectious agents flourish under immunosuppressive host conditions in monkeys and humans. This is very likely the situation in this study and the reason for dermal infections, a high incidence of mononuclear infiltrates and viral inclusions.

The following table of histological findings illustrates the point that sites of infections are diverse, and have infrequent incidences. Individually there is no dose-related increase in incidence, and they are not target organs of CP-690550, but they are sites of infection related to CP-690550 immunosuppression.

Table 71: Other Histological Findings

Dose (mg/kg/day)	0		10		50		100	
	M	F	M	F	M	F	M	F
N	5	5	3	3	5	5	3	3
Cecum								
inflammation, vascular	0	0	0	0	0	1	0	0
inflammation	0	0	0	0	0	0	0	1
Colon								
mononuclear cell infiltration	0	0	0	0	1	0	0	0
inflammation	0	0	0	0	0	0	1	1
Esophagus								
erosion/ulcer	0	0	0	0	2	0	0	0
mononuclear cell infiltration	0	0	0	0	0	1	0	0
epithelial degeneration	0	0	0	0	0	1	0	0
Jejunum								
lymphangiectasia	0	0	0	0	0	0	0	1
Ileum								
epithelium degeneration	0	0	0		0	0	2	0
Mouth								
hyperkeratosis, tongue	0	0	0	0	0	1	0	0
inflammation, necrotizing	0	0	0	0	0	1	0	0
inflammation, tongue	0	0	0	0	0	1	0	0
Salivary Gland								
inflammation, necrotizing	0	0	0	0	0	1	0	0
Skeletal Muscle								
mononuclear cell infiltration	0	0	0	0	0	1	0	0
necrosis	0	0	0	0	0	0	0	1
Skin and adnexa								
inflammation	1	0	0	0	3	0	3	1
Stomach								
erosion/ulcer	0	1	0	0	0	0	0	1

hemorrhage	0	0	0	0	0	0	0	1
inflammation, vascular	0	0	0	0	0	1	0	0
Trachea								
inflammation	0	0	1	0	1	0	0	0

Toxicokinetics

Blood samples, approximately 2 mL each, were obtained from all animals in each group at approximately 0.5 hours post 1st dose, 0.5 hours pre 2nd and 3rd doses, 0.5 hours post 2nd and 3rd doses, 2 hours post 3rd dose, 4 hours post 3rd dose, and 24 hours post 1st dose on day 1 as well as day 27 or 29. This corresponded to approximately 0.5, 6.5, 7.5, 13.5, 14.5, 16, 18, and 20 hours after administration of the first of the 3 split doses of test compound, which were administered approximately 7 hours apart. On day 19, a sample was taken 0.5 hours post 1st dose, and on day 27, a sample was taken 2.5 hours post 1st dose. Vehicle control monkeys were sampled at the same time points; however, only the 0.5 h post-1st dose samples on days 1 and 29 were analyzed.

Systemic exposure increased with dose, but dose proportionality was difficult to assess due to variability and few animals. There was no obvious difference in exposure between males and females. There was no drug accumulation over the 1 month treatment period. Tmax was highly variable ranging from 0.7 to 5.7 h. All control samples were below the lower limit of quantification, <5 ng/mL.

Toxicokinetic Parameters

Dose Sex	10		50		100	
	M	F	M	F	M	F
C_{max} (ng/mL)						
day 1	-	234	660	565	-	1740
day 27	-	-	436*	600	-	-
day 29	243	145	1430*	480	-	-
AUC₀₋₂₄ (ng-h/mL)						
day 1	-	3220	11700	7830	-	28300
day 27	-	-	7600*	9370	-	-
day 29	3440	2090	18800*	8040	-	-

* n=1 male

- no samples

Stability and Homogeneity

Stability: CP-690550-10 in 0.5% methylcellulose over a concentration range of 0.1 to 200 mg CP-690550/ml was stable for 3 days when stored either at room temperature, 5° C, or frozen.

Homogeneity: Homogeneity was determined during dose weeks 1 and 5. Dosing solutions were continuously stirred during the daily dosing period. All dosing solutions/suspensions were within an acceptable range ($\pm 10\%$) and therefore homogenous

Dose Concentration: Concentrations of the dosing solutions were verified during dose weeks 1 and 5. All dosing solutions/suspensions were within an acceptable range ($\pm 10\%$) of the intended concentrations.

Study title: CP-690550-10: 39-Week Oral Toxicity Study in the Monkey

Study no.:	2003-0301
Study report location:	4.2.3.2-repeat-dose-tox
Conducting laboratory and location:	Pharmacia & Upjohn Company, Kalamazoo, MI (subsidiary of Pfizer Inc)
Date of study initiation:	June 16, 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CP-690550-10, Lot 54422-88-I F, Purity 97.1%
	Composition: 60.1% active moiety/ potency 37.4% citric acid counterion

There was no Certificate of Analysis included with this report. A description of HPLC evaluation was included. This is the same lot as used in the 6 month duration rat repeated dose study (Report 77435) that contained a CA.

Key Study Findings

- CP-690550 was administered orally for 39 weeks as BID at 0, 0.25, 1, and 5 mg/kg/day 12 hours apart for a total daily dose of 0, 0.5, 2, and 10 mg/kg/day. There were no recovery groups.
- One female in the high dose group was euthanized on day 214. A lymphoma had infiltrated the gastric wall resulting in ulceration and erosion. At the 9 month scheduled necropsy, 2 additional animals with lymphomas were identified, one male and another female both in the 10 mg/kg/day dose group. Of the 3 lymphomas, 2 were identified as B cell origin, 1 was T cell origin, and all were in animals positive for lymphocryptovirus.
- CP-690550 treatment resulted in dose-dependent reductions in red blood cell parameters (red blood cell counts, hemoglobin concentration, and hematocrit). At 9 months, a small dose-response relationship was apparent for all three parameters, high dose means were approximately 80-90%, mid dose 91-93% and the low dose 95-96% of control means. The reticulocyte levels were more

variable, but after 9 months the mean reticulocyte levels in the high dose were 151-167% of the control means.

- White blood cell counts and lymphocytes were also reduced (up to 62% of control values) during the study by all doses of CP-690550. Although the reduction in total white cells did not always follow a dose-response relationship, the reduction in lymphocytes did exhibit dose-response dependency. By week 38, the low dose lymphocyte population was reduced to 73-74% of controls, the high dose 57-60% of control, with the mid dose intermediate and more variable (63-79% of control).
- Other than the invasive findings due to lymphomas, the histological effects of CP-690550 were limited to lymphoid tissues. Lymphoid hyperplasia involving either lymph nodes, spleen, or gut associated lymphoid tissue was present in almost all treated animals, particularly all high dose animals. Organ weights of the spleen and thymus were reduced, although thymus effects were problematic to interpret since thymus involution apparent by histopathology was present in many animals. Weights of reproductive organs of males were also reduced, but the presence of numerous immature animals in the high dose group and their lack in the control group made prevented any conclusion about CP-690550 effects.
- The heart ECG assessment and limb radiograph sections of this report were deficient in methodology or data. Therefore, they will not be used to support CP-690550 safety or regulatory decisions.
- Exposure was dose-related and similar between sexes. However, daily exposure for the low and mid dose was limited to two daily periods of 6 to 8 hours duration, since most samples beyond 6 to 8 hours were below the level of quantification of CP-690550 (<5 ng/mL). There was no systemic CP-690550 accumulation over the duration of the study.
- Due to the presence of lymph node lymphocyte hyperplasia at the low dose, 0.5 mg/kg/day (0.25 mg/kg BID), the lack of any recovery assessment, and the fact that lymphomas developed at high doses with death in one female, there was no NOAEL dose.

Methods

Doses:	0, 0.5, 2, and 10 mg/kg/day administered as divided doses BID at 0, 0.25, 1, and 5 mg/kg/dose
Frequency of dosing:	twice daily (12 hrs apart) for 39 weeks
Route of administration:	oral gavage
Dose volume:	10 mL/kg/day (5 mL/kg/dose), immediately after delivery of the dosing solution the gavage tube was flushed with 10 mL of animal drinking water to deliver the dose
Formulation/Vehicle:	Methylcellulose (0.5%)
Species/Strain:	Cynomolgus Monkey
Number/Sex/Group:	4/sex/dose
Age:	2.5-5 years (some monkeys were sexually immature)
Weight:	Males - 3.1 to 5.3 kg; females - 2.8 to 3.8 kg
Satellite groups:	None

Unique study design: Animals were pair housed except for 2 females #31 and #32 separated on day 91, and 2 males #15 and #6 separated on day 191; they were incompatibly housed together.

Test Group	Dose ^{a,b} (mg/kg/dose)	Dose ^{a,b} (mg/kg/day)	Number of Animals/Group	
			Males	Females
1	0 (vehicle)	0 (vehicle)	4	4
2	0.25	0.5	4	4
3	1	2	4	4
4	5	10	4	4

a Free base (CP-690,550) equivalents
b Monkeys were dosed twice daily with CP-690,550-10; daily doses were 12±1 hours apart.

Deviation from study protocol:

There were no deviations that affected the results or conclusions of the study. Additional studies to further characterize the findings were conducted following submission of the study to the IND, but only submitted to the Agency with this NDA submission. Appropriate protocol amendments were provided as listed here:

Amendment 1

To further characterize the observed proliferative lymphoid changes.

Amendment 2

Immunohistochemical results for the peri-thymic lymphoma observed in Monkey 32 demonstrated that the lymphoma stained positive for CD3 cells, indicating the lymphoma is of T cell origin.

Amendment 3

Additional investigation was to confirm whether the lymphoproliferative changes observed were related to lymphocryptovirus

Results

Mortality

One female, #30, in the 10 mg/kg/day dose group was euthanized on day 214 due to moribund condition. Necropsy revealed ulceration/erosions in the stomach, associated with an infiltrative lymphoma that resulted in hemorrhage into the upper gastrointestinal tract.

Clinical Signs:

Checked at least once daily, physical exam was conducted once pretest and once on day 149

There were few clinical signs attributed to CP-690550 treatment. Monkey #30 that was euthanized on day 214 had no clinical signs from day 1 to 212. On day 213 and 214 the only clinical signs were discolored urine and feces.

Body Weight:

Animals were weighed twice during the pre-dose phase, then once weekly.

There was no effect of CP-690550 on body weight or weight gain.

Food Consumption:

Not measured, but inappetence was noted

There were incidences of inappetence.

Electrocardiography:

ECG recordings were made twice during pretest and 4 times during at approximately 3-month intervals during the dosing phase (on days 9, 100, 191, and 252). Electrocardiograms (ECGs) collected during the dosing phase were taken predose and approximately 2 hours after the AM dose (corresponding to the approximate time of maximum serum concentration of CP-690550). Heart rate and PR, QRS, QT, and QTc intervals were evaluated. A board-certified veterinary cardiologist evaluated the electrocardiograms for changes in waveform morphology and presence of arrhythmias.

There were no effects of CP-690550 on cardiovascular and ECG parameters or ECG waveforms.

There was no methodological description of how the recordings were performed (sedated, restrained, freely moving, telemetered) and how they were analyzed (the method for correcting QT was not described, explained, or referenced). Heart rates were very high (>200 bpm in all animals). Data tables and graphs were submitted with insufficient explanation. A signed report from the veterinary cardiologist was not submitted. Therefore, these results will not be used for safety or regulatory decisions.

Radiography:

Distal epiphysis of femur was radiographed once pre-test and on days 149 and 261. Radiographic study was conducted as a non-[FDA]regulated portion of the study.

There were no treatment effects on the distal epiphysis of the femur based on radiographs. Most monkeys had lucent growth plates indicating an active growth plate. Three monkeys had radiographic evidence of growth plate closure during the study including control male monkeys 1 and 2 and mid-dose female monkey 28.

Since no data or representative radiographs were submitted and this analysis was conducted as a non-regulated portion of the study, these results will not be used for safety or regulatory decisions.

Ophthalmoscopy:

Eyes were examined pre-dose and on day 261

There were no effects of CP-690550 on ophthalmoscopic findings.

Hematology

Blood samples were collected twice pretest (days –22/-23 and –7/-8) and during weeks 4 (days 27/28), 13 (days 90/91), 26 (days 181/182), and 38 (days 265/266) of the dosing phase

White Blood Cells	Red Blood Cells
Hemoglobin	Hematocrit
Mean Cell Volume	Mean Cell Hemoglobin
Mean Cell Hemoglobin Concentration	Differential White Blood Cells (absolute)
Platelets	Reticulocyte Count (absolute and percent)
Red Cell Distribution Width	

Coagulation Parameters Evaluated

Activated Partial Thromboplastin Time	Prothrombin Time
Fibrinogen	

CP-690550 treatment resulted in dose-dependent reductions in red blood cell parameters (red blood cell counts, hemoglobin concentration, and hematocrit). For the high dose red cell counts were reduced compared to controls by week 4 in males and females 91% to 94% respectively, and were slowly further reduced to 80% and 90%, respectively, by week 38. There was a small dose dependent trend at week 38, with the low dose reducing red blood cells to 95-96%, with the mid dose intermediate at 91-93% of control values. A similar pattern and approximately identical levels of reduction were evident for hematocrit and hemoglobin. These indicators of anemia were unlikely to be toxicologically significant, as there were no related adverse signs in the monkeys during the known 8 month of reduced red cell parameters, but these monkeys were not subjected to events that would reveal the toxicity of anemia such as extensive exercise. In the presence of CP-690550, there is not an increase in reticulocytes until late in the study evident by the response at 39 weeks in the high dose group. This lack of or delay in reticulocyte response could also be toxicologically meaningful. The reticulocyte levels were more variable, initially lower in females than in males in females which were maintained during the study except for the high dose males and females in which an increase in reticulocytes (151-167%) occurred at week 38.

White blood cell counts and lymphocytes were also reduced (up to 62% of control values) during the study by all doses of CP-690550. Although the reduction in total white cells did not always follow a dose-response relationship, the reduction in

lymphocytes did exhibit dose-response dependency. By week 38, the low dose lymphocyte population was reduced to 73-74% of controls, the high dose 57-60% of control, with the mid dose intermediate and more variable (63-79% of control).

Table 72: Hematology Summary

Hematology (Values within table rounded by Reviewer, and not all weeks of collection are presented)								
Dose (mg/kg/day)	0		0.5		2		10	
Sex	M	F	M	F	M	F	M	F
N	4	4	4	4	4	4	4	4
RBC (10⁶/μL)								
Predose day -7/-8	6.83	6.45	6.52	6.24	6.54	6.33	6.62	6.56
Week 4 day 27/28 (% of control)	6.63	6.52	6.56 (99%)	6.53 (100%)	6.33 (95%)	6.35 (97%)	6.02 (91%)	6.12 (94%)
Week 26 day 181/182 (% of control)	6.94	6.78	6.56 (94%)	6.32 (93%)	6.34 (91%)	6.24 (92%)	5.68 (82%)	5.69 (84%)
Week 38 day 265/266 (% of control)	6.66	6.24	6.34 (95%)	6.00 (96%)	6.05 (91%)	5.78 (93%)	5.30 (80%)	5.60 (90%)
Hematocrit (%)								
Predose day -7/-8	44.0	41.7	42.1	40.0	43.3	40.0	42.3	42.0
Week 4 day 27/28 (% of control)	42.6	42.6	42.8 (100%)	41.6 (98%)	41.7 (98%)	40.6 (95%)	38.1 (89%)	39.2 (92%)
Week 26 day 181/182 (% of control)	44.3	43.6	43.0 (97%)	40.2 (92%)	42.2 (95%)	39.5 (90%)	37.1 (84%)	36.4 (83%)
Week 38 day 265/266 (% of control)	42.8	40.6	41.9 (96%)	38.6 (95%)	40.5 (95%)	37.2 (87%)	34.8 (81%)	36.0 (89%)
Hemoglobin (g/dL)								
Predose day -7/-8	13.8	13.2	13.5	12.6	13.8	12.7	13.4	13.3
Week 4 day 27/28 (% of control)	13.5	13.6	13.6 (100%)	13.4 (98%)	13.4 (99%)	13.0 (95%)	12.3 (91%)	12.7 (93%)
Week 26 day 181/182 (% of control)	13.8	13.8	13.5 (98%)	12.8 (93%)	13.3 (96%)	12.6 (91%)	11.7 (85%)	11.6 (84%)

Week 38 day 265/266 (% of control)	13.4	12.8	13.1 (98%)	12.1 (94%)	12.9 (96%)	11.9 (93%)	11.1 (83%)	11.4 (89%)
Reticulocytes (10⁶/uL)								
Predose day -7/-8	0.025	0.050	0.026	0.031	0.026	0.035	0.027	0.030
Week 4 day 27/28 (% of control)	0.026	0.053	0.026 (100%)	0.036 (68%)	0.032 (123%)	0.035 (66%)	0.023 (88%)	0.033 (62%)
Week 26 day 181/182 (% of control)	0.022	0.048	0.020 (91%)	0.037 (77%)	0.022 (100%)	0.034 (71%)	0.025 (114%)	0.043 (89%)
Week 38 day 265/266 (% of control)	0.024	0.047	0.026 (108%)	0.038 (81%)	0.032 (133%)	0.035 (74%)	0.040 (167%)	0.071 (151%)

WBC (10⁶/uL)								
Predose day -7/-8	7.02	1086	6.57	8.49	8.48	8.70	6.93	14.90
Week 4 day 27/28 (% of control)	7.84	11.06	5.38 (69%)	8.66 (78%)	6.88 (88%)	10.77 (97%)	6.34 (81%)	8.67 (78%)
Week 26 day 181/182 (% of control)	7.80	10.13	6.34 (81%)	7.51 (74%)	8.34 (107%)	6.24 (62%)	6.39 (82%)	6.85 (68%)
Week 38 day 265/266 (% of control)	7.97	8.73	5.57 (70%)	7.55 (86%)	7.88 (99%)	8.47 (97%)	7.24 (91%)	6.12 (70%)
Lymphocytes (10³/uL)								
Predose day -7/-8	3.72	4.43	3.63	4.28	4.90	4.98	4.11	4.22
Week 4 day 27/28 (% of control)	2.96	4.89	2.90 (98%)	4.33 (88%)	2.72 (92%)	3.62 (74%)	2.26 (76%)	3.05 (62%)
Week 26 day 181/182 (% of control)	3.78	4.52	3.33 (88%)	3.69 (81%)	3.56 (94%)	3.04 (67%)	2.34 (62%)	2.60 (57%)
Week 38 day 265/266 (% of control)	3.99	4.12	2.90 (73%)	3.07 (74%)	3.15 (79%)	2.61 (63%)	2.29 (57%)	2.47 (60%)

Immunophenotyping of Peripheral Blood Lymphocytes

Blood samples were collected twice pre-test and during weeks 4, 13, 26, and 38. Absolute cell count and relative proportion were determined for CD4+, CD8+, CD3+, CD20+, and CD16+ markers

Phenotypic Markers

CD3 ⁺ (total T-lymphocytes)	CD4 ⁺ CD3 ⁺ (T helper-lymphocytes)
CD8 ⁺ CD3 ⁺ (cytotoxic/suppressor T-lymphocytes)	CD20 ⁺ CD3 ⁺ (B-lymphocytes)
CD16 ⁺ CD3 ⁺ (natural killer cells)	

Immunophenotyping for lymphocyte subpopulations, revealed that T lymphocytes T-helper cells, T cytotoxic/suppressor cells, and NK cells were reduced by CP-690550 in a similar temporal pattern and to a similar extent. Maximal reductions occurred in the high dose group and although variable with each subpopulation were generally in the range of 40% to 60% of their control group.

B lymphocytes exhibited a different response, being either increased (up to 144% of control) or decreased (62% of control), without a dose-response relationship.

Table 73: T-lymphocyte Summary (10³/μL)

(Values within table were rounded by Reviewer)

Dose (mg/kg/day)	0		0.5		2		10	
Sex	M	F	M	F	M	F	M	F
N	4	4	4	4	4	4	4	4
Predose day -7/-8	2.01	2.20	2.34	2.20	2.56	2.93	2.23	2.01
Week 4 day 27/28	1.60	2.78	1.93 (121%)	2.45 (88%)	1.52 (95%)	2.28 (82%)	1.14 (71%)	1.72 (62%)
Week 26 day 181/182	2.12	2.35	2.13 (100%)	1.90 (81%)	1.90 (90%)	1.78 (76%)	1.11 (52%)	1.19 (51%)
Week 38 day 265/266 (% of control)	2.36	2.38	1.85 (78%)	1.59 (67%)	1.61 (68%)	1.55 (65%)	1.06 (45%)	1.25 (52%)
T Helper Cells (10³/μL)								
Predose day -7/-8	0.87	1.14	1.20	1.10	1.40	1.86	1.22	1.04
Week 4 day 27/28	0.77	1.57	1.04 (135%)	1.27 (81%)	0.84 (109%)	1.47 (94%)	0.68 (88%)	1.00 (64%)
Week 26 day 181/182-	0.96	1.36	1.07 (111%)	0.98 (72%)	1.04 (108%)	1.12 (82%)	0.60 (62%)	0.64 (47%)
Week 38 day 265/266 (% of control)	1.09	1.34	0.98 (90%)	0.86 (64%)	0.86 (79%)	1.04 (78%)	0.56 (51%)	0.73 (54%)

T Cytotoxic/Suppressor Cells ($10^3/\mu\text{L}$)								
Predose day -7/-8	1.00	0.86	1.02	1.02	1.00	0.96	0.88	0.79
Week 4 day 27/28	0.81	0.92	0.83 (102%)	1.06 (115%)	0.57 (70%)	0.70 (76%)	0.38 (47%)	0.57 (62%)
Week 26 day 181/182	1.06	0.88	0.95 (94%)	0.85 (96%)	0.72 (68%)	0.59 (67%)	0.44 (42%)	0.46 (52%)
Week 38 day 265/266 (% of control)	1.15	0.76	0.81 (70%)	0.68 (89%)	0.64 (56%)	0.43 (56%)	0.47 (41%)	0.46 (60%)
NK Cells ($10^3/\mu\text{L}$)								
Predose day -7/-8	1.11	1.38	0.86	1.11	1.48	1.35	1.21	1.32
Week 4 day 27/28	0.78	1.19	0.53 (68%)	0.85 (71%)	0.50 (64%)	0.54 (45%)	0.29 (37%)	0.36 (30%)
Week 26 day 181/182-	0.97	1.34	0.74 (76%)	0.87 (65%)	0.87 (90%)	0.57 (42%)	0.40 (41%)	0.62 (46%)
Week 38 day 265/266 (% of control)	1.00	0.97	0.64 (64%)	0.59 (61%)	0.83 (83%)	0.43 (44%)	0.52 (52%)	0.32 (33%)
B-lymphocytes ($10^3/\mu\text{L}$)								
Predose day -7/-8	0.52	0.66	0.35	0.80	0.68	0.54	0.51	0.72
Week 4 day 27/28	0.38	0.74	0.32 (84%)	0.88 (119%)	0.46 (121%)	0.64 (86%)	0.55 (144%)	0.82 (110%)
Week 26 day 181/182	0.49	0.52	0.34 (69%)	0.71 (136%)	0.58 (118%)	0.51 (98%)	0.68 (138%)	0.60 (115%)
Week 38 day 265/266 (% of control)	0.45	0.52	0.28 (62%)	0.62 (119%)	0.50 (111%)	0.40 (77%)	0.49 (109%)	0.66 (127%)

The hematology and immunophenotyping evaluations were adequate.

Clinical Chemistry:

Blood samples were obtained twice during pre-test (Days -22/-23 and -7/-8) and during Weeks 4 (Days 27/28), 13 (Days 90/91), 26 (Days 181/182), and 38 (Days 265/266) of the dosing phase

Clinical Chemistry Parameters Evaluated

Alanine Aminotransferase	Total Bilirubin
Alkaline Phosphatase	Direct Bilirubin
Total Cholesterol	Indirect Bilirubin
Total Protein	Albumin
Albumin/Globulin Ratio (calculated)	Globulin (calculated)
Glucose	Triglycerides
Blood Urea Nitrogen/Urea	Creatine Kinase
Creatinine	Potassium
Sodium	Calcium
Chloride	Inorganic Phosphorus
Aspartate Aminotransferase	Gamma Glutamyl Transferase

There were no CP-690550-related effects on clinical chemistry parameters.

The clinical chemistry evaluation was adequate.

Urinalysis:

Urine was collected twice pre-test and during weeks 4, 13, 26, and 38

Urine Parameters Evaluated

Specific Gravity	pH
Protein	Glucose
Ketones	Occult Blood
Bilirubin	Urobilinogen
Microscopic examination of the sediment ^a	
^a If abnormalities were observed in other urinalysis parameters	

There were no CP-690550-related effects on urinalysis parameters.

The urinalysis evaluation was adequate.

Necropsy

Abdominal findings in euthanized high dose female: The female (#30) in the 10 mg/kg/day dose group that was euthanized on day 214 had red discolored fluid in the abdominal cavity and firm tissue encompassing multiple organs in the abdominal cavity (esophagus, stomach, upper small intestine, pancreas, and right adrenal). The entire gastrointestinal tract contained red discolored contents consistent with hemorrhage. Organs that appeared enlarged included the right adrenal, kidneys, and spleen. All tissues had diffuse yellow discoloration.

Lymph Nodes: One male (#15) in the 10 mg/kg/day had enlarged bronchial and mesenteric lymph nodes and three tan circumscribed nodules in the liver. Female (#30) at 10 mg/kg/day had enlarged axillary lymph nodes at gross necropsy, as did one control group male (#4). The enlarged lymph node in female was considered to be

treatment-related because a correlate of lymphocyte hyperplasia was observed microscopically.

Heart: One male (#6) at 0.5 mg/kg/day, had an enlarged left ventricle, dilated left atrium, and hypoplasia of the septal cusp of the left atrioventricular valve. The applicant considered the atrioventricular valve insufficiency to be a congenital condition.

The Reviewer concurs with these interpretations.

Organ Weight

The weight of the following organs was measured at necropsy: adrenal glands, brain, epididymides, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, testes, thymus, and thyroid (with parathyroid). The data were not analyzed statistically by the applicant.

Thymus and adrenal weights of males and females at the high dose were lower than control weights. For the spleen there was no dose-response relationship for spleen weight, as the lowest weights occurred at the mid dose for both males and females. The effect of CP-690550 on thymic tissue is difficult to assess since histopathology identified generalized involution and atrophy of the thymus, which is a normal growth process. The incidence for males was 2 of 4, 3 of 4, 1 of 4, and 1 of 4, and for females was 1 of 4, 1 of 4, 1 of 4, and 3 of 3 corresponding to doses of 0, 0.5, 2 and 10 mg/kg/day, respectively. According to the applicant, this generalized atrophy of the thymus was related to age, however individual monkey ages were not provided in the report.

Testes and epididymides weights were reduced at all doses and ovary weight was reduced in the 10 mg/kg/day group. However, this is unlikely to be a drug related effect. Examination of the high dose group revealed 2 of the 4 animals in this group had testes weights <2 g, and 3 animals had epididymis weights <2 g., while 3 of the control males had testes weights >25 g and epididymis weights <4 g. Therefore, the apparent effect of treatment may be due to the presence of immature animals in the high dose male group. A similar situation occurred for high dose females ovarian weights, however the clinical records indicated that the females were cycling throughout the treatment period.. Whether the males were too young to mature over the course of the study, or if CP-690550 suppresses sexual function or interferes with maturation cannot be determined in this study. Therefore, an effect of CP-690550 on male reproductive organs could not be ruled out. For females the lower ovary weight did not appear to interfere with menstrual cycles.

Table 74: Organ Weights

(values rounded by Reviewer)

	Males				Female			
Dose (mg/kg/day)	0	0.5	2	10	0	0.5	102	10
Thymus g	3.11	2.68	2.75	2.29 (74%)	1.91	1.27	1.89	1.29 (68%)
% body weight	0.076	0.066	0.070	0.056 (73%)	0.57	0.037	0.054	0.034 (59%)
% brain weight	5.12	3.78	3.67	2.94 (57%)	2.75	1.84	2.75	1.84 (67%)
Spleen g	7.62	6.81	5.15 (68%)	6.10 (80%)	5.87	6.36	4.55 (77%)	5.23 (89%)
% body weight	0.166	0.160	0.128 (77%)	0.133 (80%)	0.176	0.180	0.131 (74%)	0.139 (79%)
% brain weight	9.38	9.96	6.95 (74%)	7.79 (83%)	8.49	9.05	6.63 (78%)	7.49 (88%)
Adrenal g	0.75	0.60	0.68	0.59 (79%)	0.58	0.80	0.57	0.53 (91%)
% body weight	0.016	0.014	0.017	0.013 (81%)	0.017	0.023	0.016	0.014 (82%)
% brain weight	1.01	0.88	0.92	0.76 (75%)	0.84	1.12	0.83	0.75 (89%)
Testis g	28.9	14.9 (52%)	12.6 (44%)	13.0 (46%)	-	-	-	-
% body weight	0.55	0.34 (62%)	0.31 (56%)	0.21 (40%)	-	-	-	-
% brain weight	29.6	21.7 (73%)	17.3 (58%)	16.8 (54%)	-	-	-	-
Epididymis	4.57	2.69 (59%)	2.42 (53%)	2.92 (64%)	-	-	-	-
% body weight	0.091	0.062 (68%)	0.060 (66%)	0.056 (62%)	-	-	-	-
% brain weight	4.83	3.92 (81%)	3.28 (68%)	3.79 (78%)	-	-	-	-
Ovary	-	-	-	-	0.47	0.62	0.51	0.30 (64%)
% body	-	-	-	-	0.014	0.017	0.015	0.008

weight								(57%)
% brain weight	-	-	-	-	0.67	0.85	0.75	0.42 (63%)
-, tissue is not present in that sex								

Histopathology:

Adequate battery: Yes

Peer review: No, although indicated this study was peer reviewed, this review was conducted by the applicant's pathologist, Dr. S. Peter Schmidt DVM, PhD, Pfizer Global Research & Development, Safety Sciences, Kalamazoo, MI,. A separate signed pathology report was not included, possibly due the study being conducted by a subsidiary of the applicant.

The tissues collected and processed for analysis are indicated in the table below.

The macroscopic, organ weights and histological evaluations were adequate.

Organ/Tissue	Collected	Weighed	Examined (mg/kg/day)			
			0	0.5	2	10
Aorta	X		E	E	E	E
Adrenal glands (both)	X	X	E	E	E	E
Bone, sternum (with marrow)	X		E	E	E	E
Bone marrow smear ^a	X		E	E	E	E
Brain	X	X	E	E	E	E
Epididymides (both) ^c	X	X	E	E	E	E
Esophagus	X		E	E	E	E
Eyes, optic nerve (both)	X		E	E	E	E
Gallbladder	X		E	E	E	E
Heart	X	X	E	E	E	E
Duodenum	X		E	E	E	E
Jejunum	X		E	E	E	E
Ileum	X		E	E	E	E
Cecum	X		E	E	E	E
Colon	X		E	E	E	E
Proximal tibia with joint surface	X		E	E	E	E
Kidneys (both)	X	X	E	E	E	E
Lacrimal gland	X		E	E	E	E
Liver	X	X	E	E	E	E
Lungs	X		E	E	E	E
Lymph node, bronchial	X		E	E	E	E
Lymph node, mandibular	X		E	E	E	E
Lymph node, mesenteric	X		E	E	E	E
Mammary gland ^b	X		E	E	E	E
Ovaries ^b	X	X	E	E	E	E
Pancreas	X		E	E	E	E
Pituitary	X	X	E	E	E	E
Prostate ^c	X	X	E	E	E	E
Rectum	X		E	E	E	E
Salivary glands (mandibular)	X		E	E	E	E
Sciatic nerve	X		E	E	E	E
Seminal vesicles ^c	X		E	E	E	E
Skeletal muscle	X		E	E	E	E
Skin	X		E	E	E	E
Spinal cord (cervical and lumbar)	X		E	E	E	E
Spleen	X	X	E	E	E	E
Stomach	X		E	E	E	E
Testes (both) ^c	X	X	E	E	E	E
Thymus	X	X	E	E	E	E
Thyroid glands (with parathyroid glands) ^d	X	X	E	E	E	E
Tongue	X		E	E	E	E
Trachea	X		E	E	E	E
Urinary bladder	X		E	E	E	E
Uterus ^b	X		E	E	E	E
Vagina ^b	X		E	E	E	E
Lymph node, axillary	X		E	E	E	E

Organ/Tissue	Collected	Weighed	Examined (mg/kg/day)			
			0	0.5	2	10
Gut-associated lymphoid tissue (GALT)	X		E	E	E	E
Cervix ^b	X		E	E	E	E
Oviduct ^c	X		E	E	E	E
Ureters	X		E	E	E	E
Lesions	X		E	E	E	E

a At the discretion of the Study Director, a bone marrow smear was not collected from high-dose female monkey 30 (unscheduled sacrifice).

b Females only

c Males only

d Parathyroid glands were examined microscopically if included in the section of thyroid glands

Fixatives:

Eyes: Davidson's solution

Testes and Epididymides: Bouin's solution

All Other Tissues: 10% neutral buffered formalin

The primary target organ was lymphoid tissues. There were 3 animals (females #30 and #32, and male #15) in the 10 mg/kg/day group that developed lymphomas. Two animals had malignant lymphomas with multi-organ involvement. Another female had a lymphoma in the peri-thymic fat of unknown origin. A description of the major findings in these three monkeys is presented in the table below. Ancillary studies were conducted to characterize the type of lymphoma, 2 were B-cell origin, and to determine if they were viral-mediated to do possible immunosuppression (refer to the Ancillary Pathology Studies section after the Histopathology section)

Summary of Findings in Monkeys with Identified Lymphomas

Female #30 Dose: 10 mg/kg/day Unscheduled Termination, Day 214	Female #32 Dose: 10 mg/kg/day Scheduled Termination Day 276	Male #15 Dose: 10 mg/kg/day Scheduled Termination Day 275
Lymphoma tissue was identified in the following locations and tissues: abdominal cavity adrenal duodenum esophagus jejunum kidney (malignant cells in larger vessels) lymph node, mesenteric lung (malignant cells in larger vessels) liver (malignant cells in sinusoids/vessels) stomach	Lymphoma tissue was identified in the following locations and tissues: thymus	Lymphoma tissue was identified in the following locations and tissues: lymph nodes, mesenteric and bronchial liver
The infiltration of the stomach by the	Lymphocyte hyperplasia primarily follicular,	Lymphocyte hyperplasia, primarily follicular,

lymphoma produced erosion and ulceration of the glandular mucosa which was thought to be the major factor for morbidity.	characterized by increase size and irregular shape of germinal centers, distinct mantle and marginal zones, mild in severity was present in the spleen and axilla lymph nodes	characterized by increase size and irregular shape of germinal centers, and distinct mantle and marginal zones, mild in severity was present in the spleen and the bronchial, mandibular and mesenteric lymph nodes
Within the bone marrow there was diffuse erythroid hyperplasia with an increase in immature stages erythroid series with mild severity	Within the bone marrow there was diffuse erythroid hyperplasia with an increase in immature stages erythroid series with mild severity	
In the lung, malignant cells were present in the larger vessels and there was marked alveolar edema.		
Notable other findings were mild mononuclear cell inflammation of the submucosa of the gallbladder, mild chronic inflammation with mononuclear cell infiltrate of the portal area of the liver, hepatocyte degeneration, and moderate liver degeneration indicated by moderate panlobular hepatocyte vacuolation	Notable other findings were mild mononuclear cell inflammation of the submucosa of the gallbladder, and in the kidney diffusely within the pelvis, and moderate multifocal in the medulla interstitium and collecting tubules	

Lymphoid hyperplasia in at least one of the examined lymphoid tissues (bronchial, mandibular, and mesenteric lymph nodes; gut-associated lymphoid tissue; and spleen) was present in 2 of 4 in the low dose and 4 of 4 in the mid dose and 3 of 4 in the high dose groups. For females, only one of the 2 monkeys without lymphomas had lymphoid hyperplasia. These tissues were characterized by large follicles with large irregularly-shaped germinal centers and distinct mantle and marginal zones.

At the high dose, there was an increase in bone marrow erythroid hyperplasia characterized by an increase immature erythroid precursor cells in 3 males and 3

females. Also there was 1 low dose male and 1 control male with this finding. For the high dose, but not the lower doses, this finding correlates with the reduction in on red blood cell parameters noted in the hematology assessment.

Findings were not clearly related to CP-690550 due to their single incidence in the high dose group or low incidence treatment group with no incidence in controls included degeneration and mononuclear cell inflammation in kidney collecting tubules and medulla interstitium, moderate mononuclear pelvis infiltrates in the kidney, and dilatation/ectasia of kidney tubules; lung alveolar histiocytosis; spinal cord mononuclear infiltrates of the perivascularity; vertebra bone chronic inflammation; uterine involution; chronic prostate inflammation; and testis seminiferous tubule degeneration.

There was on male in the low dose group with a congenital insufficiency of the atrioventricular valve evident by left ventricle hypertrophy, dilation of the left atrium. There were no other cardiac findings other than mononuclear infiltrates that were not dose dependent in incidence, and are a common finding in cynomolgus monkeys. Mononuclear cell infiltrates in the heart are one of the most common heart incidental finding in cynomolgus monkeys (Chamanza et al., 2010) although it cannot be ruled out that CP-690550 might exacerbate the background incidence.

Table 75: Histopathology Summary of Lymphoid Tissue

Dose (mg/kg/day)		0		0.5		2		10	
Sex		M	F	M	F	M	F	M	F
N		4	4	4	4	4	4	4	4
Tissue/Organ Finding	Severity								
Lymph Node, S Bronch									
hyperplasia lymphocyte,	mild	0	0	0	0	1	0	1	0
Lymph Node, mandibular									
hyperplasia lymphocyte,	mild	0	0	1	0	3	0	1	0
	moderate	0	0	1	0	0	0	0	0
	marked	0	0	0	0	0	0	1	0
Lymph Node, mesenteric									
hyperplasia lymphocyte,	mild	0	0	0	0	0	0	2	0
Bone Marrow, Sternum									
erythroid hyperplasia, (increase erythroid series immature stages),	mild	1	0	1	0	0	0	3	3
Spleen									
hyperplasia lymphocyte,	mild	0	0	0	0	0	0	1	1
	moderate	0	0	0	0	1	0	0	0

Gastrointestinal									
lymphoid hyperplasia	mild	0	0	0	0	0	0	1	0

Table 76: Histopathology Summary of Reproductive Tissues

Reproductive Tissues (incidences of findings)								
Sex	Male				Female			
Dose (mg/kg/day)	0	0.5	2	10	0	0.5	2	10
N	4	4	4	4	4	4	4	3
Tissue/Organ Finding								
Testis								
hypoplasia (immature)	1	1	2	3	-	-	-	-
Epididymides								
hypoplasia (immature)	1	1	1	3	-	-	-	-
Prostate								
hypoplasia (immature)	2	2	4	3	-	-	-	-
Seminal Vesicles								
hypoplasia (immature)	2	2	4	3	-	-	-	-
Ovary								
deposition, mineral (mineralization)	-	-	-	-	2	1	2	2
Uterus								
involution, endometrium	-	-	-	-	0	1	1	1
-, tissue not present in that sex								

Ancillary Pathology Studies

There were 3 amendments to the study report which had not been submitted to the Agency prior to submission of the NDA. These describe additional characterizations of lymphomas and lymphoid hyperplasia tissues observed in the study.

Lymphoproliferative tissues were evaluated by immunohistochemical methods for lymphocyte subtype (CD3, CD4, CD20, CD79a), for retroviruses by electron transmission microscopy (simian retrovirus, SRV-1, -2, and -5; simian immunodeficiency virus, SIV; simian T-cell lymphotropic virus, STLV) and for human and cynomolgus Epstein Barr Virus (EBV) by immunohistochemistry and in situ hybridization and polymerase chain reaction. Serology tested for titers to lymphocryptovirus.

In summary, two lymphomas were of B cell origin, one was of T cell origin. Serology indicated exposure to lymphocryptovirus occurred prior to the start of the study, and titers were stable. No viral particles were observed by electron microscopy and the tumors were negative for human EBV. These amendments are described here.

Amendment 1 (signed April 19 2007)

Lymphocyte Subpopulation Identification: Selected formalin-fixed, paraffin-embedded lymphoid tissues were stained for the expression of B lymphocyte (CD-79a) and T lymphocyte (CD-3) markers. The immunohistochemical staining demonstrated that the lymphoid hyperplasia observed in individual animals was the result of increased numbers of B lymphocytes in both the spleen and lymph nodes.

Lymphocryptovirus Serology: Serological analysis was conducted to determine the exposure history of the monkeys on this study to gamma herpes virus (lymphocryptovirus, LCV). Frozen serum samples were analyzed for antibodies to LCV as described by Rao et al 2000 (Journal of Clinical Microbiology, 38[9]: 3219-25). All monkeys had antibodies to LCV, indicating that LCV was endemic in the study animals. Infection had occurred prior to obtaining the first blood sample. Antibody titers were generally stable (≤ 2 -fold change) during the study, suggesting stability with regards to the infectious agent.

Table 77: Summary of Immunohistochemical Analysis of Lymphoid Tissues

Table 1. Immunohistochemistry				
animal #	tissue	CD3	CD79a	Interpretation
2	Mesenteric lymph node	Normal CD3 staining and distribution: intense paracortical staining with scattered positive cells throughout follicles, medullary cords and sinusoids	Normal CD79a staining and distribution: intense staining of follicles, some of which have defined germinal centers, and scattered CD79a+ cells throughout the subcapsular sinus, paracortex, medullary cords and sinusoids	Normal
	Spleen	Normal CD3 staining and distribution: Intense CD3 staining in PALs with scattered CD3+ cells throughout follicles, mantle and marginal zones and red pulp	Normal CD79a staining and distribution: intense staining in follicles (germinal center, mantle, marginal zones) with scattered CD79a+ cells throughout the red pulp and rare CD79a+ cells in PALs	Normal
3	Mandibular lymph node	Normal CD3 staining and distribution: intense paracortical staining with scattered positive cells throughout follicles, medullary cords and sinusoids	Normal CD79a staining and distribution: intense staining of follicles, most of which have defined germinal centers; numerous CD79a+ cells in the subcapsular sinus and medullary cords; and scattered CD79a+ cells throughout the paracortex and medullary sinusoids	Normal
5	Mandibular lymph node	Normal CD3 staining and distribution	Expansion of germinal centers with normal distribution of CD79a+ cells	B cell hyperplasia
6	Mandibular lymph node	Normal CD3 staining and distribution	Expansion of germinal centers with increased CD79a staining in the subcapsular sinus and medullary cords	B cell hyperplasia
9	Mandibular lymph node	Normal CD3 staining and distribution	Expansion of germinal centers with normal distribution of CD79a+ cells	B cell hyperplasia
10	Mandibular lymph node	Normal CD3 staining and distribution	Expansion of germinal centers with normal distribution of CD79a+ cells	B cell hyperplasia

animal #	tissue	CD3	CD79a	Interpretation
11	Spleen	Normal CD3 staining and distribution	Expansion of germinal centers with normal CD79a distribution	B cell hyperplasia
12	Mandibular lymph node	Normal CD3 staining and distribution	Expansion of germinal centers with reduced distinction between germinal centers and mantle, and increased CD79a staining in the subcapsular sinus and medullary cords	B cell hyperplasia
13	Mesenteric lymph node	Normal CD3 staining and distribution	Expansion of follicles and germinal centers with normal CD79a distribution	B cell hyperplasia
14	Mandibular lymph node	Normal CD3 staining and distribution	Expansion of germinal centers with reduced distinction between germinal centers and mantle, and increased CD79a staining in the subcapsular sinus and medullary cords	B cell hyperplasia
	Mesenteric lymph node	Normal CD3 staining and distribution	Expansion of follicles and germinal centers with normal CD79a distribution	B cell hyperplasia
15	Mandibular lymph node	Normal CD3 staining and distribution	Expansion of germinal centers with reduced distinction between germinal centers and mantle, and increased CD79a staining in the subcapsular sinus and medullary cords	B cell hyperplasia
	Spleen	Normal CD3 staining and distribution	Expansion of germinal centers with normal CD79a distribution	B cell hyperplasia
32	Spleen	Normal CD3 staining and distribution	Expansion of germinal centers with normal CD79a distribution	B cell hyperplasia

Amendment 2 (signed Nov 13 2008)

Additional studies were conducted on the thymic lymphoma of animal #32. Immunohistochemical analysis was conducted for expression of B and T lymphocyte markers (CD20, CD79a, CD3, CD4). For monkey 32, immunohistochemical staining demonstrated that the tumor was composed predominantly of CD3+ cells, consistent with T lymphocytes.

Therefore, the lymphomas were characterized in 2 animals as B cell lymphomas and in 1 animal as a T cell lymphoma.

Amendment 3 (signed Aug 24 2009)

Additional study was conducted to confirm whether the lymphoproliferative changes observed were related to lymphocryptovirus. The lymph node and spleen sections had lymphoid follicular hyperplasia with a consistent morphologic appearance characterized by increased size and number of germinal centers with mantle zones composed primarily of small, mature lymphocytes. Histologic features generally associated with post transplant lymphoproliferative disorders (PTLD) such as a dominant plasmacytic component, as seen in early PTLD or polyclonal proliferations with architecture effacement (Swerdlow, 2008) were not evident in areas of lymphoid follicular hyperplasia of these tissues, raising concern about possible causes for the findings.

The lymphomas were assessed for Epstein-Barr Virus encoded small RNA 1 (EBER-1) gene by in situ hybridization and for EBNA-2 by immunohistochemistry. Sections of spleen from Animals 15 and 30 were coincidentally stained for EBER-1 and EBNA-2 as spleen appeared on the same slides as mesenteric lymph node (B-cell lymphoma). In spleens from both animals (splenic lymphoid follicular hyperplasia for Animal 15 and normal spleen for Animal 30) a few, widely scattered EBER-1 and EBNA-2 positive cells were observed.

The lymphoma in animals #15 and #30 were positive for EBER-1 and EBNA-2 indicating an association of lymphoma with lymphocryptovirus infection. None of the lymphoid follicular hyperplastic changes contained histologic features of PTLD or were positive for EBER-1 or EBNA-2. The lymphoid follicular hyperplasia was unlikely to be unrelated to lymphocryptovirus. There was insufficient lymphoma tissue representative of the original finding from #32 to evaluate additional staining methods.

Evaluation of EBER-1 and EBNA-2 Expression in lymphoma and Lymphoid Tissue with follicular hyperplasia in cynomolgus monkeys

Tissue sections from the lymph nodes and spleen from all the animals in which lymphoid (follicular) hyperplasia were observed (Table 1).

Table 78: List of Tissue for EBER Analysis

Table 1.

Dose (mg/kg/day)	Sex	Animal Number	Tissue with Lymphoid (Follicular) Hyperplasia
0.5	Male	5	Mandib LN
		6	Mandib LN
2	Male	9	Mandib LN
		10	Mandib LN
		11	Bronch LN; Spleen
		12	Mandib LN
10	Male	13	Bronch; Mesent LN
		14	Mandib; Mesent LN; Gut lymph tissue
	Female	32	Axilla LN; Spleen

Mandib = Mandibular; LN = Lymph node; Bronch = Bronchial; Mesent = Mesenteric; Lymph = Lymphoid

The result of the EBER-1 in situ hybridization and EBNA-2 immunohistochemistry corresponded in every case. The staining results are listed in Table 2.

Table 79: Summary of EBER-2 immunostaining**Table 2.**

Dose (mg/kg/day)	Animal Number (Sex)	Tissue	Lesion	EBER-2 and EBNA-2 Staining
0	1-4 (M) 17-20(F)	MLN, spleen	-	Negative
0.5	5(M)	MLN	LFH	Negative
	6 (M)	MLN	LFH	Negative
2	9(M)	MLN	LFH	Negative
	10(M)	MLN	LFH	Negative
	11(M)	BLN, spleen	LFH	Negative
	12(M)	MLN	LFH	Negative
10	13M	BLN, MSLN	LFH	Negative
	14M	MLN, MSLN, Gut	LFH	Negative
	32F	AXLN, Spleen	LFH	Negative
	15M	-	Lymphoma	Positive
	30F	-	Lymphoma	Positive

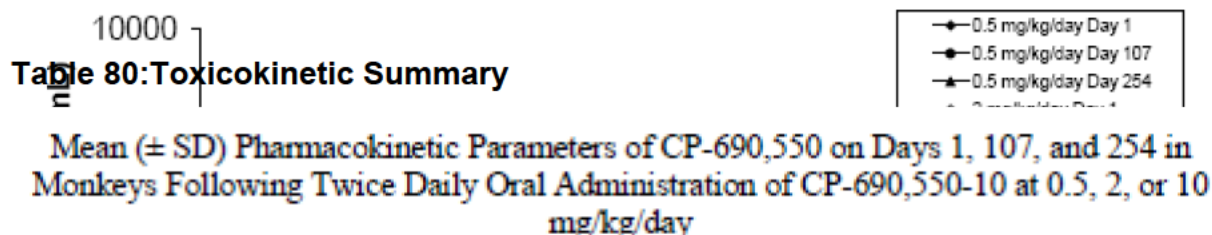
EBER-1 = Epstein-Barr Virus encoded small RNA 1; EBNA-2 = Epstein-Barr virus (nuclear antigen 2);
M = Male; F = Female; MLN = Mandibular lymph node; LFH = Lymphoid follicular hyperplasia;
BLN = Bronchial lymph node; MSLN = Mesenteric lymph node; AXLN = Axillary lymph node; Gut = Gut lymphoid tissue.

Toxicokinetics:

Blood was collected on days 1, 107 and 254 at 0, 0.5, 1, 2, 4, 8, and 12 hours after the AM dose and on days 9, 100, 191, and 252 following ECG evaluation at 0 and ~2 hours after the AM dose. Serum samples were analyzed for concentration of CP-690550 by (b) (4) (b) (4)

Toxicokinetic data were forwarded to Pharmacokinetics and Drug Metabolism (Pfizer Global Research and Development, Groton, CT) for analysis and generation of a summary report.

Exposure was dose-related. For the low and mid dose, most samples beyond 6 to 8 hours were below the level of quantification of CP-690550 (< 5 ng/mL). There was no effect of sex on toxicokinetic parameters. There was no serum CP-690550 accumulation over the duration of the study. The t_{max} was 0.50 to 0.81 hours. The AUC values from the data were calculated for a 12 hour period, the applicant then double that for the 24 hour AUC. The mean AUC₀₋₂₄ values to be used for calculating safety margins are 79, 524, and 2890 ng-h/mL for the 0.5, 2, and 10 mg/kg/doses, respectively.

Figure 8: TK Concentration-Time Profile

Dose (mg/kg/day)	Day	SEX	T _{max} (h)	C _{max} (ng/mL)	AUC ₀₋₁₂ * (ng·h/mL)	AUC ₀₋₂₄ ** (ng·h/mL)
0.5	1	M	1.0 \pm 0.7	20.9 \pm 12.9	38.1 \pm 17.5	76.3 \pm 35.0
		F	0.50 \pm 0.00	21.4 \pm 6.1	36.0 \pm 10.7	72.0 \pm 21.4
		M+F	0.75 \pm 0.54	21.1 \pm 9.4	37.1 \pm 13.5	74.1 \pm 26.9
	107	M	0.50 \pm 0.00	16.6 \pm 5.2	32.2 \pm 13.7	64.4 \pm 27.3
		F	0.63 \pm 0.25	19.0 \pm 1.3	32.3 \pm 3.6	64.6 \pm 7.1
		M+F	0.56 \pm 0.18	17.8 \pm 3.7	32.2 \pm 8.3	64.5 \pm 16.5
	254	M	0.63 \pm 0.25	17.6 \pm 6.6	33.2 \pm 20.3	66.3 \pm 40.5
		F	0.50 \pm 0.00	22.2 \pm 6.4	45.5 \pm 16.1	91.0 \pm 32.2
		M+F	0.56 \pm 0.18	19.9 \pm 6.5	39.3 \pm 18.2	78.6 \pm 36.3
2	1	M	0.50 \pm 0.00	73.0 \pm 31.2	194 \pm 51	387 \pm 102
		F	0.88 \pm 0.75	113 \pm 43	284 \pm 37	568 \pm 75
		M+F	0.69 \pm 0.53	92.8 \pm 40.7	239 \pm 64	478 \pm 127
	107	M	0.63 \pm 0.25	70.0 \pm 16.1	186 \pm 32	371 \pm 63
		F	0.50 \pm 0.00	116 \pm 25	317 \pm 64	634 \pm 128
		M+F	0.56 \pm 0.18	92.8 \pm 31.1	251 \pm 84	503 \pm 169
	254	M	0.63 \pm 0.25	82.5 \pm 26.5	198 \pm 32	397 \pm 64
		F	0.50 \pm 0.00	132 \pm 29	326 \pm 106	652 \pm 212
		M+F	0.56 \pm 0.18	107 \pm 37	262 \pm 99	524 \pm 199
10	1	M	1.0 \pm 0.7	508 \pm 170	1630 \pm 440	3250 \pm 890
		F	0.63 \pm 0.25	370 \pm 90	1040 \pm 180	2090 \pm 360
		M+F	0.81 \pm 0.53	439 \pm 146	1330 \pm 440	2670 \pm 880
	107	M	0.75 \pm 0.29	602 \pm 134	1680 \pm 330	3360 \pm 660
		F	0.63 \pm 0.25	365 \pm 85	1100 \pm 300	2200 \pm 600
		M+F	0.69 \pm 0.26	483 \pm 164	1390 \pm 430	2780 \pm 860
	254	M	0.63 \pm 0.25	491 \pm 90	1570 \pm 370	3140 \pm 740
		F	0.50 \pm 0.00	513 \pm 116	1280 \pm 340	2550 \pm 690
		M+F	0.57 \pm 0.19	501 \pm 93	1440 \pm 360	2890 \pm 730

*AUC₀₋₁₂ represents the observed exposure over the observed 12-hour dosing interval

** For consistency in data reporting, the AUC observed over the 12-hour dosing interval was extrapolated (multiplied by 2) to provide an exposure over a 24-hour period. The assumption is that the concentrations (although not measured) during the second dosing interval are equivalent to the measured concentrations during the first dosing interval.

Stability and Homogeneity

Stability: Stability was demonstrated by comparing results of the entrance (first dose of the week) and exit (last dose of the week) assays of weeks 1, 4, 8, 16, 32 (entrance assay only) and 37 (exit assay only).; assay results were within $\pm 10\%$ of label concentration in all cases and was therefore stable during the week.

For storage, the stability of the final formulation was longer than 8 weeks if stored frozen (-10°C to -20°C). At week 8, the formulation was prepared at 2 week intervals.

Homogeneity: The applicant did not determine homogeneity. They noted "At the concentrations used in this study (≤ 1 mg/mL), dosing formulations were solutions; analysis of solutions for homogeneity is not required" and cited an internal email.

As a GLP study verification of solution homogeneity is necessary The Reviewer is not aware of the status of the regulations pertaining to this issue at the time the study was conducted in 2003-2004. Given that dosing solution was stirred and administered daily for 9 months is unlikely the lack of verification had any impact on the findings.

Concentration Verification: Samples from the middle of the container of each dose formulation on the day of preparation from weeks 1, 4, 8, 16, 32 and 37 were analyzed for concentration. Samples were within $\pm 10\%$ of nominal values, ranging from 90.0% to 107.0%.

STUDIES IN JUVENILE MONKEY

Study title: Tofacitinib (CP-690550) 39-week oral (gavage) administration toxicity study in the juvenile cynomolgus monkey with a 26-week recovery phase

Study no.:	09GR248 (b) (4) 2501-010)
Study report location:	Mod 4.2.3.5.4 (the interim report was submitted with the initial NDA, the final report was submitted Feb 16 2012)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Feb 25, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CP-690550, Lot E010010198, Purity 99.5%

Key Study Findings

- Juvenile cynomolgus monkeys, 13-14 months of age, were administered CP-690,570 twice daily at a total daily dose of 0, 0.5, 2 and 10 mg/kg/day for 39 weeks. A 6-month recovery period followed the dosing phase for all doses except for the lowest dose 0.5 mg/kg/day group.
- There were no effects of CP-690550 on mortality, body weights or weight gain, clinical chemistry, coagulation parameters, bone growth, and cardiovascular and ECG parameters.
- The mid and high doses resulted in reduced thymus (50% of control) and spleen (66% of control) weights, with full recovery in females and partial recovery in males. There were no histopathological correlates to these changes.
- Compared to effects in the control group, CP-690550 treatments resulted in an increased incidence in inflammatory cell foci of the heart at the high dose, an increase in ulcers of the tail at the mid and high doses, and in increase in lymphoid hyperplasia at the mid and high doses. A full histopathology assessment was not conducted due to the lack of findings in previous toxicology studies of adult monkeys at these doses.
- There were CP-690550 dose-related changes in both red blood cell and white blood cell parameters.
 - Red blood cell, hemoglobin and hematocrit were 84-86% of control values at the high dose.
 - Total white blood cells were not altered, but total lymphocytes were reduced 31% (69% of control for males, 81.5% for females). Immunophenotyping indicated that most lymphocyte subsets were reduced (NK cells 9% of predose levels; CD4+ T cells 52%, CD8+ T cells 49%, CD4+ naïve T cells 42%, CD8+ naïve T cells 32%, memory central CD8+ T cells 48% and memory effector CD8+ T cells 69%). There was full or partial recovery of lymphocyte subsets over the 6 month recovery phase with females demonstrating full recovery or more complete recovery than males.
- Bone marrow analysis found no changes in cellular morphology or evidence of substantial erythroid or myeloid cellular lineage, although at the high dose there was a reduction in M:E ratio (77% of control value) due to an increase in erythroid precursors.
- Additional studies to address immunotoxicity included a T-cell dependent antibody response study using keyhole limpet hemocyanin (KLH). CP-690550 reduced the anti-KLH IgM response and completely inhibited the anti-KLH IgG response at the high dose. The response to KLH was restored when tested during the recovery phase in previously untested monkeys. The response to a second KLH challenge was evaluated in vitro from peripheral blood mononuclear cells (PBMC) collected at necropsy. There was no effect of CP-690550 to suppress the PBMC proliferative response or the response to a mitogen, concanavalin A.
- The NOAEL was 0.5 mg/kg/day, corresponding to AUC₀₋₂₄ of 62 ng-h/mL at week 36 (2 x AUC₀₋₁₂ of 31.1 ng-h/mL), which is approximately 0.11-fold of the systemic exposure as the maximally recommended human dose of 10 mg bid.

This study provided toxicological support for the safety of some (b) (4) impurities to supplement the previous PharmTox Review filed March 9 2012.

Methods

Doses: 0, 0.5, 2, and 10 mg/kg/day
(0, 0.25, 1, and 5 mg/kg BID)

Frequency of dosing: Twice daily (12 hours \pm 1 hour apart) for at least 39-weeks excluding the day of necropsy and the days of a 26-week recovery phase

Route of administration: Orally by gavage

Dose volume: 10 mL/kg/day (5 mL/kg/dose)

Formulation/Vehicle: 0.5% (w/v) Methylcellulose, 4000 cps, in reverse osmosis (RO) water

Species/Strain: Cynomolgus monkey (*Macaca fascicularis*) of Mauritian origin

Number/Sex/Group: 4/sex/dose

Age: 13 to 14 months of age

Weight: 1.6 to 2.4 kg

Satellite groups: Recovery: 3/sex/dose, except no low dose 0.5 mg/kg/day animals

Unique study design: Animals of the same sex were pair- or group-housed.
Blood samples (approximately 1 mL) were taken from all available animals before the start of the predose phase and analyzed by the Pfizer DSRD Immunotoxicology CoE under non-GLP conditions to determine the presence of anti-Lymphocryptovirus (LCV) antibodies of each animal. Only animals positive for LCV were selected for study. (A previous monkey toxicology study, Report 2003-0301, indicated that lymphomas had developed in animals that were positive for lymphocryptovirus antibodies.)

Group number	Group description	Dose level (mg/kg/dose)	Dose level (mg/kg/day)	Animals/group		Necropsy after ...	
				Male	Female	39 weeks	65 weeks
1	Control	0	0	7	7	4 M / 4F	3 M / 3 F
2	Low	0.25	0.5	4	4	4 M / 4F	-
3	Intermediate	1.0	2	7	7	4 M / 4F	3 M / 3 F
4	High	5.0	10	7	7	4 M / 4F	3 M / 3 F

Deviation from study protocol: There were no study deviations that affected the overall interpretation or conclusions of the study.

Observations and Results

Mortality examined at least twice daily

There were no mortalities.

Clinical Observations observed at each dose for behavior, appearance, and feces (normal/soft/fluid/none), detailed fur inspection once a week.

There was no effect of CP-690550 on clinical observations of behavior, appearance or feces. Some clinical signs that were recorded during the dosing phase (e.g. hair thinning/loss, soft/fluid feces, minor lesions or injuries on extremities) common background observations unrelated to dose or duration of dosing.

Body weights recorded once weekly

Body weights increased without an effect of CP-690550. Their rate of gain was considered to be normal growth for juvenile animals of this age.

Food Consumption not determined

Due to pair- or group housing, food consumption was not determined.

EKG performed on all animals, non-anesthetized but temporarily restrained, about 2 to 4 hours after dosing, once during the predose phase and in weeks 1, 13, and 39. Fridericia's formula was used for QTc calculations.

There was no effect of CP-690550 on ECG, arrhythmia, or cardiovascular parameters monitored at times during the study. The mean heart rates for all groups ranged from 225 to 265 beats/min. The mean QT interval ranged from 0.15 to 0.18 sec, and the QTc ranged from 0.25 to 0.28 sec. There were minor changes throughout the dosing phase of isolated cases of QTc lengthening, slight tachycardia, slight bradycardia or atrioventricular block grade 1 in single animals that were either consistent with similar findings at pretreatment intervals, or also observed in animals of the control group. Therefore, none of these findings were considered to be related to CP-690550 treatment.

The EKG assessment was acceptable.

Bone assessment Radiographs for determining bone length of the radius and tibia were taken from anesthetized animals once during the predose phase, in week 38/39, and at the end of the recovery phase in week 63/64.

Increased bone length occurred over time in all dose groups, with no effect of CP-690550 treatment.

Ophthalmoscopy not assessed

There were no previous concerns in adult monkey toxicological studies of these parameters. There were no developmental abnormalities in studies of rats, and no abnormal findings in a 6-month toxicity study or after 1 year of dosing in the 2 year rat carcinogenicity study. Therefore, the absence of an ophthalmic assessment is acceptable although not optimal.

Hematology

Blood samples were obtained in the pre-dose phase, weeks 4, 13, 26, and 39 of the dosing phase and from recovery animals in weeks 43, 52, and 65 (there was no recovery group for the low dose). Samples from males were obtained on day 176 and from females on day 180 of the dosing and recovery phases (indicated in the tables as Day 176/180).

Parameters	Abbreviation	Units
White blood cell count	WBC	10E9/L
Red blood cell count	RBC	10E12/L
Hemoglobin	HGB	mmol/L
Hematocrit	HCT	%
Mean corpuscular volume	MCV	fL
Mean corpuscular hemoglobin	MCH	fmol
Mean corpuscular hemoglobin concentration	MCHC	mmol/L
Platelet count	PLT	10E9/L
Lymphocytes	LYM	%
Monocytes	MONO	%
Neutrophils	NEUT	%
Eosinophils	EOSI	%
Basophils	BASO	%
Absolute lymphocytes	ALYM	10E9/L
Absolute monocytes	AMON	10E9/L
Absolute neutrophils	ANEU	10E9/L
Absolute eosinophils	AEOS	10E9/L
Absolute basophils	ABAS	10E9/L
Reticulocyte count	RETI	0/00
Absolute reticulocyte count	TRET	10E12/L

Coagulation Parameters

Parameters	Abbreviation	Units
Prothrombin time	PT	s
Activated partial thromboplastin time	APTT	s

In the 10 mg/kg/day group from day 22 onwards, there were decreases in red blood cell parameters (red blood cells, hemoglobin, and hematocrit) of both sexes. Decreased erythrocyte numbers also occurred in the 2 mg/kg/day male group. The largest reduction occurred in males at week 26 (day 176) of the high dose group (expressed as % of control: red blood cells 84%, hemoglobin 84% and hematocrit 86%). These changes returned to control levels by the end of the recovery phase.

The applicant commented that the lowest values detected in week 26 (day 176) and elevated reticulocyte counts may have been influenced by the extended blood sampling for PBMC purification as part of the immunotoxicity assessment. The Reviewer concurs with this assessment.

Reviewer comment on Tables: values were rounded by the reviewer, % of control were calculated based on vehicle control for that day of collection, which differs from the statistical analysis of the applicant, in which analysis was sometimes based on predose values. Not all days of collection are presented. For selected results, changes from controls were calculated as %of control.

Table 81: Hematology Summary in Juvenile Rats

RBC (10¹²/L)								
Dose (mg/kg/day)	0		0.5		2.0		10	
Sex	M	F	M	F	M	F	M	F
Predose day 11	6.9	6.8	7.0	6.7	6.8	7.0	6.9	7.1
Day 22	6.7	6.8	6.7	6.4	6.4	6.5	6.4 (96%)	6.5 (96%)
Day 176/180	6.8	6.1	6.5	6.0	6.3	6.0	5.7 (83%)	5.7 (93%)
Day 270	7.0	6.9	0	6.6	6.6	6.7	6.0 (86%)	6.4 (93%)
Recovery Day 176/181	6.8	6.8	-	-	6.5	6.8	6.5 (96%)	6.9 (101%)

Hematocrit (%)								
Dose (mg/kg/day)	0		0.5		2.0		10	
Sex	M	F	M	F	M	F	M	F
Predose day 11	45.2	46.6	47.6	45.5	46.2	46.8	45.5	46.7
Day 22	44.9	46.6	45.3	44.5	45.0	44.1	41.7 (93%)	43.2 (93%)
Day 176/180	46.1	42.6	45.6	42.3	44.2	41.5	39.5 (86%)	38.6 (84%)
Day 270	45.6	46.6	47.4	44.9	45.8	44.7	40.5 (89%)	42.2 (90%)
Recovery Day 176/181	45.9	45.7	-	-	45.5	43.4	43.1 (94%)	43.7 (96%)

Hemoglobin (mmol/L)								
Dose (mg/kg/day)	0		0.5		2.0		10	
Sex	M	F	M	F	M	F	M	F
Predose day 11	8.2	8.5	8.6	8.4	8.3	8.4	8.2 (100%)	8.5 (100%)
Day 22	8.1	8.6	8.3	8.1	8.0	7.8	7.8	7.8

							(96%)	(91%)
Day 176/180	8.1	7.4	8.0	7.4	7.6	7.2	6.8 (84%)	6.8 (92%)
Day 270	8.2	8.5	8.7	8.2	8.1	8.0	7.2 (88%)	7.7 (90%)
Recovery Day 176/181	8.3	8.4	-	-	8.2	8.2	8.0 (96%)	8.1 (96%)

Reticulocytes (%)								
Dose (mg/kg/day)	0		0.5		2.0		10	
Sex	M	F	M	F	M	F	M	F
Predose day 11	4.1	5.1	3.2	5.5	3.8	2.8	2.6 (63%)	4.1 (80%)
Day 22	5.5	5.6	4.4	5.7	6.1	4.3	4.0 (73%)	4.5 (80%)
Day 176/180	5.7	12.9	5.7	14.1 (109%)	7.8	10.5	6.6 (116%)	13.5 (105%)
Day 270	3.5	5.3	4.4	6.5	7.0	3.8	5.3 (151%)	7.5 (142%)
Recovery Day 176/181	3.2	4.4	-	-	5.3	3.7	3.4 (106%)	6.8 (152%)
% Lymphocytes								
Predose day 11	52.0	49.6	63.5	53.8	57.7	37.6	56.4	50.9
Day 22	50.3	60.4	34.0 (68%)	55.0	41.6	54.0	27.0 (54%)	39.3 (65%)
Day 176	71.4	63.3	66.9	66.7	48.7	60.5	49.1 (69%)	51.6 (82%)
Day 270	64.1	46.9	55.9	51.6	50.5	37.7 (80%)	40.2 (63%)	36.5 (78%)
Recovery Day 176/181	60.2	60.4	-	-	54.6 (91%)	68.3 (113%)	59.2 (98%)	61.1 (101%)
WBC (10⁹/L)								
Dose (mg/kg/day)	0		0.5		2.0		10	
Sex	M	F	M	F	M	F	M	F
Predose day 11	10.2	12.8	10.0	9.5	9.5	3.4	11.9	10.3
Day 22	7.2	7.9	7.4	6.8	5.9 (82%)	8.1 (102%)	8.0 (111%)	9.3 (118%)
Day 176/180	10.5	11.0	8.8	8.8	9.0 (86%)	8.1 (74%)	8.6 (82%)	9.3 (84%)

Day 270	9.8	12.5	8.8	10.5	7.4 (76%)	9.8 (78%)	8.0 (82%)	10.2 (82%)
Recovery Day 176/181	11.2	9.0	-	-	7.6 (68%)	9.4 (104%)	6.0 (54%)	8.7 (97%)
Neutrophils (%)								
Dose (mg/kg/day)	0		0.5		2.0		10	
Sex	M	F	M	F	M	F	M	F
Predose day 11	43.6	44.0	30.0	41.6	34.9	55.2	36.8	44.5
Day 22	45.1	31.8	62.8	35.8	53.1	37.8	67.8 (150%)	53.1 (167%)
Day 176/ 180	21.3	27.9	26.3	24.9	42.7 (200%)	31.8 (114%)	42.9 (201%)	40.1 (144%)
Day 270	29.1	45.3	36.4	40.1	38.0 (130%)	55.5 (122%)	51.4 (177%)	53.9 (119%)
Recovery Day 176 / 181	33.6	32.0	-	-	35.2 (105%)	26.1 (82%)	33.7 (100%)	27.3 (85%)

On day 22 for all dose groups including the controls, there were minimal to moderate decreases in absolute lymphocyte counts, relative to pre-dose values (-22% to -68%) described further in the immunophenotyping section below. From week 13 to 39, decreased absolute lymphocyte counts, relative to pre-dose value, occurred in males in the 2 and 10 mg/kg/day dose groups, and females in the 10 mg/kg dose group, with the greatest decrease occurring at week 39 in the 10 mg/kg/day dose group. The magnitude of the mean decrease was greatest in the males of the 10 mg/kg/day dose group in week 39.

Dose related reduction in absolute total lymphocyte counts, relative to pre-dose levels occurred in week 13 for male animals in the 0.5 mg/kg/day dose group (-24%), and from week 13 to 39 for males in the 2 mg/kg/day (-42 to -47%) and 10 mg/kg/day (-44 to -54%) dose groups, and for females in the 10 mg/kg/day dose group (-32 to -35%). The largest reduction in lymphocytes occurred at the highest dose at the end of the dosing period (week 39). This high dose male group had only partial recovery throughout the recovery phase, whereas lymphocyte counts of males at 2 mg/kg/day and females at 10 mg/kg/day were fully recovered.

The hematological assessments were acceptable.

Bone Marrow Evaluation

Bone marrow smear full myelogram evaluation was conducted on slides of main study and recovery

animals of the control and the mid and high dose groups. Three smears per animal were provided; only one smear per animal was required for examination. Differential count was based on 500 cells in bone marrow smears of all animals, which is adequate.

There were no substantial CP-690550-related changes in numbers of individual or total erythroid and myeloid cell compartments or M:E ratios. In addition, there were no changes in cellular morphology or evidence of erythroid or myeloid cellular lineage toxicity either at the end of 39 weeks of dosing or after a 26 week recovery phase. Females in the high dose, 10 mg/kg/day, had a lower M:E ratio (75% of control) at the end of the dosing period due to an increase in erythroid precursor cells, proerythroblasts (228%) and early erythroblasts (144%). At the mid dose, a similar change in erythroid cells proerythroblasts (214%) and early erythroblasts (129%) was balanced by an increase in myeloid cells myeloblasts (133%) and metamyelocytes (109%), and increased eosinophils (118 %). There were no consistent changes for males.

Following a 26 week recovery period, the 10 mg/kg/day male and female groups had a general increase in the percentage of most erythroid compartments and slightly lower myeloid component relative to controls, with corresponding decreases in M:E ratios in 2 mg/kg/day females (83%) and 10 mg/kg/day males (77%).

Over all the CP-690550-related changes were not toxicologically significant.

Myeloid/Erythroid Ratio (and % of control)								
Dose (mg/kg/day)	0		0.5		2.0		10	
Sex	M	F	M	F	M	F	M	F
End of Dosing Phase	1.16	1.38	-	-	1.10 (95%)	1.37 (99%)	1.09 (93%)	1.04 (75%)
End of Recovery Phase	1.33	1.51	-	-	1.21 (91%)	1.25 (83%)	1.02 (77%)	1.27 (84%)
- There was no bone marrow evaluation for the 0.5 mg/kg/dose								

Clinical Chemistry

Blood samples were obtained in the pre-dose phase, weeks 4, 13, 26, and 39 of the dosing phase and from recovery animals in weeks 43, 52, and 66.

Parameter	Abbreviation	Units
Total bilirubin	TBIL	μmol/L
Creatinine	CREA	μmol/L
Blood urea	BU	mmol/L
Glutamate dehydrogenase	GLDH	U/L
Aspartate aminotransferase	AST	U/L
Alanine aminotransferase	ALT	U/L
Alkaline phosphatase	ALP	U/L
Gamma glutamyl transferase	GGT	U/L
Glucose	GLU	mmol/L
Total cholesterol	CHOL	mmol/L
Triglycerides	TRIG	mmol/L
Inorganic phosphorus	PHOS	mmol/L
Calcium	CA	mmol/L
Sodium	NA	mmol/L
Potassium	K	mmol/L
Chloride	CL	mmol/L
Total protein	TP	g/L
Albumin	ALB	g/L
Globulin	GLOB	g/L
Albumin/globulin ratio	A:G	

There was no effect of CP-690-550 on clinical chemistry and coagulation parameters.

The assessment of clinical chemistry parameters was acceptable.

Urinalysis

no samples were collected

Gross Pathology

Animals received an intramuscular injection with ketamine hydrochloride followed by intravenous sodium pentobarbitone prior to exsanguination. A complete macroscopic examination was conducted.

There were no CP-690550-related macroscopic findings.

The evaluation of macroscopic findings was acceptable.

Organ Weights

Weighed organs are listed in the table of Tissues and Organs for Pathology (below). They included adrenal, brain, heart kidney, liver, lungs, spleen, and thymus.

Thymus weight (expressed as absolute weight, relative to body weight, and relative to brain weight) was reduced at 2 mg/kg/day and 10 mg/kg/day in males and at 10 mg/kg/day in females (refer to the Table, below). Spleen weights (expressed as absolute weight, relative to body weight, and relative to brain weight) were also reduced compared to controls in the 2 mg/kg/day and 10 mg/kg/day males and females dose

groups. Spleen and thymus weights recovered for females, but only partially recovered for males.

Spleen and Thymus Weights (g and % of body weight) (Treatment effect as % of Control; Values were rounded by the Reviewer)

Dose (mg/kg/day)		Males				Female			
		0	0.5	2	10	0	0.5	2	10
Spleen	g	4.94	5.60 (113%)	4.40 (89%)	4.16 (84%)	6.601	4.891 (74%)	4.04 (61%)	4.36 (66%)
% body weight	0.2		0.20 (102%)	0.17 (85%)	0.16 (80%)	0.268	0.196 (0.73%)	0.17 (63%)	0.19 (70%)
% brain weight	7.20		7.30 (101%)	6.26 (87%)	6.02 (84%)	9.74	7.04 (72%)	5.83 (60%)	6.79 (70%)
Recovery	6.62		-*	9.80 (148%)	5.02 (75%)	5.189	-*	7.00 (135%)	7.12 (137%)
g									
%body weight	0.20		-*	0.26 (126%)	0.17 (85%)	0.192	-*	0.24 (123%)	0.24 (126%)
% brain weight	9.41		-*	13.60 (144%)	6.46 (69%)	7.86	-*	10.12 (129%)	10.57 (134%)
Thymus	g	5.13	4.54 (88%)	2.61 (50%)	2.71 (53%)	5.016	4.488 (89%)	4.51 (90%)	2.49 (50%)
% body weight	0.21		0.17 (79%)	0.10 (48%)	0.11 (51%)	0.206	0.180 (87%)	0.19 (92%)	0.11 (53%)
% brain weight	7.51		5.87 (78%)	3.70 (49%)	3.94 (52%)	7.40	6.51 (88%)	6.48 (88%)	3.88 (52%)
Recovery	6.09		-*	5.38 (88%)	3.99 (65%)	3.508	-*	4.99 (142%)	4.89 (139%)
g									
%body weight	0.19		-*	0.15 (80%)	0.13 (69%)	0.131	-*	0.17 (130%)	0.16 (126%)
% brain weight	8.66		-*	7.46 (86%)	5.17 (60%)	5.28	-*	7.23 (137%)	7.23 (137%)

* there was no Recovery group for the 0.5 mg/kg/day dose

Histopathology

Adequate Battery: Yes, refer to the table below. Not all tissues were examined as would be expected in a general toxicological assessment. However, based on findings in the toxicology studies in adult monkeys and rats, all affected organs in those studies were examined here, along with the major organs.

Peer Review: Yes, by the applicant's pathologist.

Tissues and Organs for Pathology

Ref. no.	Tissue / organ	(*)	(†)	(§)	Ref. no.	Tissue / organ	(*)	(†)	(§)
1	skin/animal identification/chip	*			30	salivary glands, mandibular	*		
2	glands, mammary	*			31	salivary glands, lingual	*		
3	spleen	*	†	§	32	parotids	*		
4	pancreas	*			33	thyroid + parathyroids	*		
5	stomach	*			34	tongue	*		
6	duodenum	*			35	trachea	*		
7	jejunum	*			36	esophagus	*		
8	lymph nodes, mesenteric	*		§	37	thymus	*	†	§
9	ileum, with Peyer's patch	*e		§	38	rib			
10	cecum	*			39	heart	*	†	§
11	colon	*			40	lungs (with mainstem bronchi)	*	†	§
12	rectum	*			41	aorta (arch and anterior abdominal)	*		
13	adrenals	*	†		42	eyes + optic nerves	*b		
14	kidneys	*	†	§	43	lacrimal glands	*		
15	liver	*	†	§	44	spinal cord cervical	*		
16	gall bladder	*			45	spinal cord thoracic			
17	ovaries	*			46	spinal cord lumbar			
18	uterus/cervix	*			47	brain (cerebral cortex, thalamus, midbrain, medulla, cerebellum)	*	†	§
19	vagina	*			48	pituitary	*		
20	urinary bladder	*			49	injection site (i.m., KLH injection site)	*		
21	testes	*c			50	application site			
22	epididymides	*c			51	bone marrow smear (sternum)	*a,d		
23	seminal vesicles	*			52	blood sample	*h		
24	prostate	*			53	draining lymph nodes (inguinal)	*f		
25	sciatic nerve	*			54	nasal cavity with nasopharynx and paranasal sinus	*		
26	sternum with bone marrow	*		§	55	Lymphomas if present-need to be frozen (unscheduled and scheduled necropsies)	*g		§ /§2
27	femur with bone marrow and articular surface	*			56	Brown adipose tissue	*		
28	skeletal muscle	*			57	eyelid	*		
29	lymph nodes, mandibular	*		§	58	gross lesions	*		§ /§2

Fixative 10% neutral buffered formalin except where stated as:

a - methanol

b - Davidson's fluid

c - modified Davidson's fluid

d - see terminal procedure section

g - embedded in OCT

h - see *Ex-vivo* KLH stimulation of Peripheral Blood Mononuclear Cells (PBMC) section

* - tissues preserved

† - organs weighed

e - Peyer's patches only for oral administration

§ - tissues examined for main study animals

f - draining lymph nodes for KLH immunization

§ - tissues examined for recovery animals

2

Paired organs were weighed together

Bone tissue designated for histopathological examination was decalcified using Kristenson's fluid.

Histological Findings

In contrast to the applicant's interpretation that "histopathological findings were generally similar in control and dosed animals and consistent with the expected background pathology in cynomolgus monkeys," there were findings that were at greater incidence in the mid and high doses than in the control groups. These include adverse findings in the heart, lymphoid organs, and skin.

Heart: There was an increase in inflammatory cell foci, at the mid and high doses. All were focal with minimal severity. The heart findings are unlikely to be background pathology as suggested by the applicant as there were findings in 7 of 8 animals at the high dose compared to 1 of 8 animals in the control group. Historical background data was not provided. However, there was no associated cardiac muscle pathology. Also, there were no obvious cardiovascular-related clinical signs, and the EKG findings were similar for all groups.

Inflammatory cell foci: Other organs with Inflammatory cell foci were also noted in other major organs, kidney, liver and lung and in one incidence in the brain at the end of the dosing phase. However for these organs, there was no association of greater incidence with higher doses as observed with the heart. Recovery animals were not examined, except for gross lesions, due to the applicant's interpretation, there were no CP-690550-related effects at the end of the dosing phase.

Lymph Nodes, Spleen, Thymus, Bone Marrow: Lymphoid hyperplasia occurred in various lymphoid tissues. The Applicant considered these findings as not CP-690550 related since it "often affected only a single follicle within those tissues, was never present in more than one lymphoid tissue per animal and is within normal historical variation from control animals within the facility." The Reviewer does not agree with the above rationale as it is unlikely that the entire organ was histologically examined. It's possible that challenging the animals with KLH in the TDAR assessment confounded these lymphoid tissue findings, but how that would affect the incidence in the mid and high doses, but not the control or low dose is not obvious. The lymphoid hyperplasia is considered adverse in part to the development of lymphomas in the 9-month toxicological study in more mature monkeys (2-5 years of age).

Skin: Evaluation of skin at the macroscopic examination found 3 of 8 animals at the mid dose (2 males, 1 female) and 4 of 8 animals (2 males, 2 females) with skin lesions, while there were no instances in the control and low dose groups. These lesions comprised focal tail ulcers of slight to severe in severity or tail skin crusts or scabs. Their incidences in the mid and high dose group may be related to slower healing from CP-690550 immunosuppression in combination with group housing and their juvenile age providing a greater opportunity for continuous irritation. Nevertheless, their presence or persistence is associated with the higher doses of CP-690550.

Cecum: Evaluation of the ileocecal valve due to red discoloration observed in the macroscopic examination, revealed GALT hyperplasia, focal ulcer of moderate severity

with minimal multifocal hemorrhage in one high dose male, and one low dose female recovery.

Spleen and Thymus: The applicant reported no adverse pathology associated with these organs although the spleen was reduced in size to 70-80% of the control weight and the thymus reduced in size up to approximately 52% of the control weight based on % of body weight.

Histopathological Findings

Group		1		2		3		4	
Dose (mg/kg/day)		0		0.5		2		10	
Sex		M	F	M	F	M	F	M	F
N examined (unless indicated otherwise)		4	4	4	4	4	4	4	4
Heart									
inflammatory cell foci		0	2	0	0	0	1	4	3
Lymph Nodes, Mandibular									
hyperplasia, lymphocytes		0	0	0	0	0	0	1	0
extramed. hematopoiesis		0	0	0	0	0	0	0	1
Sternum with Bone Marrow									
lymphoid follicle		0	0	0	0	0	0	0	1
Spleen									
hyperplasia, lymphocytes		0	0	0	0	0	1	0	1
Findings related to lesions observed in macroscopic examination									
Skin									
N examined		0	0	2	3	2	2	3	4
ulcer		-*	-*	0	0	2	1	2	2
hemorrhage				0	0	0	0	1	0
scab				0	0	0	0	0	1
* these groups were not evaluated									

Special Evaluation

Immunophenotyping by flow cytometry

Blood samples were withdrawn from all animals twice during predose and in weeks 4, 13, 26, and 39 of the dosing phase and from all surviving animals in study weeks 43, 52, and 65 (during the recovery phase).

Immunophenotyping with specific monoclonal antibodies (refer to the table below) were used to evaluate the populations of T-cell subsets (CD4+ and CD8+ naïve, central and effector memory T-cells), B-cells, and natural killer (NK) cells. Total lymphocyte counts were determined on the same day. Absolute numbers of each lymphocyte subpopulation were computed from the percentages of each lymphocyte population determined by flow cytometry (relative numbers) and the absolute counts determined from hematology data. Fold-changes in absolute count from baseline were calculated per individual. Effects were determined by comparing absolute lymphocyte subset numbers on specific blood collection dates against the predose values closest to first CP-690550 administration. In addition, the inter-animal variation, as well as, longitudinal variability of absolute lymphocyte subset numbers of vehicle control animals were taken into consideration when determining CP-690550-related changes.

The following combinations of antibodies were used:

Antibody combinations	Determination of
Isotype controls	background
CD3, CD16 and CD20	CD3- CD20+ B-cells, CD3- CD16+ NK-cells
CD3, CD4, CD95, CD28 CD3, CD8, CD95, CD28	CD3+T-cells, CD3+CD4+ T-helper cells, CD3+CD8+ cytotoxic T-cells, CD3+CD4+ CD95 ^{low} CD28+ naïve CD4+ T-cells CD3+CD4+CD95 ^{high} CD28+ central memory CD4+ T-cells CD3+CD4+CD95 ^{high} CD28- effector memory CD4+ T-cells CD3+CD8+ CD95 ^{low} CD28+ naïve CD8+ T-cells CD3+CD8+CD95 ^{high} CD28+ central memory CD8+ T-cells CD3+CD8+CD95 ^{high} CD28- effector memory CD8+ T-cells

At week 4, numbers of total lymphocytes and lymphocyte subsets for the vehicle and tofacitinib-treated monkeys all decreased compared to prestudy values, but there was no reduction when expressed as % lymphocytes of total WBC. The types of lymphocytes were reduced (control group B cells decreased 54% IN males and 53% in females at week 4; and control group T cells decreased 53% in males and 27% in females). The reason for this decrease was not known. Therefore, effects were only evaluated on samples collected in weeks 13 (day 85), 26/28 (day 176 in males and day 194 in females), and 39 (day 270). A comparison of controls with CP-690550-treated groups was therefore not conducted; rather a comparison was conducted with pre-dose values within each dose group. Pre-dose and week 13, 26 and 39 measurements for

the control group were fairly similar and effects of CP-690550 were more readily apparent.

The table below indicates the percentage of prestudy values (group means) on day 270 (end of the dosing phase) and each time point during the recovery phase (recovery days 22 to recovery days 176/181) for each lymphocyte subset that was decreased during the dosing phase.

		Group means (percent of prestudy value)							
		10 mg/kg/day				Vehicle control			
		D270	R22	R85	R176/181	D270	R22	R85	R176/181
NK cells	Males	9.8	70.1	64.4	50.2	87.0	106.6	133.0	98.4
	Females	7.4	52.3	46.8	55.0	62.7	72.8	47.0	90.7
CD3+ T cells	Males	52.2	61.9	75.3	56.2	101.6	94.3	113.1	106.7
	Females	47.0	83.4	65.7	82.7	86.8	118.6	100.7	128.6
CD4+ T cells	Males	54.4	66.17	87.0	65.1	103.2	95.8	111.8	108.8
	Females	56.0	91.3	74.9	94.3	92.4	122.7	104.0	129.5
CD8+ T cells	Males	58.5	59.7	69.4	42.5	105.1	90.2	118.1	100.5
	Females	40.7	78.7	53.9	74.5	86.4	128.6	102.7	137.1
Naïve CD4+	Males	43.9	50.7	80.7	64.7	108.5	99.1	113.0	110.9
	Females	40.7	60.2	61.6	90.7	96.1	137.7	111.3	140.1
Naïve CD8+	Males	34.7	38.4	74.6	50.6	119.75	99.2	134.4	120.6
	Females	29.5	56.0	58.7	99.9	97.4	149.1	118.4	155.7
Central CD8+	Males	57.5	88.7	123.6	72.1	101.6	85.4	106.8	80.4
	Females	42.2	76.4	81.2	98.7	86.6	115.9	143.1	141.8
Effector CD8+	Males	82.7	72.6	59.3	33.2	91.6	77.5	100.0	81.1
	Females	53.7	89.4	45.9	56.3	76.8	105.8	69.7	108.4

D=Dosing Phase Day; R = Recovery Phase Day

T cells: Decreased absolute total T cells and CD4+ and CD8+ T cell subsets occurred in weeks 13 through 39 of the dosing phase in both males and females of the 10 mg/kg/day dose group. For CD4+ T cells, decreases were observed in the 10 mg/kg/day dose group in both genders throughout the dosing period with decreases generally greater for females than males expressed as a % of predosing values. There were no treatment effects on T cells at the mid or low dose. For CD8+ T cells, decreases were observed for females in the 10 mg/kg/day dose group from weeks 13 through 39 and males in the 2 and 10 mg/kg/day dose groups. Decreases were observed for both naïve CD4+ and naïve CD8+ T cell populations in both males and females in the 10 mg/kg/day dose group from week 13 through 39 and these generally paralleled the decreases observed for total CD4+ and CD8+ populations respectively. At the end of the dosing period (day 270) the group mean percent of predose values for total T cells were 52.2% and 47.0% for males and females respectively, and for CD4+ T cells were 54.4% and 56.0% for males and females respectively. Partial to complete recovery of both total T cell and CD4+ T cell subsets occurred.

Central or effector memory CD4+ and CD8+ T cells: For central memory CD8+ T cells, decreased cell numbers occurred in the 10 mg/kg/day dose group for females (weeks 13-39) and males (mainly on weeks 26 and 39). Decreases in effector memory CD8+ T cells were noted for some animals (3 of 7 males / 3 of 7 females) of the 2 mg/kg/day

dose groups. and some animals (3 of 7 males /3 of 7 females) of the 10 mg/kg/day dose group (reduced 13-19% of predose values at day 22 of dosing, but then increased during the rest of the dosing phase; 1 female had a 13% of predose values, but not until day 85). There were no effects on central or effector memory CD4+ T cells.

NK cells: A dose-dependent decrease in absolute NK cell numbers occurred at weeks 13, 26 and 39 in males and females of the 2 and 10 mg/kg/day dose groups. NK cell numbers were 34.5% and 20.5% of prestudy values for the 2 mg/kg/day dose group and 9.8% and 7.4% of prestudy values for the 10 mg/kg/day dose group (males and females, respectively) at week 39.

Partial to complete recovery of NK cells was observed for males and females in the 2 and 10 mg/kg/day dose groups during the recovery phase. The mean percent of prestudy NK cell values in the 10 mg/kg/day dose group increased from 9.8-7.4% to 50.2-70.1% and 46.8-55.0% in males and females respectively during the recovery period.

B cells: There were no effects on B cells at any dose or gender throughout the dosing period.

Lymphocyte proliferation assays

Blood samples at predose, weeks 13, 26, and 39 of the dosing phase, and weeks 52 and 65 from all surviving animals (recovery phase). Peripheral blood mononuclear cells (PBMCs) were isolated on the day of blood collection and used to assess mitogen stimulation using Concanavalin A (ConA) as described in the study phase plan C22-007 and amendment C22-007-A1, as a GLP activity with quality assurance auditing.

CP-690550 did not reduce functionality of the remaining T cells when tested *in vitro* by proliferation in response to ConA stimulation. A higher stimulation index for males at the 10 mg/kg/day dose compared to the male vehicle-group following 39 weeks of treatment occurred at 5.0 and 1.0 µg/mL ConA. This was not observed in females, and was not due to an increased responsiveness to ConA, but rather to lower background proliferation. There were other statistically significant effects on T lymphocyte proliferation upon *in vitro* stimulation of PBMCs with ConA, however these were not considered to be CP-690550 related due to the lack of ConA concentration dependence, lack of CP-690550 dose dependence, and the sporadic nature of these effects.

Stimulation of PBMC by Concanavalin A
Predosing Phase

Table 1 Con A stimulation of PBMC pre dosing. Group means and standard deviations of Stimulation indices (SI) at indicated Con A concentrations as well as results of the unpaired two sided t test between the vehicle group and each dose group of the respective gender are given.

Group	Con A stimulation [$\mu\text{g/mL}$]			
	5,0	1,0	0,2	0,0
Group 1, f	48 \pm 26	21 \pm 8	4 \pm 1	1
Group 1, m	92 \pm 27	42 \pm 18	7 \pm 3	1
Group 2, f	88 \pm 48	21 \pm 16	3 \pm 1	1
p=	0,414 n.s.	0,989 n.s.	0,270 n.s.	
Group 2, m	72 \pm 42	29 \pm 15	5 \pm 2	1
p=	0,359 n.s.	0,278 n.s.	0,144 n.s.	
Group 3, f	45 \pm 13	23 \pm 8	4 \pm 1	1
p=	0,800 n.s.	0,579 n.s.	0,758 n.s.	
Group 3, m	89 \pm 64	44 \pm 38	6 \pm 5	1
p=	0,906 n.s.	0,885 n.s.	0,643 n.s.	
Group 4, f	82 \pm 38	30 \pm 10	4 \pm 1	1
p=	0,066 n.s.	0,082 n.s.	0,565 n.s.	
Group 4, m	64 \pm 35	35 \pm 20	6 \pm 3	1
p=	0,115 n.s.	0,564 n.s.	0,682 n.s.	

n.s.: not significant ($p > 0,05$); *: $0,05 \geq p > 0,01$; **: $0,01 \geq p > 0,001$; ***: $p \leq 0,001$.

Dosing Phase

Table 4 Con A stimulation of PBMC dosing phase week 39. Group means and standard deviations of Stimulation indices (SI) at indicated Con A concentrations as well as results of the unpaired two sided t test between the vehicle group and each dose group of the respective gender are given.

Group	Con A stimulation [$\mu\text{g/mL}$]			
	5,0	1,0	0,2	0,0
Group 1, f	722 \pm 271	161 \pm 123	3 \pm 3	1
Group 1, m	825 \pm 245	211 \pm 52	5 \pm 4	1
Group 2, f	835 \pm 363	168 \pm 59	2 \pm 1	1
p=	0,570 n.s.	0,922 n.s.	0,329 n.s.	
Group 2, m	886 \pm 69	198 \pm 58	5 \pm 4	1
p=	0,644 n.s.	0,700 n.s.	0,923 n.s.	
Group 3, f	894 \pm 331	253 \pm 121	3 \pm 1	1
p=	0,309 n.s.	0,185 n.s.	0,726 n.s.	
Group 3, m	1026 \pm 326	200 \pm 74	3 \pm 2	1
p=	0,218 n.s.	0,738 n.s.	0,260 n.s.	
Group 4, f	622 \pm 185	173 \pm 62	3 \pm 2	1
p=	0,438 n.s.	0,815 n.s.	0,766 n.s.	
Group 4, m	1427 \pm 390	422 \pm 178	8 \pm 5	1
p=	0,005 **	0,011 *	0,292 n.s.	

n.s.: not significant ($p > 0,05$); *: $0,05 \geq p > 0,01$; **: $0,01 \geq p > 0,001$; ***: $p \leq 0,001$.

Recovery Phase

Table 6 Con A stimulation of PBMC recovery phase week 65. Group means and standard deviations of Stimulation indices (SI) at indicated Con A concentrations as well as results of the unpaired two sided t test between the vehicle group and each dose group of the respective gender are given.

Group	Con A stimulation [µg/mL]			
	5,0	1,0	0,2	0,0
Group 1, f	910 ±287	371 ±116	10 ±4	1
Group 1, m	1050 ±301	338 ±39	8 ±7	1
Group 3, f	946 ±308	338 ±96	11 ±7	1
p=	0,890 n.s.	0,727 n.s.	0,905 n.s.	
Group 3, m	1364 ±393	201 ±241	2 ±1	1
p=	0,334 n.s.	0,386 n.s.	0,197 n.s.	
Group 4, f	954 ±493	387 ±153	23 ±19	1
p=	0,899 n.s.	0,976 n.s.	0,322 n.s.	
Group 4, m	1105 ±321	152 ±85	2 ±0	1
p=	0,841 n.s.	0,026 *	0,188 n.s.	

n.s.: not significant ($p > 0,05$); *: $0,05 \geq p > 0,01$; **: $0,01 \geq p > 0,001$; ***: $p \leq 0,001$.

T-cell dependent antibody response (TDAR)

KLH vaccinations were used to model the primary antibody response. Each animal received a single i.m. injection of 1 mg of KLH in 0.9% sodium chloride on day 183 (week 27) and for recovery animals on day 365 (week 53). Recovery animals that got the day 365 vaccination had not been previously immunized by KLH. Blood for IgG and IgM determination was obtained before and 3, 7, 14, 21, and 28 days after each vaccination. IgG and IgM quantification was conducted by ELISA assays at (b) (4). Since pre-immunization levels of anti-KLH IgM and IgG antibodies were detected before KLH immunization (baseline values), the data are normalized by subtracting the group mean levels of anti-KLH IgM and IgG antibodies (U/mL) measured before immunization from the Day 14 post-immunization levels. The normalized data was used to calculate the percent decreased anti-KLH IgM and IgG levels relative to control groups.

Anti-KLH IgM and IgG antibody responses were observed in vehicle control animals immunized with KLH during the dosing or recovery phases of the study, and for both isotypes, peak responses were generally observed on day 14 post-immunization. Mean anti-KLH IgM antibody levels (group mean day 14 post-immunization levels, including dosing and recovery), increased to 419-1407% relative to pre-immunization values. Mean anti-KLH IgG levels (day 14 values) increased to 451-1430% relative to preimmunization values.

Response during the Dosing Phase in Naive Monkeys

CP-690550 at 10 mg/kg/day, reduced by 86-97% the anti-KLH IgM (day 14 antibody measurement) and inhibited of the anti-IgG response (tested during the dosing phase) in both male and female animals compared to the vehicle control groups at the same post-immunization time point. Administration of tofacitinib at ≤ 2 mg/kg/day had no effect

on anti-KLH specific IgM and IgG production following immunization with KLH, in male and female animals.

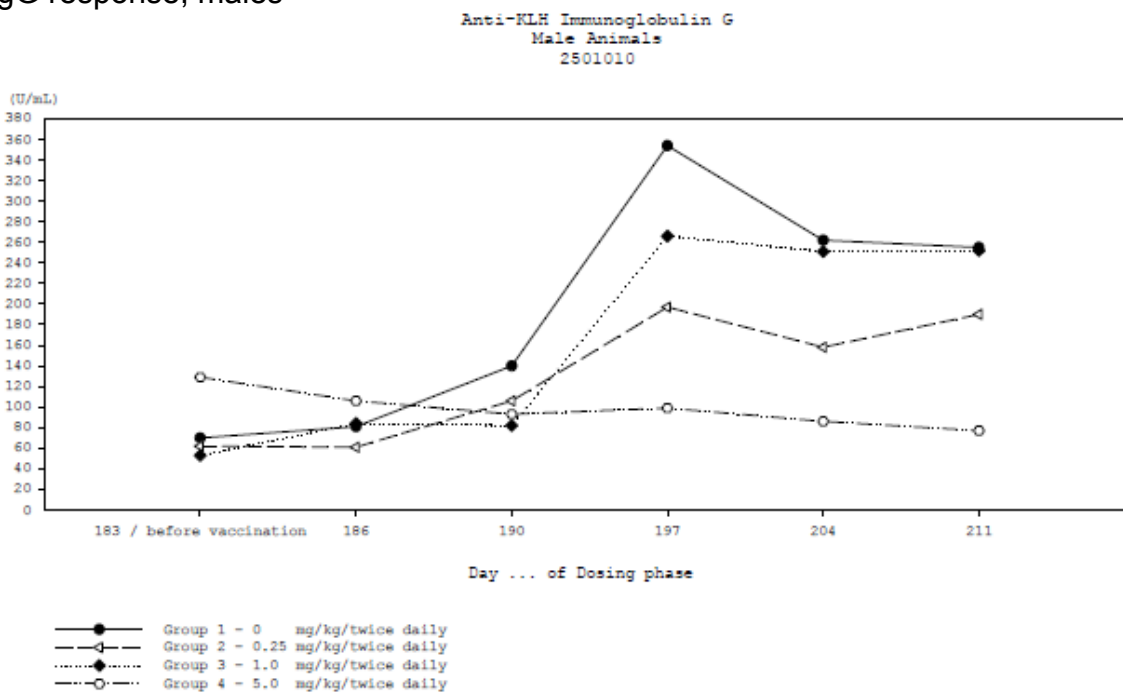
Response during the Recovery Phase in Naive Monkeys:

For both males and females in the 10 mg/kg/day dose group, the primary response to KLH (both anti-KLH IgM and IgG) was similar to that observed in vehicle control animals when evaluated during the recovery phase.

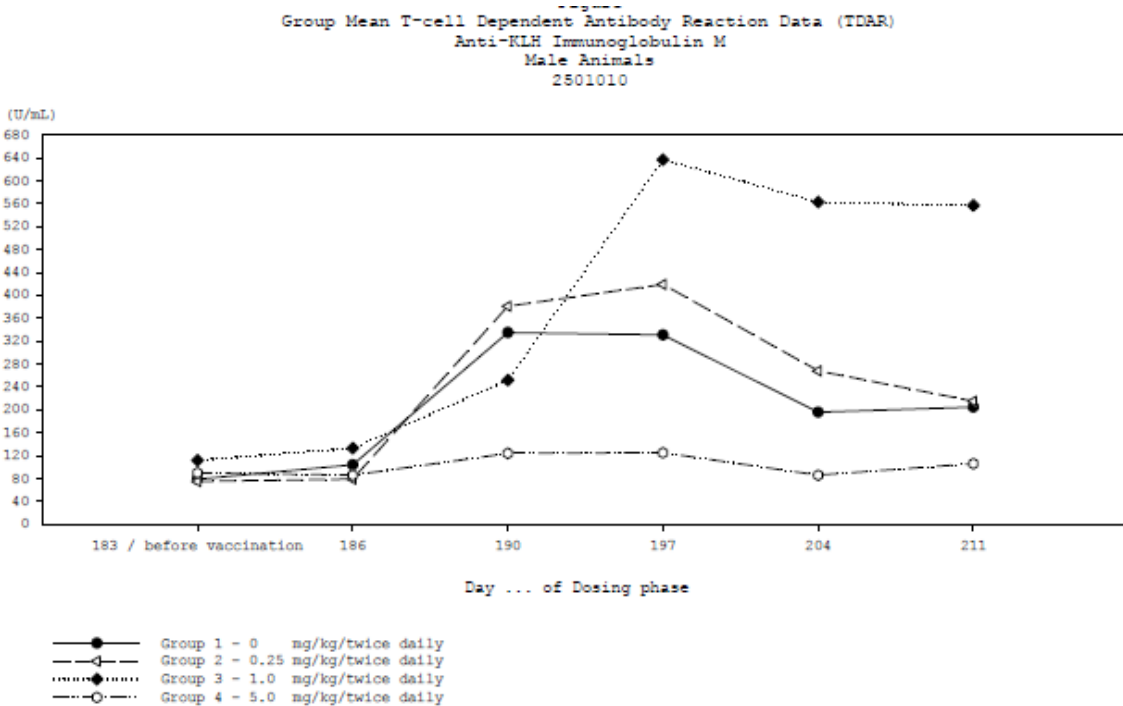
Anti- KLH IgM and IgG levels following KLH challenge in vivo

Anti-KLH isotype	Sex	Dose group (mg/kg/day)	Group mean (U/mL)		
			Pre-immunization value	Day 14 post-immunization	Day 14 minus pre-immunization
IgM	Males	0	79	331	252
		10	90	125	35
		Percent decreased relative to control group			<u>86%</u>
	Females	0	126	567	441
		10	83	97	14
		Percent decreased relative to control group			<u>97%</u>
IgG	Males	0	70	354	284
		10	129	99	-30
		Percent decreased relative to control group			<u>100%</u>
	Females	0	55	248	193
		10	99	94	-5
		Percent decreased relative to control group			<u>100%</u>

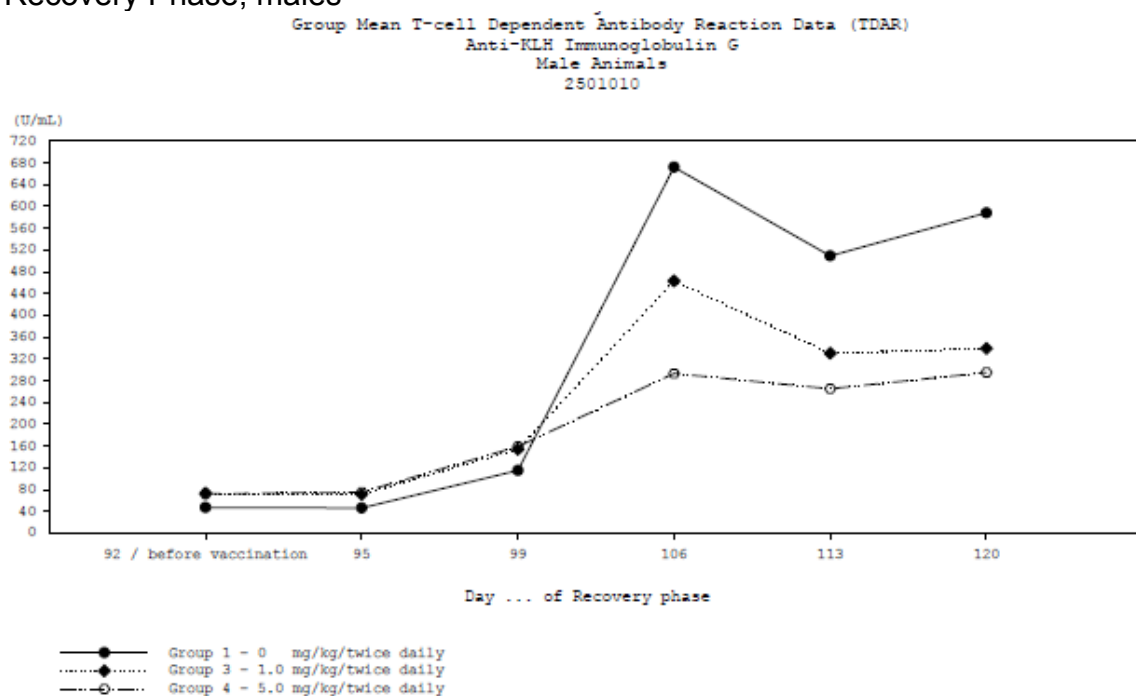
Dosing Phase IgG response, males



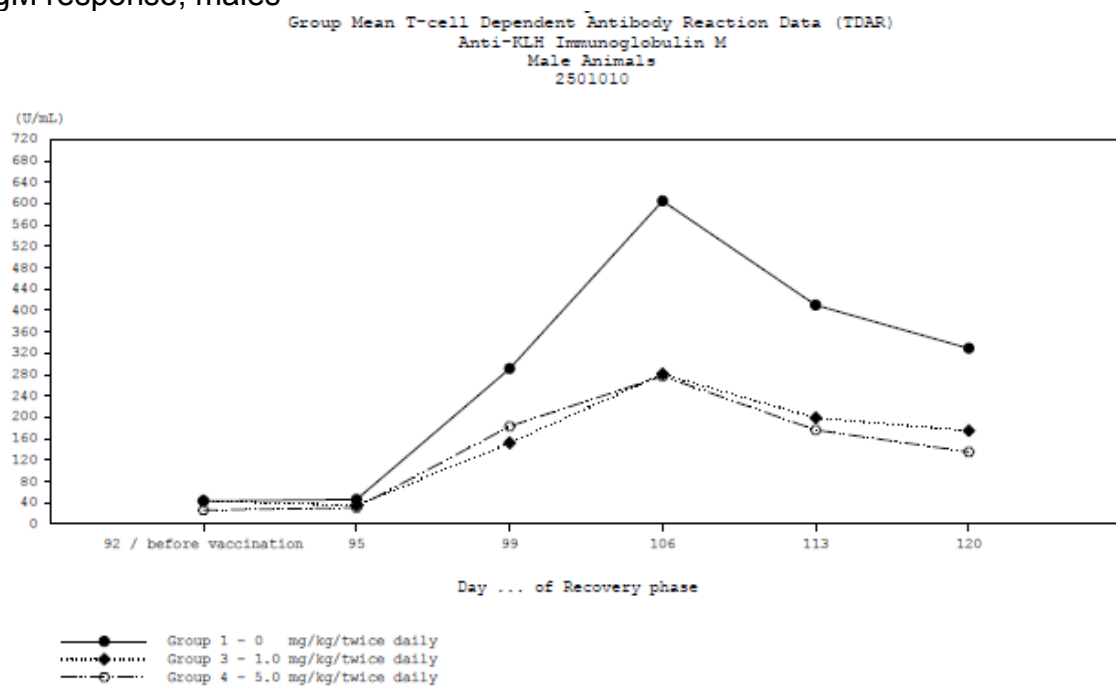
IgM response, males



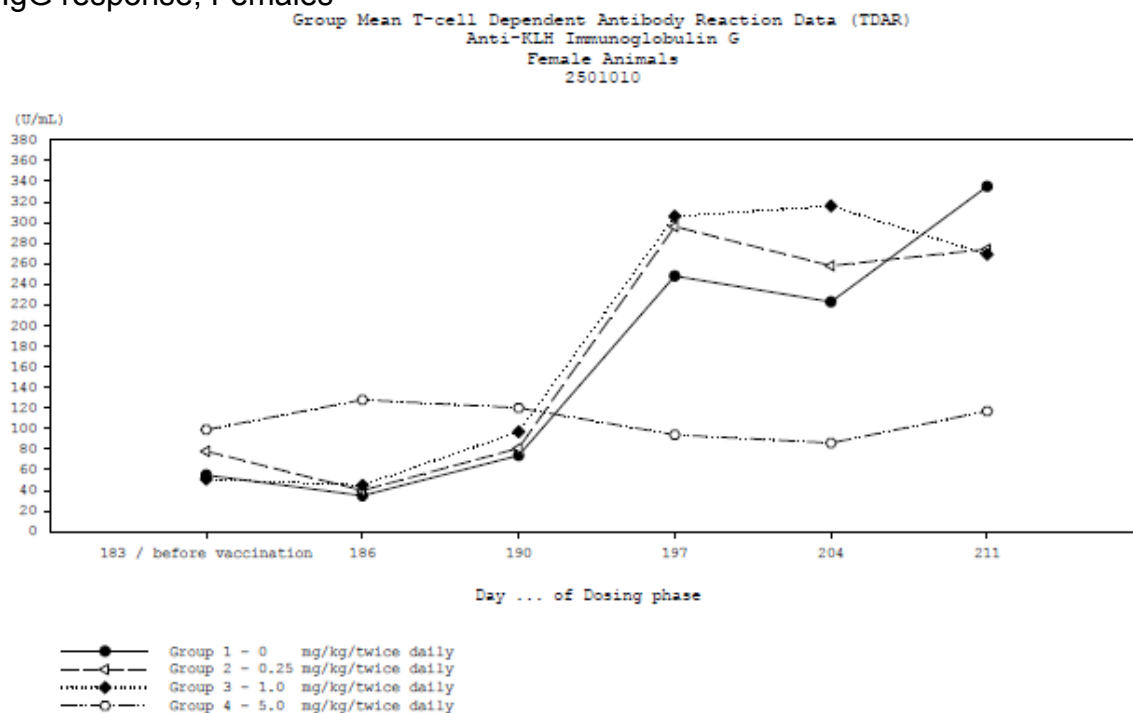
Recovery Phase, males



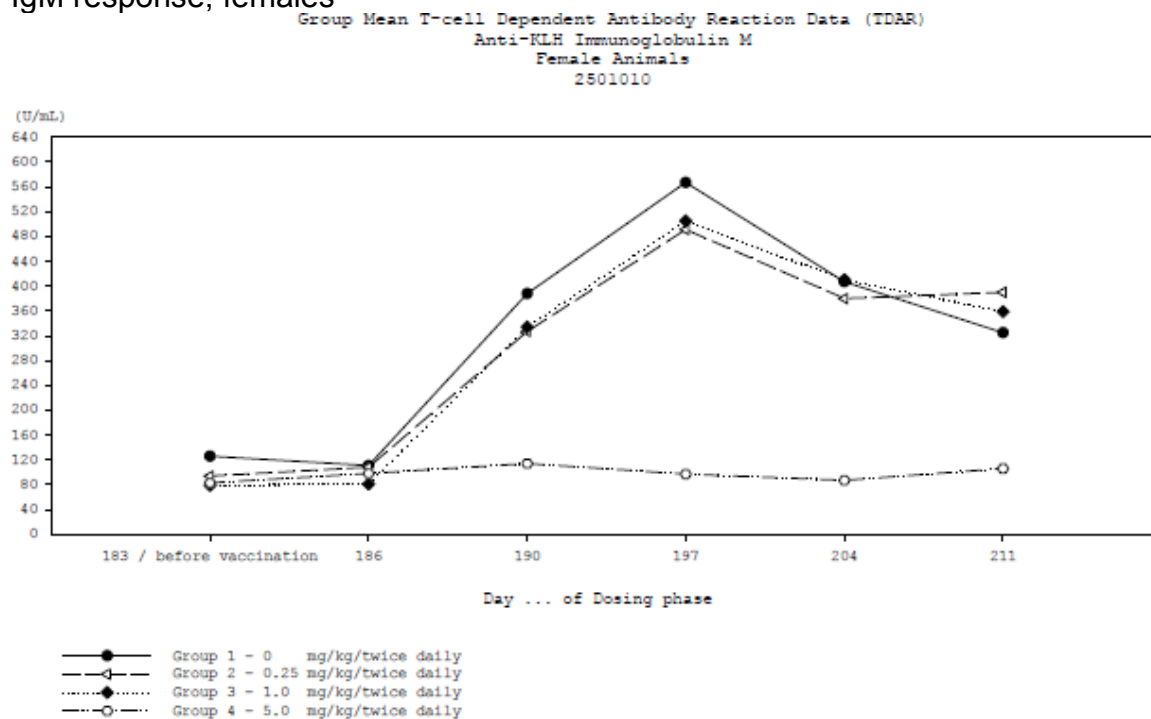
IgM response, males



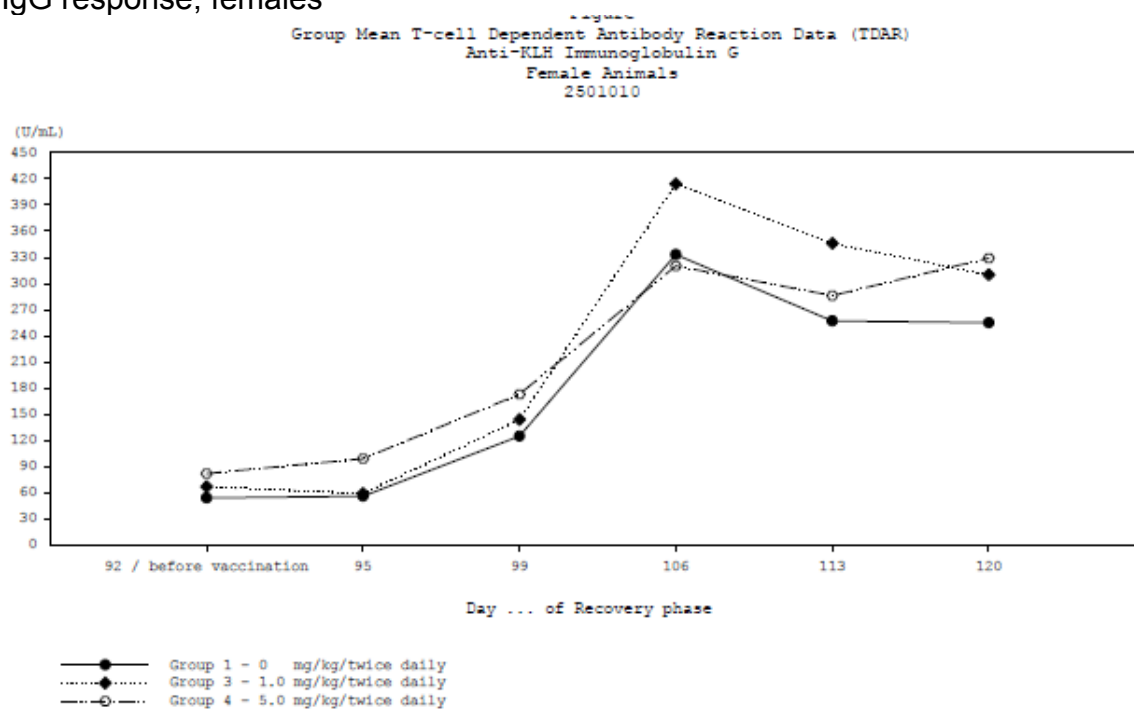
Dosing Phase IgG response, Females



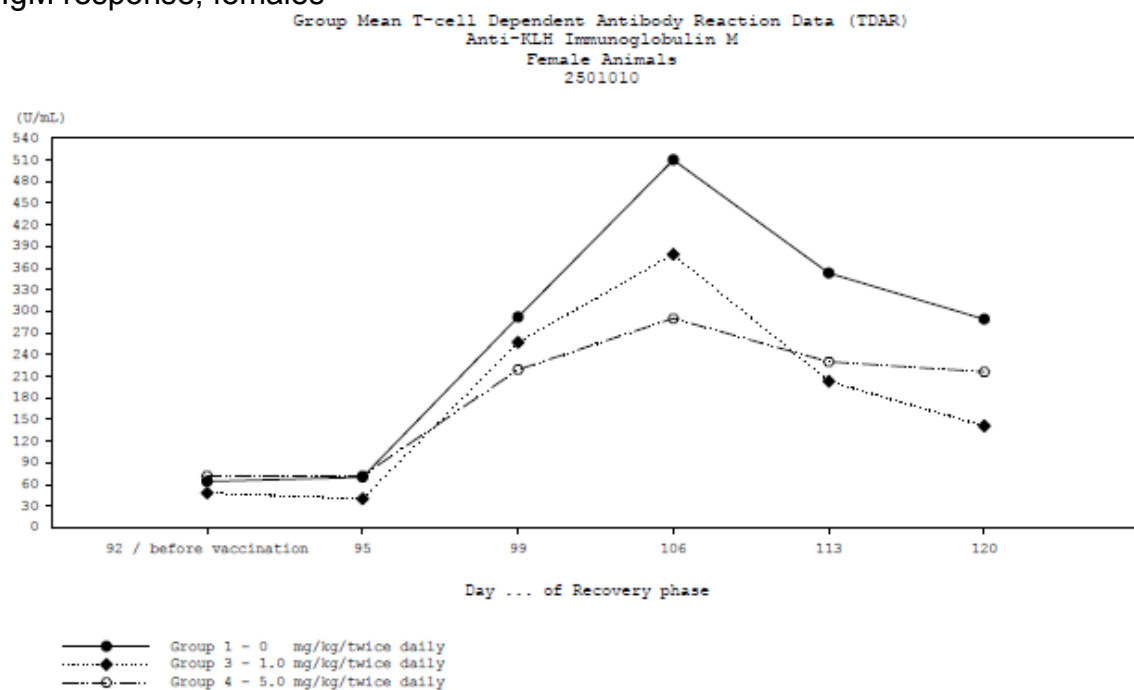
IgM response, females



Recovery Phase IgG response, females



IgM response, females



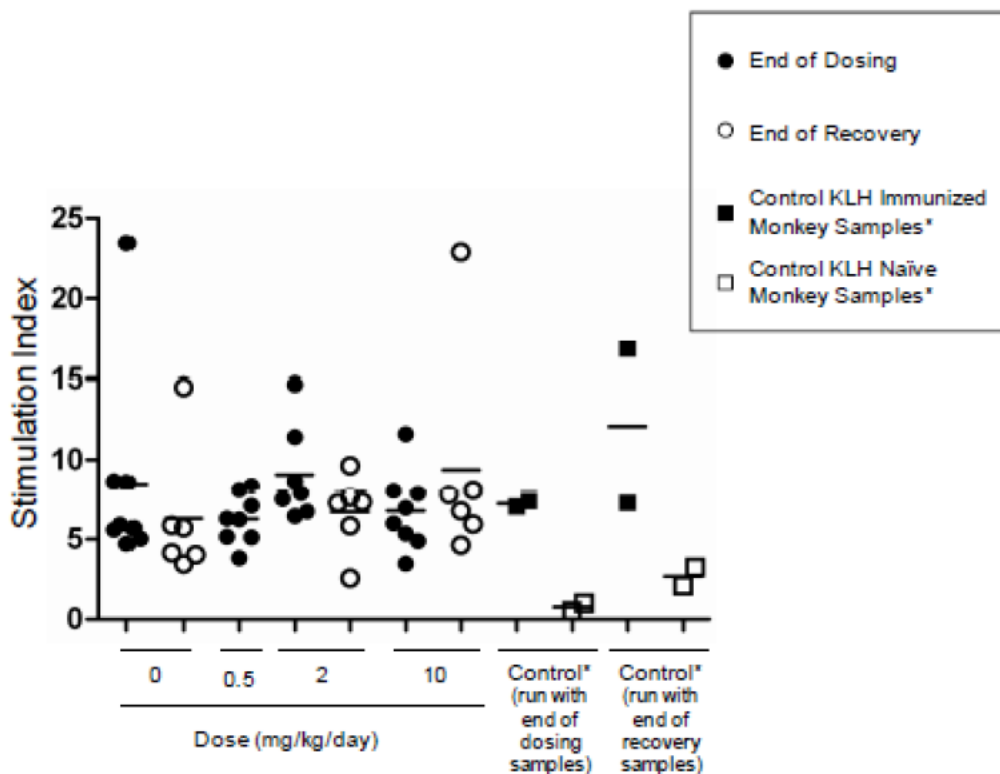
Ex-vivo KLH stimulation of peripheral blood mononuclear cells (PBMC)

The *ex vivo* proliferative response induced by stimulation of PBMCs with KLH *in vitro* evaluated the recall or secondary response since all animals tested had been immunized with KLH 3 months earlier.

At necropsy, blood was obtained from each sedated animal, and shipped to the (b) (4) facility, within 4 hours of sampling. PBMCs were isolated and cryopreserved on the day of blood collection. PBMCs were prepared for each individual animal, according to (b) (4) SOP ATM-3-0014. PBMC isolation and cryopreservation were performed as a GLP activity. PBMCs from each animal were tested for their ability to respond to the addition of exogenously added KLH *in vitro*. Since all animals were exposed to KLH three months prior to necropsy, this represented a recall or secondary immune response. PBMCs were incubated with KLH and proliferation determined by (b) (4) incorporation. The *ex-vivo* KLH stimulation assay was conducted as a non-GLP activity. The Stimulation index (SI) [OD450-540nm KLH stimulated/OD450-540nm unstimulated] was determined for both KLH immunized monkey control samples and KLH naïve monkey control samples.

CP-690550 had no effect on the response to KLH stimulation. The SI for PBMCs from KLH immunized animals ranged from 7.1-16.8 and for KLH naïve animals ranged from 0.5-3.2 and indicated that a robust secondary KLH response was observed only for PBMCs from KLH immunized animals and not for PBMCs isolated from KLH naïve animals. The mean SI in the juvenile animal vehicle control group (SI = 8.5, males and females combined) was similar to that observed for the control KLH immunized animals (SI = 7.2).

Figure 1. In Vitro KLH Stimulation of PBMCs from Juvenile Cynomolgus Monkeys Treated with Tofacitinib



* Control PBMCs obtained from KLH-immunized and KLH-naïve adult animals are from (b) (4) Study 8230337 and are described in Section 3, Test Method, of this study phase report.

Stimulation Index for In Vitro KLH Stimulation of PBMC's (results for male and female were combined in the applicants data table)

Group	1	2	3	4	KLH immunized control	KLH naive control
Dose (mg/kg/day)	0	0.5	2	10	-	-
end of dosing	8.5	6.3	9.1	6.8	7.2	0.8
end of recovery	6.3	-	6.7	9.4	12.1	2.7

Toxicokinetics

Blood samples were obtained on day 1 and in week 36 at 0 (predose), 0.5, 1, 3, 6, and 12 hours after dosing (last collection prior to next dose). Analysis by LC-MS/MS was conducted at (b) (4). The lower limit of quantification was 5 ng/mL.

Control (vehicle) group had no quantifiable concentrations of CP-690550. There were no sex-related differences in exposure. T_{max} increased with an increase in dose. Mean

T_{max} for combined males and females following administration of 0.25, 1, and 5 mg/kg CP-690550 was observed at 0.50, 0.96, and 1.0 hours on day 1, and at 0.63, 0.61, and 0.93 hours at week 36. Systemic exposure (assessed by C_{max} and AUC_{0-12}) increased with increasing dose and was approximately dose proportional, with no accumulation from day 1 to week 36.

Toxicokinetic Parameters

Dose (mg/kg/day)		0.5	2	10
(mg/kg/12 hr)		0.25	1	5
C_{max} (ng/mL)	week 1	36.8	116	531
	week 36	33.2	119	427
T_{max} (h)	week 1	0.5	0.96	1.0
	week 36	0.63	0.61	0.93
AUC_{0-12} (ng-h/mL)	week 1	36.9	209	1140
	week 36	31.1	212	1180

Stability and Homogeneity

Stability: Tofacitinib (CP-690550) was stable in 0.5% methylcellulose over a concentration range of 0.05 to 200 mg tofacitinib/mL for up to 8 days when stored at room temperature or refrigerated (b) (4) project number 894-003-01 and 1100-003).

Homogeneity and Concentration Verification: Formulations were prepared at least weekly. Standard analysis of the formulations for homogeneity and concentration verification by HPLC was performed before the beginning of the study and analysis for concentration only in weeks 6, 12, 18, 26, 30, and 36 by (b) (4). All analyzed formulations of dosing were between 96.3% and 102.9% of nominal concentration

6 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells

Study title: Genetic Toxicology Report, CP-690550-10, Microbial Reverse Mutation Assays

Study no.:	01-2063-11
Study report location:	4.2.3.3.1
Conducting laboratory and location:	Drug Safety Evaluation Department, Pfizer Global Research and Development, Pfizer Inc., Groton, CT 06340
Date of study initiation:	Mar 14, 2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CP-690550-10 (citrate salt), Lot # 43798-2- 1H, Purity 97.4% (LC)

There was no Certificate of Analysis included with this report. A description of LC evaluation was included. Purity was indicated in Table 2.6.7.4 in Module 2 as being 96.9%.

Compound Purity:	LC Analysis: 97.4% as CP-690,550-10 on an anhydrous, solvent free basis TLC Analysis: No individual impurity > 0.5% and the total amount of impurities not > 2.0%.
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Key Study Findings

- CP-690550 (citrate salt) at concentrations up to 5 mg/plate was not mutagenic in the in vitro reverse mutation assay using strains of *Salmonella typhimurium* and *Escherichia coli* with or without enhanced metabolic enzymes.

Methods

Strains:	<i>Salmonella typhimurium</i> strains, TA 1535, TA 1537, TA 98, TA 100, and <i>Escherichia coli</i> strain WP2uvrA pKM101
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Concentrations in definitive study:	Doses tested were 0.05, 0.15, 0.5, 1.5, and 5 mg per plate, incubated in the presence or absence of the metabolic activation system.
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It was not mentioned if the dose was prepared as mg of drug substance or mg of active moiety. If the former, then the doses are approximately 40%

lower than stated the study should be repeated.

Basis of concentration selection:

A preliminary toxicity study using strain TA100 was performed in the presence and absence of S9 mix in order to establish dose levels for the mutation tests.

The negative controls, positive controls, and test article were tested on duplicate plates both in the presence and absence of S9 at dose levels of 0.01, 0.05, 0.2, 1, and 5 mg per plate.

There was no toxicity to the bacteria in either the presence or absence of S9 mix, as evidenced by equivalent or higher numbers of revertant colonies on drug-treated plates than on vehicle control plates. There was no evidence of precipitation in the overlay agar at any of the concentrations tested, either in the presence or the absence of S9.

Formulation/Vehicle:

Dimethylsulfoxide (DMSO)

Negative control:

Dimethyl sulfoxide was tested at 100 µL per plate both in the presence and absence of S9 mix.

Positive control:

With metabolic activation using rat S9 and cofactors:

Control	Dose level (mg per plate)	Bacterial strain
2-aminoanthracene (2-AAN)	0.10	<i>E. coli</i> WP2 <i>uvrA</i> pKM101
2-AAN	0.005	<i>S.t.</i> TA 1535 and TA 100
2-AAN	0.01	<i>S.t.</i> TA 98 and TA 1537

Without metabolic activation:

Control	Dose level (mg per plate)	Bacterial strain
<i>N</i> -Ethyl- <i>N</i> -nitro- <i>N</i> -nitrosoguanidine	0.005	<i>E. coli</i> WP2 <i>uvrA</i> pKM101
Sodium nitrite	2.0	<i>S.t.</i> TA 1535
2-Nitrofluorene	0.005	<i>S.t.</i> TA 98
9-Aminoacridine	0.04	<i>S.t.</i> TA 1537
Nitrofurantoin	0.002	<i>S.t.</i> TA 100

Additional quality control experiments were performed during each mutation test, including the testing of each strain for resistance to ampicillin (indicating the presence of the pKM101 plasmid in TA 98 and TA 100), the exogenous histidine- and tryptophan- dependence of strains (indicating the lack of histidine biosynthetic capability in *Salmonella* and lack of tryptophan biosynthetic capability in *E. coli*), and the sensitivity of strains to UV light and crystal violet (indicating persistence of the *uvrB* and *rfa* mutations).

Incubation & sampling time:

The plate incorporation method was used. The definitive mutation test was conducted using the plate incorporation method. Triplicate plates for

positive controls, negative controls, and CP-690550 concentrations were prepared for each bacterial strain in the presence and absence of S9 mix. When the agar had set, the plates were inverted and incubated for 2 or 3 days at 37° C, observed for compound insolubility and cytotoxicity, and scored.

Stability: The stability of CP-690550 in the presence of vehicle was not assessed.

Metabolic Activation System: S9 fraction was prepared from livers obtained from Aroclor 1254-pretreated male rats (Mol Tox, Inc., Lot 1143; total protein 43.6 mg/mL). The S9 percentage was approximately 20% (v/v) in the final test medium.

Analysis: After incubation, revertant colonies were counted using an automatic colony counter or manually. Details regarding the colony counter or method of data collection for this study were not described. Plates were also examined for test article precipitation as well as cytotoxicity. A plate was scored as toxic if it had no background lawn or revertant colonies, or it has a less dense background lawn and no revertant colonies and/or less than one-half as many revertant colonies as the average revertant count of the corresponding negative control plates for that strain.

Study Validity

The study met the acceptance criteria as described below and was valid.

Criteria for Determining Test Results:

Test acceptance criteria-

- 1) For an individual plate count value to be considered acceptable, the following criteria needed to be met for each strain:
 - a. The plate was not visibly contaminated
 - b. The background lawn of cells appeared to be the same density as the negative controls
 - c. There was an even distribution of revertant colonies across the plate
 - d. The revertant count was at least one-half that of the mean for the negative controls
- 2) The results for any single concentration of test or control article in the definitive assay are considered to be acceptable if at least two of the three plates are acceptable as determined above.
- 3) For the results of a test article to be considered acceptable, all of the following criteria needed to be met:
 - a. Criteria 1 and 2 are satisfied
 - b. Data from at least 3 concentrations of the test article are acceptable
 - c. If concentration analyses are performed, the highest concentration must be within 90-
- 4) 110% of the intended dose (for concentrations of 5 mg/plate), or toxicity or limiting
- 5) insoluble compound must be present

- 6) For the results of a given strain to be considered successful, the following criteria needed to be met:
- Criterion 3 is satisfied
 - The expected response of the negative controls is within the historical range for the strain
 - The positive control article produces at least a four-fold increase over the background frequency
 - The bacteria demonstrate their typical responses in the quality control tests
 - At least one concentration of the test article produces evidence of cytotoxicity, compound insolubility, or is tested up to 5 mg/plate
 - In the case that optical density is not determined or is less than expected, cell titer is $\geq 1.0 \times 10^9$ cells/mL

Positive mutagenic response criteria-

For a significant mutagenic response to be recorded, the following criteria all needed to be met:

- For *S. typhimurium* strains, TA 98, TA 100, and *E. coli* WP2uvrA pKM101, a doubling (or more) of the mean concurrent vehicle control values at some concentration of the test item is necessary. For strains TA 1535 and TA 1537, a 3-fold increase over the control value is considered significant.
- A dose response. At high-levels, it is possible that this relationship could be inverted or obscured because of, for example, 1) toxicity to the bacteria generally, 2) specific toxicity to the mutants, and 3) inhibition of foreign compound metabolizing enzymes where mutagens require metabolic activation by the liver.
- A reproducible effect in independent tests.
- Equivocal or positive findings are confirmed in an independent confirmatory assay using the strain(s) and activation condition(s) in question.

Results

The highest CP-690550 dose tested with or without S9 metabolic enzymes was 5 mg/plate. No precipitation or cytotoxicity was observed at any dose level.

CP-690550 did not result in an increase in revertant bacteria colonies in any strain and dose which would be indicative of its mutagenic potential.

Table 82: Definitive Assay Results

Table 2

CP-690,550-10, Lot No. 43798-2-1H

Study Number: 01-2063-11

BACTERIAL MUTATION ASSAY; SUMMARY OF TEST RESULTS

DEFINITIVE PLATE INCORPORATION ASSAY

<u>Compound</u>	<u>Conc/Vol</u>	<u>S9</u>	<u>Test No</u>	NO OF REVERANT COLONIES PER PL			
				<u>WP2 uvrA</u> <u>pKM101</u>	<u>TA98</u>	<u>TA100</u>	<u>TA1538</u>
DMSO	0.10 ml/plate	No S9	3A	149 ± 26			
	0.10 ml/plate		3B		30 ± 4	138 ± 9	18 ± 1
DMSO	0.10 ml/plate	20% Induced Rat Liver S9	3A	157 ± 5			
	0.10 ml/plate		3B		34 ± 9	155 ± 11	17 ± 9
CP-690,550-10	0.050 mg/plate	No S9	3A	138 ± 20			
	0.050 mg/plate		3B		34 ± 1	154 ± 9	20 ± 8
CP-690,550-10	0.15 mg/plate	No S9	3A	143 ± 6			
	0.15 mg/plate		3B		29 ± 9	138 ± 14	21 ± 5
CP-690,550-10	0.50 mg/plate	No S9	3A	139 ± 20			
	0.50 mg/plate		3B		33 ± 7	140 ± 13	21 ± 5
CP-690,550-10	1.5 mg/plate	No S9	3A	140 ± 11			

<u>Compound</u>	<u>Conc/Vol</u>	<u>S9</u>	<u>Test No</u>	NO OF REVERANT COLONIES PER P			
				<u>WP2 uvrA</u> <u>pKM101</u>	<u>TA98</u>	<u>TA100</u>	<u>TA15</u>
CP-690,550-10	1.5 mg/plate	No S9	3B		24 ± 4	159 ± 6	20 ± 10
CP-690,550-10	5.0 mg/plate	No S9	3A	146 ± 8			
	5.0 mg/plate		3B		26 ± 7	158 ± 9	29 ± 9
CP-690,550-10	0.050 mg/plate	20% Induced Rat Liver S9	3A	159 ± 9			
	0.050 mg/plate		3B		39 ± 5	167 ± 14	22 ± 5
CP-690,550-10	0.15 mg/plate	20% Induced Rat Liver S9	3A	166 ± 5			
	0.15 mg/plate		3B		36 ± 4	154 ± 13	18 ± 2
CP-690,550-10	0.50 mg/plate	20% Induced Rat Liver S9	3A	159 ± 11			
	0.50 mg/plate		3B		40 ± 4	180 ± 12	17 ± 0
CP-690,550-10	1.5 mg/plate	20% Induced Rat Liver S9	3A	151 ± 12			
	1.5 mg/plate		3B		36 ± 9	173 ± 13	16 ± 5

<u>Compound</u>	<u>Conc/Vol</u>	<u>S9</u>	<u>Test No</u>	NO OF REVERANT COLONIES PER PLATE ± SD				
				<u>WP2 uvrA</u> <u>pKM101</u>	<u>TA98</u>	<u>TA100</u>	<u>TA1535</u>	<u>TA1537</u>
CP-690,550-10	5.0 mg/plate	20% Induced Rat Liver S9	3A	126 ± 26				
	5.0 mg/plate		3B		33 ± 9	166 ± 17	17 ± 1	11 ± 4
2-Nitrofluorene	0.0050 mg/plate	No S9	3B		1017 ± 66			
9-Aminoacridine	0.040 mg/plate	No S9	3B					61 ± 13
ENNG	0.0050 mg/plate	No S9	3A	1766 ± 42				
Nitrofurantoin	0.0020 mg/plate	No S9	3B			1300 ± 55		
Sodium Nitrite	2.0 mg/plate	No S9	3B				200 ± 15	
2-Anthramine	0.0050 mg/plate	20% Induced Rat Liver S9	3B			1479 ± 523	277 ± 12	

<u>Compound</u>	<u>Conc/Vol</u>	<u>S9</u>	<u>Test No</u>	NO OF REVERANT COLONIES PER PLATE ± SD				
				<u>WP2 uvrA</u> <u>pKM101</u>	<u>TA98</u>	<u>TA100</u>	<u>TA1535</u>	<u>TA1537</u>
2-Anthramine	0.010 mg/plate	20% Induced Rat Liver S9	3B		2617 ± 96			377 ± 14
2-Anthramine	0.10 mg/plate	20% Induced Rat Liver S9	3A	1390 ± 39				

7.2 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

Study title: Genetic Toxicology Report, CP-690550: In Vitro Cytogenic Assays

Study no.: 01-2063-10

Study report location: 4.2.3.3.1

Conducting laboratory and location: (b) (4)

Date of study initiation: March 12, 2001

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: CP-690550-10, Lot # 43798-2-1H,
Purity 98.7% (TLC) listed in the report

There was no Certificate of Analysis included with this report. A description of LC evaluation was included. Purity was also indicated in Table 2.6.7.4 in Module 2 as being 96.9%.

Key Study Findings

- CP-690,550 produced a statistically significant increase in structural chromosome aberrations in human lymphocytes in the 3-hour test in the presence of enhanced rat metabolic enzymes at doses ≥ 1700 $\mu\text{g/mL}$ that produced approximately $>48\%$ mitotic suppression (cytotoxicity). No increase in chromosome aberrations was seen in the 3-hour or 24 hour treatments without metabolic activation. There was also no increase in polyploidy.

Methods

Cell line: Human peripheral lymphocytes were obtained from healthy human volunteers (number of volunteers was not indicated) and cultured in Williams medium E containing phytohaemagglutinin (PHA, 1%).

Concentrations in definitive study:

3-hour test:

without S9: 236, 393, 960, 1200, and 3000

with S9: 403, 1540, 1920, 2400, and 3000 $\mu\text{g/mL}$

2nd test: 1600, 1700, 1800, 1900, and 2000 $\mu\text{g/mL}$

24-hour test

without S9: 25.1, 41.8, 116, 540, and 900 $\mu\text{g/mL}$

Several tests were performed in the presence of S9 mix; because of a steep dose-toxicity response, the concentration range had to be adjusted after the first

test to allow a sufficient number of evaluable concentrations. A total of four 3-hour tests with metabolic activation were performed in order to fully characterize the relationship between CP-690550-induced cytotoxicity and chromosome aberrations. The doses used in the final evaluation are indicated above.

The stability of the test article in DMSO was not reported; however, all stock solutions were prepared immediately before use. The stability of the test article in the culture medium was not reported. There was no evidence of test article insolubility in any of the treated cultures. Evidence of insolubility was only seen in the 300 mg/mL stock solution of CP-690550 prepared for the 3-hour test with metabolic activation. There were no apparent changes in pH of any of the solutions, as evidenced by the lack of a color change of the pH indicator in the dosing medium. It is not clear whether the osmolality of the solution was assessed.

Metabolic activation system: Rat liver S9 homogenate (R-610 and R-639) was purchased from (b) (4), where it had been prepared and tested. The enzymatic activity of each batch of S9 was characterized by testing its ability to catalyze the transformation of cyclophosphamide to an active mutagenic metabolite.

Basis of concentration selection:	In a preliminary experiment (Tests 1, 2, and 3), CP-690550 produced minimal to moderate mitotic suppression under all exposure conditions over a dose range of 7.8 to 500 µg/mL. Doses were adjusted in the definitive tests so as to result in 3 or more analyzable concentrations, with the highest dose showing a greater than 50% reduction in mitotic index.
Negative control:	Dimethyl sulfoxide was tested at 1% (v/v) final concentration.
Positive control:	cyclophosphamide, 10 µg/mL, used in the presence of metabolic activation S9 mix mitomycin C, 0.4 µg/mL, used in the absence of metabolic activation S9 mix
Formulation/Vehicle:	The test article was dissolved in dimethylsulfoxide (DMSO).
Incubation & sampling time:	Exposure conditions: Tests were conducted both in the presence and absence of S9 mix. Treatments with

test articles or vehicle control were performed on duplicate cell cultures. In tests without S9 mix, cultures were exposed to test article or control for 3 hours followed by a 21-hour recovery period in fresh culture medium, or for 24 hours continuously. In tests with metabolic activation, cultures were exposed for 3 hours to either test or control article in serum free medium in the presence of the S9 mixture followed by a 21-hour recovery in complete medium. Approximately 3 hours prior to the end of the treatment and recovery periods, Colcemid® was added to each culture at a final concentration of 0.075 µg/mL.

Harvesting of cultures and slide preparation: At the end of the treatment period, the cells were collected by centrifugation, treated with a hypotonic solution of 1% sodium citrate, then fixed with 2-3 changes of methanol: acetic acid. Cell preparations were stained and coverslips mounted.

Analysis: The mitotic index was first determined by systematically scoring 1000 consecutive nuclei for the presence of metaphase figures. At least three concentration levels, where possible, were selected for assessment of chromosomal aberrations. Two hundred metaphase figures from each test concentration were analyzed for chromosome aberrations (100 metaphase cells per culture; 50 cells per culture if aberrations were numerous) where possible.

Study Validity

The study was valid, meeting the required criteria listed below.

Criteria for determining test results:

Test acceptance criteria

For a particular experiment to be considered successful, the following criteria need to be met:

- 1) The test should produce a minimum of three analyzable test article concentrations (*i.e.* sufficient metaphase cells present for analysis), the highest of which should meet one of the following criteria:
 - a. Produces approximately a 50% reduction of the mitotic index relative to the negative controls
 - b. Shows evidence of incomplete solubility
 - c. Has “technical limitations”
 - d. Is tested at 5000 µg/mLIf none of these criteria are met, and there is evidence of a steep cytotoxicity response, then the highest concentration for analysis is selected as the

- highest level that does not express excessive ($\geq 75\%$ reduction) mitotic inhibition, but is within a 1.25-fold factor of the cytotoxic concentration.
- 2) The number of test article concentrations is sufficient to demonstrate a positive or negative response and/or reproducibility of the induction of chromosome damage at one or more concentrations.
 - 3) The sample size is sufficient to demonstrate statistical significance of a positive response.

Clastogenicity criteria

- 1) A positive clastogenic response is generally indicated by a statistically significant dose-related increase in aberrant cells in the treated groups relative to the concurrent negative control. These increases should be reproducible between replicate cultures and between independent tests.
- 2) An equivocal response is generally indicated by a statistically significant increase in aberrant cells at one concentration of the treated groups, typically the highest concentration evaluated.
- 3) A test is considered negative if no statistically significant increase in the number of aberrant cells is observed at any dose level of the treated groups relative to the concurrent negative control.

Results

Dose concentration analysis indicated the doses were appropriately prepared, 103%-104% of the expected concentration.

In the 3 hour test without metabolic activation, the high dose of 3000 $\mu\text{g/mL}$ CP-690550 resulted in toxicity, thus the doses of 393, 960 and 1200 $\mu\text{g/mL}$ were evaluated. There was no significant increase in chromosomal aberrations. In the 3-hour test with metabolic activation, there was difficulty obtaining a non-toxic dose that also met the criteria of mitotic index suppression of at least 50%. The dose range was narrowed to account for the apparent steep dose-toxicity curve (1730 $\mu\text{g/mL}$ produced 57% mitotic suppression and 1540 $\mu\text{g/mL}$ produced 22% mitotic suppression). Doses of 403, 1540, 1920 and 2400 $\mu\text{g/mL}$ producing 0 to 66% reduction in the mitotic index resulted in no effect up to 1920 $\mu\text{g/mL}$, and an increase in abnormal cells (8.0%) at 2400 $\mu\text{g/mL}$ compared to DMSO control. Another assay with doses of 1700, 1800 and 1900 $\mu\text{g/mL}$ resulted in chromosomal aberrations of 10.0, 14.0, and 8.5%, respectively.

In the 24 hours test without metabolic activation, doses of 41.8, 116, and 540 $\mu\text{g/mL}$ producing 8 to 49% mitotic suppression ($\geq 900 \mu\text{g/mL}$ was cytotoxic) resulted in similar incidences of chromosomal aberration as the DMSO (negative) control.

The chromosomal assay was positive for clastogenicity only in the presence of enhance preparation of metabolic enzymes at near the dose corresponding to 50% mitotic suppression, a criteria, actually 48%.

Table 83: Chromosomal Aberration, 3-Hour Test Without Metabolic Activation

3 HOUR TREATMENT
WITHOUT METABOLIC ACTIVATION

Treatment ^a	Mean (%) Mitotic Suppression ^b	Total Cells Analyzed	Mean (%) Abnormal Cells ^c	p- Value ^d	Mean (%) Polyploid Cells ^e
<u>Negative Control:</u> DMSO					
1.00%	0	200	0.0	--	0.1
<u>Test Article:</u> CP-690,550-10 [^] (µg/ml)					
236*	--	--	--	--	--
393	0	200	0.5	0.500	0.7
960	44	200	0.0	>0.500	0.8
1200	52	200	0.5	0.500	0.9
3000**	T	--	--	--	--
<u>Positive Control:</u> Mitomycin-C					
0.400	24	100	30.0	<0.001	--

a: Two replicate cultures evaluated for each treatment group; when possible 100 cells per culture are analyzed for chromosome damage.

b: Mean (%) Mitotic Suppression = [One minus the quotient (Mean Test Article Mitotic Index / Mean Negative Control Mitotic Index)] (x) 100.

c: Mean (%) Abnormal Cells = Sum of % Abnormal Cells for duplicate cultures / 2.

d: Data analyzed by 1-tailed Fisher's Exact test for increases in abnormal cells compared to the negative control values.

e: Mean (%) Polyploid = Sum of % Polyploidy for duplicate cultures / 2.

Abbreviations:

--: Dashes indicate data not available or determined.

*: Cultures treated with test article concentrations ≤ 236 µg/ml were not evaluated.

**: Cultures treated with test article concentration 3000 µg/ml were not evaluated due to toxicity.

T: Toxic (≥ 75 % mitotic suppression by visual evaluation).

^: Precipitate present in 100X stock preparation of 300 mg/ml.

Table 84: Chromosomal Aberration, 3-hour test with Metabolic Activation

3 HOUR TREATMENT WITH METABOLIC ACTIVATION					
Treatment ^a	Mean (%) Mitotic Suppression ^b	Total Cells Analyzed	Mean (%) Abnormal Cells ^c	p- Value ^d	Mean (%) Polyploid Cells ^e
<u>Negative Control: DMSO</u>					
1.00%	0	200	0.5	--	0.2
<u>Test Article: CP-690,550-10 (µg/ml)</u>					
1600*	17	--	--	--	--
1700	48	200	10.0	<0.001	2.1
1800	54	200	14.0	<0.001	2.7
1900	64	200	8.5	<0.001	1.6
2000**	--	--	--	--	--
<u>Positive Control: Cyclophosphamide</u>					
10.0	0	200	6.0	0.001	--

a: Two replicate cultures evaluated for each treatment group; when possible 100 cells per culture are analyzed for chromosome damage.

b: Mean (%) Mitotic Suppression = [One minus the quotient (Mean Test Article Mitotic Index / Mean Negative Control Mitotic Index)] (x) 100.

c: Mean (%) Abnormal Cells = Sum of % Abnormal Cells for duplicate cultures / 2.

d: Data analyzed by 1-tailed Fisher's Exact test for increases in abnormal cells compared to the negative control values.

e: Mean (%) Polyploid = Sum of % Polyploidy for duplicate cultures / 2.

Abbreviations:

--: Dashes indicate data not available or determined.

*: Cultures treated with test concentrations ≤ 1600 µg/ml were not evaluated.

**: Cultures treated with test concentrations ≥ 2000 µg/ml were not evaluated since sufficient mitotic suppression was achieved at lower concentrations.

Table 85: Chromosomal Aberration, 24 Hour Test Without Metaboic Activation

HUMAN LYMPHOCYTE ABERRATION ASSAY: SUMMARY OF TEST

24 HOUR TREATMENT
WITHOUT METABOLIC ACTIVATION

Treatment ^a	Mean (%) Mitotic Suppression ^b	Total Cells Analyzed	Mean (%) Abnormal Cells ^c	p- Value ^d	Mean (%) Polyploid Cells ^e
<u>Negative Control: DMSO</u>					
1.00%	0	200	2.5	--	0.2
<u>Test Article: CP-690,550-10</u> (µg/ml)					
25.1*	--	--	--	--	--
41.8	8	200	2.0	>0.500	0.4
116	21	200	1.0	>0.500	0.4
540	49	200	3.0	0.500	3.5
900**	T	--	--	--	--
<u>Positive Control: Mitomycin-C</u>					
0.050	16	100	28.0	<0.001	--

a: Two replicate cultures evaluated for each treatment group; when possible 100 cells per culture are analyzed for chromosome damage.

b: Mean (%) Mitotic Suppression = [One minus the quotient (Mean Test Article Mitotic Index / Mean Negative Control Mitotic Index)] (x) 100.

c: Mean (%) Abnormal Cells = Sum of % Abnormal Cells for duplicate cultures / 2.

d: Data analyzed by 1-tailed Fisher's Exact test for increases in abnormal cells compared to the negative control values.

e: Mean (%) Polyploid = Sum of % Polyploidy for duplicate cultures / 2.

Abbreviations:

--: Dashes indicate data not available or determined.

*: Cultures treated with test concentrations ≤ 25.1 µg/ml were not evaluated.

**: Cultures treated with test concentrations ≥ 900 µg/ml were not evaluated due to toxicity.

T: Toxic (≥ 75 % mitotic suppression by visual evaluation).

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

Study title: *In Vivo* Rodent Micronucleus, Oral Route in Rats: CP-690550

Study no.:	01-2063-12
Study report location:	4.2.3.3.2
Conducting laboratory and location:	Drug Safety Evaluation Department, Pfizer Global, Research and Development, Pfizer Inc., Groton, CT 06340
Date of study initiation:	April 11, 2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CP-690550-10, Lot # 43798-2-1H, Purity 98.1% (HPLC, TLC), Composition: 60% active moiety/potency

There was no Certificate of Analysis included with this report. Purity was also indicated in Table 2.6.7.4 in Module 2 as being 96.9%.

Key Study Findings

- CP-690550 tested negatively for the induction of micronuclei in immature erythrocytes of CD rats after oral administration of up to an MTD dose, 250 mg/kg.

Methods

Doses in definitive study:	0, 62.5, 125, 250 mg/kg
Frequency of dosing:	Administered mg/kg dose was based on the mg of active moiety of the drug substance. once daily for 3 days
Route of administration:	orally by gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% methylcellulose.
Species/Strain:	CD [strain CRL:CD(SD)IGSBR] rats
Number/Sex/Group:	6 rats/sex/dose, but only the first 5/sex sufficiently dosed surviving rats in each group were used for micronucleus evaluation.
Satellite groups:	Toxicokinetics group of 250 mg/kg, 5 rats/sex, Blood was obtained from 3/sex/timepoint after the third dose (Day 3) at 0.5, 1, 2, 4, 8, and 24 hours after dosing.
Basis of dose selection:	The highest dose of 250 mg/kg was selected as the MTD based on data from the acute dose rat

Negative control: study (Study 01-2063-07) in which all rats died at 1000 mg/kg and 1 of 6 died at 500 mg/kg.
Positive control: 0.5% methylcellulose
Mitomycin C, 1.25 mg/kg, intraperitoneally administered at 1.25 mg/mL and a dose volume of 10 mL/kg for three days

Micronucleus evaluation: Approximately 24 hours after the third treatment, 5 surviving rats in each group were euthanized and femur bone marrow was obtained. Two smears were made for each animal, fixed in absolute methanol, and stained with acridine orange. Slides were scored blindly and independent of treatment group order, under fluorescence optics using an oil immersion objective $\geq 63\times$. For each preparation, 2000 PCE were scored for the presence of micronuclei and the proportion of PCE to NCE in 1000 erythrocytes was determined as an index of target organ toxicity.

Study Validity

The study met the criteria specified for a valid test described below and was a valid assessment for CP-690550-related micronuclei formation.

Positive mutagenic response criteria: A positive response is defined as a statistically significant, dose related, and reproducible elevation in the number of micronucleated PCE (MNPCE) in the treated animals relative to the negative controls.

Test acceptance criteria:

- 1) Data should be available for at least 3 different dose levels in both sexes with at least 5 rats in each dose group. A minimum of 3 surviving animals in a dose group may be considered acceptable by the Study Director on a study-by-study basis.
- 2) The highest dose should be at or near the estimated maximum tolerated dose (MTD) for that species or the maximum practical dose (MPD). The MPD is defined as 2000 mg/kg for relatively nontoxic compounds or as the highest dose that can be administered due to the limits of compound solubility, vehicle toleration, or dose volume considerations.
- 3) The % MNPCE observed in control animals in response to negative and positive controls should be within the expected range for animals of similar strain, sex, age, and treatment regimen for this laboratory as suggested by the historical negative and positive control data.

Results

The doses were appropriately made ranging from 95 to 101% of the expected concentrations. No mortality or adverse clinical signs attributed to CP-690550 treatment occurred over the 3 days of treatment. There was a statistically significant reduction in body weight gain of male (-24.5%) but not female animals dosed at 250 mg/kg. The toxicokinetic results for the 250 mg/kg dose are presented below.

The negative and positive controls produced their expected responses. Polychromatic erythrocytes in the bone marrow were not increased and there was no CP-690550-related increase in micronucleated polychromatic erythrocytes at any dose.

Table 86: Micronucleus Assay, CP-690550 Concentration in Rats

Gender	T _{max} (h)	C _{max} (ng/mL)	AUC _{0-Tlast} (ng·h/mL)
M	4	7700	47500
F	2	9850	84600
M+F	2	8480	51700

Table 87: Summary of the In Vivo Micronucleus Assay**RAT MICRONUCLEUS ASSAY: SUMMARY OF TEST RESULTS**

Oral Route MALES				
COMPOUND	ANIMAL #	% Wgt gain ^a	% PCE ^b	% MNPCE ^c
0.5% Methylcellulose	1	21.4	83.8	0.20
	2	14.5	85.8	0.10
	3	18.3	84.1	0.10
	4	14.9	91.3	0.00
	5	16.5	89.3	0.10
	Mean	17.1	86.9	0.10
	(SD) ±	2.8	3.3	0.07
Mitomycin C 1.25 mg/kg	7	-4.5	70.3	2.10
	8	5.2	77.5	2.40
	9	8.2	67.7	2.25
	10	3.4	79.3	3.50
	11	-2.6	66.3	5.85
	Mean	1.9	72.2	3.22
	(SD) ±	5.3	5.9	1.57
CP-690,550-10 62.5 mg/kg	13	17.0	91.9	0.40
	14	15.9	88.0	0.30
	15	13.5	86.6	0.05
	16	15.4	84.7	0.20
	17	18.3	83.7	0.30
	Mean	16.0	87.0	0.25
	(SD) ±	1.8	3.2	0.13
CP-690,550-10 125 mg/kg	19	16.5	70.8	0.20
	20	17.2	84.4	0.25
	21	17.0	80.3	0.15
	22	15.2	75.4	0.15
	23	16.8	89.8	0.30
	Mean	16.5	80.1	0.21
	(SD) ±	0.8	7.4	0.07
CP-690,550-10 250 mg/kg	26	15.0	85.3	0.15
	27	10.2	78.8	0.20
	28	13.0	82.0	0.15
	29	14.4	76.7	0.20
	30	12.2	75.7	0.20
	Mean	12.9	79.7	0.18
	(SD) ±	1.9	4.0	0.03

a. Statistical analysis of % body weight gain; p=0.0042

Statistically significant decrease in % body weight gain

b. Statistical analysis of % PCE; p=0.0129

Statistically significant decrease in % PCE

c. Statistical analysis of % MNPCE; p=0.1927

Day 1 = 4/24/01

Protocol No.: 048

Study No.: 01-2063-12

RAT MICRONUCLEUS ASSAY: SUMMARY OF TEST RESULTS

Oral Route FEMALES				
COMPOUND	ANIMAL #	% Wgt gain ^a	% PCE ^b	% MNPCE ^c
0.5% Methylcellulose	37	7.6	62.8	0.15
	38	1.1	81.3	0.05
	39	9.1	83.4	0.40
	40	-1.6	69.7	0.10
	41	9.4	82.6	0.15
	Mean	5.1	76.0	0.17
	(SD) ±	5.0	9.2	0.14
1.25 mg/kg Mitomycin C	43	-3.5	67.6	2.20
	44	-11.6	35.3	3.40
	45	-2.2	61.5	1.85
	46	-9.5	58.5	1.05
	47	-6.2	51.3	2.55
	Mean	-6.6	54.8	2.21
	(SD) ±	4.0	12.4	0.87
62.5 mg/kg CP-690,550-10	49	11.1	69.0	0.25
	50	7.0	62.5	0.25
	51	11.2	77.6	0.15
	52	11.3	81.4	0.05
	53	9.3	90.8	0.15
	Mean	10.0	76.3	0.17
	(SD) ±	1.9	11.0	0.08
125 mg/kg CP-690,550-10	55	11.5	79.6	0.30
	56	6.9	88.6	0.25
	57	11.5	86.7	0.20
	58	6.8	89.2	0.35
	59	5.9	73.7	0.05
	Mean	8.5	83.6	0.23
	(SD) ±	2.7	6.7	0.12
250 mg/kg CP-690,550-10	61	12.3	67.6	0.35
	62	9.0	76.0	0.10
	63	9.4	75.2	0.25
	64	5.4	67.4	0.20
	65	7.9	76.9	0.10
	Mean	8.8	72.6	0.20
	(SD) ±	2.5	4.7	0.11

a. Statistical analysis of % body weight gain; p=0.2224

b. Statistical analysis of % PCE; p=0.6229

c. Statistical analysis of % MNPCE; p=0.5355

Day 1 = 4/24/01

Protocol No.: 048

Study No.: 01-2063-12

7.4 Other Genetic Toxicity Studies

Study title: Mammalian mutation assays (forward mutation assay, HGPRT locus)

Study no.:	01-2063-16
Study report location:	4.2.3.3.1
Conducting laboratory and location:	Drug Safety Evaluation Department, Pfizer Global Research and Development, Groton, CT 06340
Date of study initiation:	September 26, 2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CP-690550-10, Lot # 43798-2-1H, Purity = 97.4% (LC) Composition: 60% active moiety/potency 38.1% citrate counterion

There was no Certificate of Analysis included with this report. Purity was also indicated in Table 2.6.7.4 in Module 2 as being 96.9%.

Key Study Findings

- CP-690550-10 did not induce forward mutations at the X-linked hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus in Chinese hamster ovary cells when tested up to cytotoxic levels 3000 µg/mL with or without the presence of metabolic enzymes

Methods

Strains:	Chinese hamster ovary cells (CHO-K1-BH4 subclone, karyotypically stable with a low spontaneous mutant frequency at the HGPRT locus)
Concentrations in definitive study:	with metabolic activation: 600 to 1100 µg/mL without metabolic activation: 1300 to 3400 µg/mL
Basis of concentration selection:	Preliminary cytotoxicity assays conducted with and without metabolic activation with concentrations of CP-690550-10 ranging from 16 to 5000 µg/mL. Substantial cytotoxicity (<50% relative survival) was observed in the tests conducted with metabolic activation at concentrations ≥ 1000 µg/mL and in the tests conducted without metabolic activation at the highest concentration of 5000 µg/mL.
Negative control:	DMSO at 1%
Positive control:	with metabolic activation: 3-methylcholanthrene (3-MCA) at 4 µg/mL

Formulation/Vehicle:
Incubation & sampling
time

without metabolic activation:

ethylmethanesulfonate (EMS) at 400 µg/mL

DMSO at 1%

- Cultured cells were exposed to CP-690550 at appropriate doses or control compounds for 5 hours in culture medium with reduced serum content, with and without S9.
- Cells were subsequently harvested, washed, and replated for 7 days to allow colony development and cytotoxicity measurements. An additional 10^6 cells per culture were plated after treatment with CP-690550 in hypoxanthine-free medium for 7 days to allow the expression of induced mutations.
- Mutant selection was performed on day 7 in the presence of 6-thioguanine (heritable loss of HGPRT activity through structural gene mutations at the *hgp* locus confers cellular resistance to the cytotoxicity of 6-thioguanine).
- Mutants per 10^6 survivors are calculated by dividing the total number of 6-TG-resistant colonies by the number of cells plated (normally 10^6), while correcting for the absolute cloning efficiency (ACE) of each culture determined at the time of selection.

The assay treatment conditions consist of duplicate solvent control cultures, duplicate positive control cultures and at least 6 to 9 different test article dose levels using 2 (duplicate) cultures per dose level.

Study Validity

The study was judged to be valid.

The applicant provided the following points they used to assess study validity and evaluation:

Dose Selection

- The highest test level for the preliminary range finding assay should extend to either:
 - the limits of solubility,
 - the highest concentration that can be obtained due to technical limitations, or
 - a maximum of 5 mg/ml (final concentration) if solubility limits are not reached first.

- An additional 5-8 subsequent concentrations are selected for testing.
- The lowest concentration selected should not reduce either the relative cell survival or cloning efficiency by more than 30%.
- Cytotoxicity is defined on the basis of the following factors assessed on Day 2 or 3 of the preliminary cytotoxicity assay: % relative cell survival, substantial cell lysis, and/or altered morphological appearance under an inverted microscope.
- For noncytotoxic compounds that precipitate during the preliminary range finding assay, the highest concentration should be the lowest dose level that precipitates in medium.
- When possible, the highest concentration chosen for the definitive mutation assays should produce an 80-90% reduction in either of the following:
 - Day 0 and/or Day 2-3 mean relative cell counts (% survival); or
 - Day 0 average relative cloning efficiency.

Controls

- The background mutant frequency (mean of the negative controls) is calculated separately for S9 activation and nonactivation assays, even though the same population of cells may be used for concurrent assays, and is generally 0 to 20×10^{-6} .
 - The acceptability of assays with mean backgrounds greater than 20×10^{-6} will be determined by the Study Director on a case-by-case basis.
- At least one positive control is included with each assay to demonstrate the validity and sensitivity of the test system (one for the direct assay, one for the assay with metabolic activation) in detecting mutagenic activity.
 - A test is valid if at least one replicate of each positive control per test produces an acceptable positive response (Arlett et al., 1989).
 - An appropriate positive control response is defined as a mutation frequency that equals or exceeds the lower 95% confidence limit for the historical positive controls for each control tested.

Cloning Efficiency

- The average cloning efficiency (ACE) of negative controls should be between 70% and 120%.
 - All assays with mean negative control cloning efficiencies below 50% are unacceptable.
 - A value greater than 100% is possible because of minor experimental errors in cell counts (usually + 10%) and dilutions during cloning.
 - Assay variables can lead to artificially low cloning efficiencies in the range of 50% to 70% and still yield internally consistent and valid results. Assays with cloning efficiencies in this range are conditionally acceptable
- If the test article appears to have either negligible or equivocal mutagenic activity, the assay is not considered acceptable unless at least one concentration of test article either
 - 1) reduces the relative Day 0 or Day 2-3 cell survival and/or colony survival at Day 0 to approximately 10-20% of the control values;
 - 2) reaches a maximum applied concentration of 5 mg/ml when tested analytically; or

- 3) produces evidence of compound insolubility in the treatment medium.
- Mutant frequencies are calculated from a minimum of 3 mutant selection dishes and 2 cloning efficiency dishes, normally this is calculated from sets of five dishes for mutant colony count and at least three dishes for viable colony count. The acceptability of the reduction in numbers of dishes is to allow for contamination losses.
 - Mutant frequencies for five concentrations of treated cultures are normally determined in each assay, a minimum of three analyzed cultures is considered necessary under the most favorable test conditions in order to accept a single assay for evaluation of the test article.

Criteria for a Negative Response

Evaluated as nonmutagenic if:

1. the test met criteria for assay acceptability,
2. there is no substantial increase in mutant frequency ($MF < 20$ mutants per 10^6 survivors), and
3. there is no evidence of a linear dose-related increase.

Criteria for a Positive Response

Evaluated as mutagenic if:

- At least one concentration should produce a mean mutant frequency > 20 mutants per 10^6 survivors with a statistically significant dose-related trend.
- Other factors are also considered may include, but are not limited to the following:
 - Reproducibility: Concordance between replicate cultures, while a spurious increase in only one replicate at a single concentration is not considered to be sufficient evidence of a positive response.
 - Repeatability: The biological significance of a weak increase in mutant frequency may be further evaluated by a repeat test for reproducibility over a similar concentration range or over a different range as needed to achieve an acceptable cytotoxicity response curve. If an acceptable repeat assay does not confirm an earlier, equivocal response, the test article will be considered to be nonmutagenic in this test system.

Results

The positive and negative controls produced the expected mutation responses and these were within the acceptable limits of the historical control range. Cytotoxicity was observed at concentrations of 3000 and 3400 $\mu\text{g/mL}$ with average relative cell survivals at day 2 of 43% and 4%, respectively.

In the assay without metabolic activation, cytotoxicity resulted in a mean relative cloning efficiency of less than 20% at the highest concentration tested 3400 $\mu\text{g/mL}$. Doses above 950 $\mu\text{g/mL}$ were therefore excluded by the Reviewer since the criteria for validity included the requirement that relative cell survival be $>30\%$. However, this did not affect

the overall interpretation of the study. The mean mutation frequency was 2 per 10^6 survivors, greater than 0.5 of the negative control, but since the historical negative control ranged to 5 ± 6 , the assay was judged negative.

In the assay with metabolic activation, micronuclei frequency was variable at doses above 900 $\mu\text{g/mL}$, ranging from 0.5 to 5.0, up to 2-fold above the negative control value (2.5), but there was no dose dependence in this response. Cytotoxicity was also high, >50%. For these reasons the assay with metabolic activation was judged negative. Since the criteria for validity included the requirement that relative cell survival be >30%, the inclusion of doses above 950 $\mu\text{g/mL}$ without metabolic activation were excluded by the Reviewer for the study conclusions, in contrast to the applicants presentation. However, this did not affect the overall interpretation of the study.

Table 88: CHO:HGPRT Assay Summary, Without Metabolic Activation

TABLE 3A CP-690,550-10 CHO/HGPRT ASSAY: RELATIVE CELL SURVIVAL MUTAGENICITY TEST WITHOUT METABOLIC ACTIVATION							
DAY 0 CELL COUNTS					DAY 2 CELL COUNTS		
Treatment	Replicate Number	Counts per ml (X10 ⁵)	Mean Count per ml (X10 ⁵)	%Relative Count	Counts per ml (X10 ⁵)	Mean Count per ml (X10 ⁵)	%Relative Count
Negative control: DMSO							
1%	1	1.71	1.72	100	3.25	3.14	100
	2	1.73			3.03		
Test Article: CP-690,550-10							
(µg/ml)							
1300	1	1.22	1.20	70	3.15	3.17	101
	2	1.18			3.18		
2600	1	0.68	0.63	37	3.00	2.78	89
	2	0.58			2.55		
3000	1	0.54	0.52	30	1.34	1.35	43
	2	0.50			1.35		
3400	1	0.69	0.73	42	0.12	0.13	4
	2	0.77			0.13		
3800	1	1.06	1.00	58	0.08	0.08	3
	2	0.94			0.07		
4200	1	1.08	1.11	65	0.10	0.09	3
	2	1.14			0.08		
4600	1	1.15	1.13	66	0.12	0.13	4
	2	1.11			0.14		
5000*	1	1.12	1.07	62	0.17	0.15	5
	2	1.02			0.12		

Abbreviations:

*Incomplete compound solubility in DMSO stock concentrations (100X)

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Table 89: CHO/HRPRT Assay Summary Without Metabolic Activation

TABLE 3B CP-690,550-10 CHO/HGPRT ASSAY: SUMMARY OF RESULTS MUTAGENICITY TEST WITHOUT METABOLIC ACTIVATION							
Treatment	Replicate Number	DAY 0 CYTOTOXICITY		DAY 7 MUTANT SELECTION		Mutants per 10 ⁶ Survivors	Mean Mutant Frequency
		Cloning Efficiency %Absolute	%Relative	Cloning Efficiency %Absolute	%Relative		
<u>Negative Control: DMSO</u>							
1%	1	89	100	86	100	0	0.5
	2	82	100	88	100	1	
<u>Test Article: CP-690,550-10</u>							
(µg/ml)							
1300	1	94	110	90	103	2	1.0
	2	93	109	91	105	0	
2600	1	94	110	81	93	2	1.5
	2	80	94	85	98	1	
3000	1	72	84	71	82	3	2.0
	2	66	77	77	89	1	
3400	1	5	6	66	76	0	0.0
	2	6	7	69	79	0	
3800	1	0	0	T	NA	T	NA
	2	0	0	T	NA	T	
4200	1	0	0	T	NA	T	NA
	2	0	0	T	NA	T	
4600	1	0	0	T	NA	T	NA
	2	0	0	T	NA	T	
5000*	1	0	0	T	NA	T	NA
	2	0	0	T	NA	T	
<u>Positive Control: Ethylmethanesulfonate</u>							
(µg/ml)							
400	1	72	84	75	86	485	470.5
	2	77	90	75	86	456	

Abbreviations: T-cultures terminated due to excessive cytotoxicity.

NA-not applicable

*Incomplete compound solubility in DMSO stock concentrations (100X)

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Table 90: CHO/HGPRT Assay with Metabolic Activation

TABLE 4A CP-690,550-10 CHO/HGPRT ASSAY: RELATIVE CELL SURVIVAL MUTAGENICITY TEST WITH METABOLIC ACTIVATION							
DAY 0 CELL COUNTS					DAY 3 CELL COUNTS		
Treatment	Replicate Number	Counts per ml (X10 ⁵)	Mean Count per ml (X10 ⁵)	%Relative Count	Counts per ml (X10 ⁵)	Mean Count per ml (X10 ⁵)	%Relative Count
Negative control: DMSO							
1%	1	1.48	1.49	100	3.04	2.98	100
	2	1.50			2.92		
Test Article: CP-690,550-10 (ug/ml)							
600	1	1.40	1.35	91	3.13	3.12	105
	2	1.30			3.11		
700	1	1.23	1.23	83	2.91	2.71	91
	2	1.22			2.51		
800	1	0.88	0.92	62	2.38	2.49	84
	2	0.95			2.59		
850	1	0.90	0.98	66	1.42	1.70	57
	2	1.06			1.98		
900	1	0.81	0.91	61	1.62	1.69	57
	2	1.00			1.75		
950	1	0.78	0.68	46	1.51	1.29	43
	2	0.58			1.06		
1000	1	0.98	0.84	56	0.78	0.86	29
	2	0.69			0.93		
1100	1	0.96	0.90	60	0.35	0.51	17
	2	0.83			0.66		

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Table 91: CHO/HGRPT with Metabolic Activation

TABLE 4B							
CP-690,550-10							
CHO/HGPRT ASSAY: SUMMARY OF RESULTS							
MUTAGENICITY							
TEST WITH METABOLIC ACTIVATION							
		DAY 0 CYTOXICITY		DAY 7 MUTANT SELECTION			
	Replicate	Cloning Efficiency		Cloning Efficiency		Mutants	Mean
Treatment	Number	%Absolute	%Relative	%Absolute	%Relative	per 10 ⁶	Mutant
						Survivors	Frequency
<u>Negative Control: DMSO</u>							
1%	1	82	100	90	100	3	
	2	79	100	98	100	2	2.5
<u>Test Article: CP-690,550-10</u>							
(µg/ml)							
600	1	86	107	96	102	2	
	2	89	111	102	109	3	2.5
700	1	71	88	T	NA	T	
	2	72	89	T	NA	T	NA
800	1	52	65	93	99	2	
	2	53	66	109	116	3	2.5
850	1	36	45	T	NA	T	
	2	48	60	T	NA	T	NA
900	1	37	46	82	87	7	
	2	43	53	95	101	3	5.0
950	1	42	52	79	84	1	
	2	24	30	75	80	1	1.0
1000	1	27	34	75	80	0	
	2	23	29	74	79	1	0.5
1100	1	6	7	83	88	4	
	2	17	21	76	81	5	4.5
<u>Positive Control: 3-Methylcholanthrene</u>							
(µg/ml)							
4	1	85	106	99	105	96	
	2	90	112	110	117	71	83.5

Abbreviations: T-Cultures were not needed for cytotoxicity range and were terminated

NA-not applicable

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Study Title: CP-690550 In Vivo/In Vitro Unscheduled DNA Synthesis in Rat Primary Hepatocyte Cultures at 2 Timepoints

Study no.: 01-2063-17
Study report location: Mod 4.2.3.3.1
Conducting laboratory and location: (b) (4)
Date of study initiation: Oct 4, 2001
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: CP-690550-10, Lot 43798-2-1H, Purity 96.9%;
Composition: 60% active moiety/potency

There was no Certificate of Analysis included with this report.

Key Study Findings

- Using the unscheduled DNA synthesis assay, there was no evidence for hepatocyte DNA repair as a reflection of DNA damage tested at 2-4 h or 14-16 h after a single oral dose of 125, 250, or 500 mg/kg of CP-690550.
- The high dose, 500 mg/kg dose, produced mean serum concentrations of CP-690550 of 21800 ng/mL at 2-4 h and 2600 ng/mL at 14-16 h after dosing with corresponding liver concentrations of 53200 and 8220 ng/gram liver, respectively.

Methods

Species/Strain: Rat, males, CrI:CD®(SD) BRJ
~8 weeks of age

Concentrations in definitive study: 125, 250, or 500 mg/kg

Basis of concentration selection: Concentration analysis indicated doses were 97.9% to 117.4% of expected concentrations
The highest dose level of 500 mg/kg was selected as the MTD based on a single dose oral acute study in which all rats died at 1000 mg/kg and one out of six rats died at 500 mg/kg (Pfizer Study No. 01-2063-07).

The highest dose selected for analysis of nuclear labeling will be the highest dose with sufficient cells of normal morphology to permit a meaningful assessment of UDS. Doses which induce evidence of overt cytotoxicity in the hepatocytes (i.e. pyknotic nuclei, reduced levels of radiolabeling) will be excluded from evaluation.

Frequency of dosing: Once
Route of administration: Oral gavage
Dose volume: 10 ml/kg
Formulation/Vehicle: 0.5% aqueous methylcellulose
Number/Sex/Group: UDS test: 4 or 5 males/treatment group/time (timepoints 2-4 h and 14-16 post treatment)
Satellite groups: Toxicokinetics: 9 males/500 mg/kg
Blood samples collected pre-dose (0 hour), 0.5, 1.0, 2.0-4.0 (just before sacrifice and liver collection), 8 and 14 to 16 hours (just before sacrifice and liver collection) after dosing.
Liver samples were collected approximately 2 to 4 hours and 14 to 16 hours after dosing.

Positive control: Dimethylnitrosamine,
10 mg/kg, IP, for the 2-4 hour timepoint
15 mg/kg, IP, for the 14-16 h timepoint

Formulation/Vehicle: 0.5% methylcellulose
Incubation & sampling time: Sampling time 2-4 and 14-16 hours postdose
Hepatocytes from rats treated in vivo were isolated and cultured in the presence of methyl-⁽³⁾H thymidine (³HTdr) in five cultures/animal/timepoint (3 for the UDS assay, 1 to assess attachment, and 1 backup). After 2-4 or 14-16 h of incubation, cells were processed for autoradiography and the amount of radiolabeled nucleotide was measured used as a measure of DNA damage.

Group Designation and Number Of Animals Used in The UDS Assay

Group No.	Treatment	Sacrifice Times After Treatment	
		2 - 4 Hours	14 - 16 Hours
1	Positive Control ^a	4	4
2	Vehicle Control	4	4
3	125 mg/kg	4	4
4	250 mg/kg	4	4
5	500 mg/kg	6 ^b	6 ^b
6*	500 mg/kg	9	

The route of administration will be oral gavage, which is historically the most common route of administration for this test procedure. The dosing volume will not exceed 10 mL/kg.

*Dimethylnitrosamine will be used as the positive control. It will be dissolved in deionized water and administered by intraperitoneal injection once at approximately 10 mg/kg for the 2-4-hour sacrifice and 15 mg/kg for the 14-16-hour sacrifice.

*Six animals per timepoint will be dosed and four will be perfused for hepatocyte collection. Two additional animals will be included in the event of any deaths. Any animals not used for perfusion will be sacrificed by an appropriate barbiturate followed by incision of the diaphragm and exsanguination.

*Livers will be collected and flash frozen for shipment 2-4 hours and 14-16 hours after a single oral dose. Blood will be collected for serum drug concentration and flash frozen for shipment at the following timepoints: pre-dose (0 hour), 0.5, 1.0, 2.0-4.0 (just before sacrifice and liver collection), 8 and 14-16 (just before sacrifice and liver collection) hours after a single oral dose. The satellite animals might not be treated at the same time as the remaining animals from the same timepoint.

Additional Methods Description

Attachment efficiency, an estimate of the number and viability of cells attaching to the dishes, was determined for one culture from each animal using trypan blue dye exclusion and in situ analysis.

After a labeling period of approximately 4 hours, medium was removed, cultures were rinsed, and hepatocyte cultures were re-fed with WMEI 0.25 mM thymidine and incubated an additional 16 to 20 hours. Following incubation of hepatocyte cultures, the medium is removed and cell nuclei are swollen by addition of 1% sodium citrate and then fixed in an acetic acid:methanol (1:3) solution. Slides were then air dried and autoradiograms were prepared with a 7-10 day exposure period.

Analysis

- The quality of the autoradiography, the number and distribution of cells on the slides, and cellular morphology were considered in the evaluation.
- Three treatment groups from each timepoint were analyzed for nuclear labeling. Three animals from each group were analyzed.
- The cells were examined microscopically at approximately 1500x magnification under oil immersion and the field displayed on the video screen of an automatic counter.
- Only normally-appearing nuclei were scored, and any occasional nuclei blackened by grains too numerous to count were excluded as cells in which replicative DNA synthesis occurred rather than repair synthesis.
- The net nuclear grain count was determined by counting nuclear grains and subtracting the average number of grains in three nuclear-sized areas adjacent to each nucleus (cytoplasmic count).
- The net nuclear grain count was routinely determined for ~50 randomly selected cells on two or three coverslips (~100 total nuclei) for each animal. The average mean net nuclear grain count (k standard deviation) was determined from the coverslips (~100 total nuclei) for each animal and averaged for each treatment condition. In addition, the frequency of S-phase cells (determined by the number

of S-phase cell nuclei per total nuclei) was determined for each culture based on a ~500 cell sample.

Study Validity

The study satisfied the criteria listed below and was a valid assay.

Criteria for an acceptable assay

- Inclusion of a minimum of two analyzable test concentrations at two dose times with a minimum of 3 surviving animals per treatment level.

Vehicle Control

- The viability of the vehicle control hepatocytes collected from the perfusion process must be at least 50%. Normally the perfusion viability exceeds 70%. The toxicity of the treatment with the test material may be reflected in perfusion viability, therefore no lower limit will be set for cultures from test material treated animals.
- The average net nuclear labeling in the vehicle control cultures is typically in the range of -5.00 to 1.00. In addition, no more than 10% of the cells should be in repair (contain five or more net nuclear grains).

If the analyzed vehicle control animals fail to meet these criteria, the assay will normally be considered invalid.

Positive Control

- The positive control is used to demonstrate that the cell population employed was responsive and the methodology was adequate for the detection of UDS. The average response to the positive control treatments must exceed either criteria used to indicate UDS.
- Two dose levels will be analyzed for nuclear grain counts at each timepoint.
- Grain count data obtained per animal will be acceptable as part of the evaluation if obtained from at least two replicate cultures and at least 100 cells per animal. Grain count data should be available from two of the three animals.

Criteria for evaluation for a positive response

- 1) An increase in the group average of the mean net nuclear grain count to at least three grains per nucleus above the concurrent vehicle control group average leading to a positive number or
 - 2) An increase in the group average of the percent of nuclei with five or more net grains such that the percentage of these nuclei in test cultures is 10% above the percentage observed in the group average of the concurrent vehicle controls.
- Generally, if the first condition is satisfied, the second often will be met. However, satisfaction of only the second condition can also indicate UDS activity. DNA-damaging agents can give a variety of nuclear labeling patterns; agents may strongly affect only a minority of the cells or weakly affect a majority of cells.

- In cases where increases are not observed in all three of the animals chosen for analysis, the test material will be considered active for that condition if cells from two of the three animals show increases.
- When results are neither clearly positive nor clearly negative, the presence of a dose response, the frequency distribution of cellular responses, and the reproducibility of data among animals is considered.
- A group average net nuclear grain count greater than zero, but not meeting the criteria for a positive response as described above may be considered in the evaluation of a dose response.
- Groups in which one out of three animals chosen for analysis show increases will be decided on a case by case basis depending on the level of activity in cells from the active animal, the level of activity in cells from the inactive animals, and the presence or absence of activity in surrounding dose groups.

Criteria for evaluation for a negative response

- The test article is considered negative if none of the above criteria are met.

Results

There were no mortalities. Clinical signs in CP-690550-treated animals consisted hypoactivity, labored breathing and/or squinted eyes observed in the 500 mg/kg group.

Hepatocyte viability was appropriate, ranging from 82.7% to 94.1% for the 2- to 4-hour time point, and from 83.6% to 95.9% for the 14 to 16-hour timepoint. The mean % cells in S phase were similar among the treatment groups for both time points. The positive and negative control responses confirmed the assay was valid. There were no CP-690550-related increases in the mean net nuclear grain count or in the percentage of cells with >5 net nuclear grains/nucleus for either time point, thus the assay was judged negative.

Follow-up analysis of the concentration of CP-690550 in the rats, found that the liver concentrations of CP-690550 were 2.4 and 3.2 times higher than the concentration in serum 2-4 h and 14-16 h, respectively.

Table 92: Serum and Liver Concentrations of CP-690550 (mean \pm sd) following a 500 mg/kg oral dose

Time	2-4 h	14-16 h
Liver (ng/g)	53200 \pm 10600	8220 \pm 3190
Serum	C _{max} at 2 h 21800 ng/mL AUC _{0-t} 163000 ng-h/mL	

Table 93: Unscheduled DNA Synthesis Assay Summary for 2-4 Hours

Summary of Test Results: 2- to 4-Hour Treatment Period CP-690,550						
Study No.: 23178				Test No.: 1		
Compound	Animal No.¹	Mean Nuclear Grains²	Mean Net Nuclear Grains³	Mean Cytoplasmic Grains⁴	%Cells with ≥ 5 NNG⁵	Mean Cells in S-Phase per 500
0.5% Methylcellulose 10 mL/kg	5834	7.86	-0.28	8.14	8.00	4.00
	5836	6.19	-0.19	6.38	1.33	3.33
	5837	4.88	-0.84	5.72	1.33	3.67
	Mean (SD) ±	6.31 1.52	-0.44 0.44	6.75 1.45	3.56 3.97	3.67 1.94
Dimethylnitrosamine 10 mg/kg	5830	30.31	24.82	5.49	99.33	2.33
	5831	31.18	25.59	5.59	100.00	4.65
	5832	26.22	20.51	5.71	98.00	3.67
	Mean (SD) ±	28.99 3.57	23.40 3.62	5.60 0.37	99.00 1.51	3.41 1.73
CP-690,550 125 mg/kg	5838	6.30	-0.65	6.95	2.50	4.83
	5840	6.38	-0.98	7.36	2.67	4.00
	5841	5.99	-0.93	6.92	2.00	2.67
	Mean (SD) ±	6.22 0.60	-0.85 0.26	7.08 0.69	2.39 1.27	3.83 1.84
CP-690,550 250 mg/kg	5842	4.50	0.08	4.42	2.00	3.00
	5844	6.76	-0.77	7.53	6.00	1.33
	5845	5.79	-0.69	6.48	1.33	3.00
	Mean (SD) ±	5.68 1.11	-0.46 0.47	6.14 1.51	3.11 2.67	2.44 1.13
CP-690,550 500 mg/kg	5846	6.73	-0.06	6.79	7.33	3.33
	5847	5.71	-0.54	6.25	2.00	4.67
	5849	5.87	-0.76	6.63	2.67	3.67
	Mean (SD) ±	6.11 0.61	-0.45 0.48	6.56 0.32	4.00 3.46	3.89 1.27

¹ Three animals per dose level were analyzed.² Average nuclear grain counts (≥50 cells/slide or ≥100 cells/animal).³ Average of net nuclear grain counts (≥50 cells/slide or ≥100 cells/animal) with standard deviation (SD) between coverslips. Net nuclear grains (NNG) = Nuclear grain count - Average cytoplasmic grain count.⁴ Average of cytoplasmic grain counts (≥50 cells/slide or ≥100 cells/animal).⁵ Average percentage of cells with greater than or equal to 5 net nuclear grains (≥50 cells/slide or ≥100 cells/animal).

Table 94: Unscheduled DNA Synthesis Assay Summary for 14-16 Hours

Summary of Test Results: 14- to 16-Hour Treatment Period CP-690,550						
Study No.: 23178			Test No.: 1			
Compound	Animal No.¹	Mean Nuclear Grains²	Mean Net Nuclear Grains³	Mean Cytoplasmic Grains⁴	%Cells with ≥ 5 NNG⁵	Mean Cells in S-Phase per 500
0.5% Methylcellulose 10 mL/kg	5858	5.88	0.25	5.63	6.00	3.00
	5860	6.51	0.10	6.41	4.00	5.67
	5861	5.63	0.97	4.67	11.33	4.00
	Mean	6.01	0.44	5.57	7.11	4.22
	(SD) \pm	1.03	0.57	1.02	5.93	1.72
Dimethylnitrosamine 15 mg/kg	5855	16.16	11.83	4.33	85.33	5.67
	5856	15.51	9.78	5.73	66.00	8.00
	5857	17.19	12.23	4.94	79.33	12.67
	Mean	16.28	11.28	5.00	76.89	8.78
	(SD) \pm	3.38	3.60	0.72	16.28	4.49
CP-690,550 125 mg/kg	5862	6.83	-0.79	7.62	5.33	10.67
	5863	5.57	-0.87	6.43	0.67	5.33
	5864	6.69	-0.20	6.89	4.00	4.67
	Mean	6.36	-0.62	6.98	3.33	6.89
	(SD) \pm	0.88	0.95	1.18	3.74	3.10
CP-690,550 250 mg/kg	5866	4.93	-0.45	5.39	2.67	4.67
	5867	5.17	0.45	4.72	4.67	4.33
	5868	7.07	-0.79	7.86	5.33	6.33
	Mean	5.73	-0.26	5.99	4.22	5.11
	(SD) \pm	1.44	0.78	1.78	3.80	2.32
CP-690,550 500 mg/kg	5870	5.81	-0.51	6.33	5.33	2.00
	5871	5.39	0.25	5.15	3.33	3.67
	5872	4.85	0.48	4.37	6.00	2.33
	Mean	5.35	0.07	5.28	4.89	2.67
	(SD) \pm	0.86	0.55	1.07	2.26	1.87

¹ Three animals per dose level were analyzed.² Average nuclear grain counts (≥ 50 cells/slide or ≥ 100 cells/animal).³ Average of net nuclear grain counts (≥ 50 cells/slide or ≥ 100 cells/animal) with standard deviation (SD) between coverslips. Net nuclear grains (NNG) = Nuclear grain count - Average cytoplasmic grain count.⁴ Average of cytoplasmic grain counts (≥ 50 cells/slide or ≥ 100 cells/animal).⁵ Average percentage of cells with greater than or equal to 5 net nuclear grains (≥ 50 cells/slide or ≥ 100 cells/animal).

7 Carcinogenicity

STUDIES IN RATS

Study title: 2-Year Oral Gavage Carcinogenicity and Toxicokinetic Study with CP-690550 in Rats

Study no.: 07GR439
 Study report location: module 4.2.3.4
 Conducting laboratory and location: (b) (4)
 Date of study initiation: Jan 31 2008
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: CP-690550-10, Lot E010006488, Purity 99.9%%
 CAC concurrence: Yes, Fax to Applicant May 2 2007 for SPA
 Yes, with the Rat carcinogenicity findings on March 6, 2012

Key Study Findings

In a 2-year carcinogenicity study, rats were administered CP-690550 in 0.5% methylcellulose in deionized water by oral gavage at doses of 0, 10, 30, and 75 mg/kg/day. The female high dose was initially 100 mg/kg/day, but then reduced to 75 mg/kg/day during week 19 due to mortalities related to bacterial infections at the 100 mg/kg/dose. Surviving males administered 75 mg/kg per day were killed during week 94 and all surviving males from the remaining groups during week 98 of dosing. The 26-week toxicokinetic values for the 75 mg/kg/day dose in this study were similar to values in the male and female 100 mg/kg/day dose of the 6-month general toxicology study (02-2063-20). In both studies, CP-690550 AUC exposure was higher in females than males.

CP-690550-related neoplastic findings were gender specific in the rat and included:

- interstitial cell adenomas in testis at ≥ 30 mg/kg/day
- benign thymomas in thymus for females at 100/75 mg/kg/day
- malignant hibernomas for females at ≥ 30 mg/kg/day.

Table 95: Incidences of Malignancies

Gender	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose (mg/kg/day)	0	10	30	75	0	10	30	100/75*
Malignancy								
number examined	70	60	60	70	67	60	57	63

Testis interstitial cell adenoma	1	2	4	14	-	-	-	-
Body, whole/cavity malignant hibernoma	1	0	1	2	0	2	5	4
number examined	69	59	58	66	67	60	57	63
Thymus benign thymoma	1	0	0	1	0	1	1	4
malignant thymoma	0	0	1	0	1	0	1	0
combined	1	0	1	1	1	1	2	4

Comparison of drug exposure between the dose at which there were no malignancy findings in the rat (using the AUC values for males, 3880 ng-h/mL, which is the lower of the mean male and female AUC values) with the estimated human AUC₀₋₂₄ of 550 ng-h/mL for the maximal dose of 10 mg BID (20 mg/day), resulted in an approximate 7-fold exposure margin.

CP-690550-related, non-neoplastic microscopic findings included increased incidences of decreased cellularity of lymphocytes in lymphoid tissues (spleen, thymus, mesenteric and inguinal lymph nodes, and Peyer's patch of the intestine); decreased extramedullary hematopoiesis, decreased pigment, and increased sinusoidal dilatation in the spleen; marginally decreased cellularity in the bone marrow (sternum only); and increased incidence and severity of alveolar proteinosis and alveolar macrophage infiltrates in the lung of males and females. Decreased lymphocyte cellularity was attributed to an expected pharmacologic immunomodulatory effect of CP-690550 on the lymphoid/hematopoietic tissues.

Adequacy of Carcinogenicity Study

The study was adequately conducted and interpreted.

Appropriateness of Test Models

The test model was appropriate.

Evaluation of Tumor Findings

The evaluation of tumor findings was adequate. The review by Dr. Matthew Jackson in the Division of Biometrics (Jan 31, 2012) arrived at similar conclusions regarding the data. Differences in interpretation are attributed to whether the tumors are common or rare since a significant finding is based on a different set of probability value thresholds.

Table 2.7: Critical p-values used to determine statistical significance

Type of test	Rare tumor	Common tumor
Trend	0.025	0.005
Pairwise test between placebo and high dose	0.05	0.01

The statistical review of Dr. Matthew Jackson listed the Applicant's findings of significant effects in the following table:

Table 96: Statistical Summary of Tumor Incidences

Table 2.1: Sponsor's significant results

Organ	Tumor or combination	Significant test
Body, Whole/Cav	benign angiomas	in the male low dose group.
Body, Whole/Cav	benign angioma and malignant hemangiosarcoma combination	in the male low dose group.
Pituitary	benign adenomas	in the male high dose group.
Skin/Subcutis	benign lipomas	in the male high dose group (using trend test)
Testis	benign interstitial cell tumors	in the male high dose group
Thymus	benign thymomas and thymomas combination	in the female high dose group
Body, Whole/Cav	malignant hibernomas	in the female mid and high dose groups
Cervix	benign endometrial stromal polyps	in the female high dose group (using trend test)

Discussion with ECAC on March 6 2012, further refined the significant tumor findings accounting for common tumors and historical control data to include only interstitial cell (Leydig) adenomas, benign thymomas, and malignant hibernomas.

Methods

Doses:	Males: 0, 10, 30, 75 mg/kg/day Females: 0, 10, 30, 100/75 mg/kg/day (dose reduced from 100 to 75 mg/kg/day on day 133)
Frequency of dosing:	Once daily for at least 94 weeks
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% (w/v) methylcellulose, 4000cps, in reverse osmosis water
Basis of dose selection:	results of the 6-month toxicology study (Report 02-2063-20)
Species/Strain:	CrI:CD(SD) rats
Number/Sex/Group:	60 or 70/sex/dose (see table below)
Age:	46-53 days of age (178-299 g males and 148-230 g for females)
Animal housing:	Individually housed in solid bottom polycarbonate cages with paper bedding
Paradigm for dietary restriction:	Not dietary restricted
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	No
Deviation from study protocol:	There were numerous minor deviations that were not expected to affect the data, interpretation, or conclusions of the study. Three additional deviations that could impact the interpretation are listed here, the last two points being necessary changes to enable the study to be interpreted appropriately.

1) Lack of evaluation of bone marrow smears

1) Lack of evaluation of bone marrow smears

There is one deviation that could significantly impact on the conclusions concerning drug safety. Bone marrow smears prepared from the femur of each animal at scheduled and unscheduled sacrifices were not evaluated; The Applicant noted "evaluation was not warranted based on other findings." without further elaboration. Due to the possibility of myeloid or erythroid hyperplasia and subsequent development of malignancies, those tissue should have been examined. No lymphosarcomas occurred in the rat or mouse toxicology studies, and there were 7 in CP-690550 treated male and female rats, evenly spread across the dose groups, with 1 in the control animals. The overall lack of lymphoproliferative findings in the rat provide some assurance that these processes were not occurring.

There is one deviation that could significantly impact on the conclusions concerning drug safety. Bone marrow smears prepared from the femur of each animal at scheduled and unscheduled sacrifices were not evaluated for myeloid-erythroid composition and ratios. The applicant noted "evaluation was not warranted based on other findings." without further elaboration. Due to the possibility of myeloid or erythroid hyperplasia and subsequent development of malignancies, those tissue should have been examined. The histopathology of sternal and femur bone examined marrow and did not find evidence of hyperplasia or neoplasia. Also no lymphoproliferative changes occurred in the rat or mouse general toxicology studies, and in this carcinogenicity study there were 7 in CP-690550 treated male and female rats, evenly spread across the dose groups, and 1 in the control animals. Collectively, these findings suggest, at least in the rat, neoplasia was unlikely to occur in bone marrow.

2) Dosing Adjustment

The Applicant provided information in IND 70903 (paper volume SD-138, submitted Aug 20 2008, received Aug 21 2008) and cross-referencing to the carcinogenicity study being conducted under IND (b) (4) SD-204 (Aug 20 2008) that the dose in the high dose treatment group was reduced due to mortality in the high dose female group. This was a notification after the dose was reduced.

3) Early Termination of Groups:

From the Applicant's summary "Based on the early termination plan established by the applicant in conjunction with the FDA, all surviving males given 75 mg/kg/day were sacrificed during Week 94, all surviving males from the remaining groups were sacrificed during Week 98, and all surviving females from all groups were sacrificed during Week 103 of the dosing phase. Sufficient numbers of animals survived to adequately evaluate carcinogenicity with at least 15/sex/group surviving to the terminal sacrifice."

Study Design

Table 97: Study Design

Group ^a	No. of Animals		Dose Level (mg/kg/day)		Dose Concentration (mg/mL)	
	Male	Female	Male	Female	Male	Female
Carcinogenicity Animals						
1 (Control)	70	70	0	0	0	0
2 (Low)	60	60	10	10	1	1
3 (Mid)	60	60	30	30	3	3
4 (High) ^c	70	70	75	100/75	7.5	10/7.5
Sentinel Animals						
5 (Sentinel)	10	10	b	b	b	b
^a Group 1 received control article [0.5% (w/v) methylcellulose, 4000 cps, in reverse osmosis water] only. ^b Sentinel animals were not dosed. ^c Group 4 females were dosed at the 10-mg/mL concentration from Days 1-132 and then dosed at the 7.5-mg/mL concentration from Day 133 through terminal sacrifice.						

Observations and Results

Mortality

Animals were checked twice daily (a.m. and p.m.) for mortality

The number of surviving animals at the end of the study is indicated in the table below. All surviving males of the high dose group (n=16) were sacrificed during week 94, with the remaining male groups sacrificed during week 98. The increased mortality in males given 75 mg/kg/day was attributed, partly to bacterial infection attributed to CP-690550-related immunosuppression.

All surviving females from all groups were sacrificed during week 103. Bacterial infections were also a cause of death in females at 100 mg/kg/day. Six females treated with 100 mg/kg/day developed signs of *Clostridium piliforme* infection (Tyzzer's disease) and died between weeks 15 and 22. Lowering the dose level to 75 mg/kg/day was effective at preventing any further Tyzzer's disease outbreaks. The bacterial infections were attributed to immunosuppression, a pharmacodynamic effect of CP-690550, but also indicated the maximum tolerated dose had been exceeded. Therefore, the dose level for high-dose females was lowered to 75 mg/kg/day beginning in week 19 (day 133) of the dosing phase. After the dose was decreased to 75 mg/kg/day in week 19, a few females at 75 and 30 mg/kg/day still had bacterial infections resulting in death.

Table 98: Survival to Study Termination

Gender	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose (mg/kg/day)	0	10	30	75	0	10	30	100/75*
n, day1	70	60	60	70	70	60	60	70
week that group was terminated	98	98	98	94	103	103	103	103
n at termination	21	22	28	16	21	19	16	14
% survival	30%	37%	47%	21%	30%	32%	27%	20%

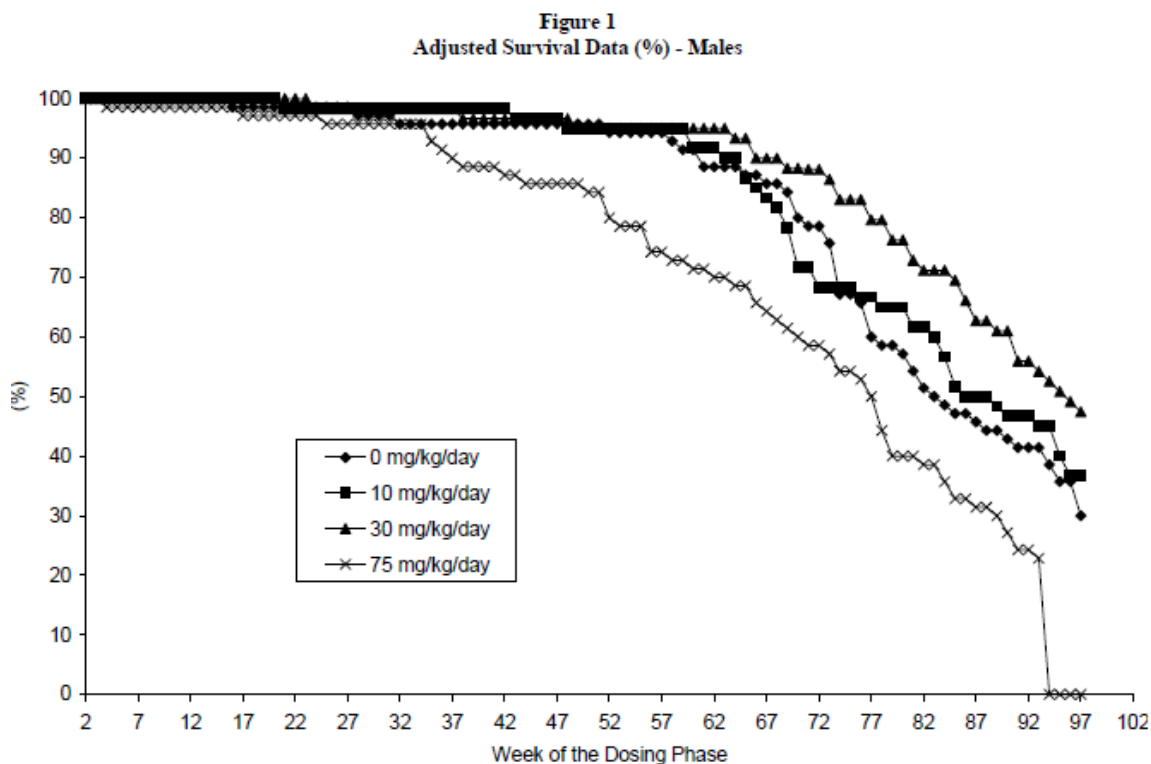
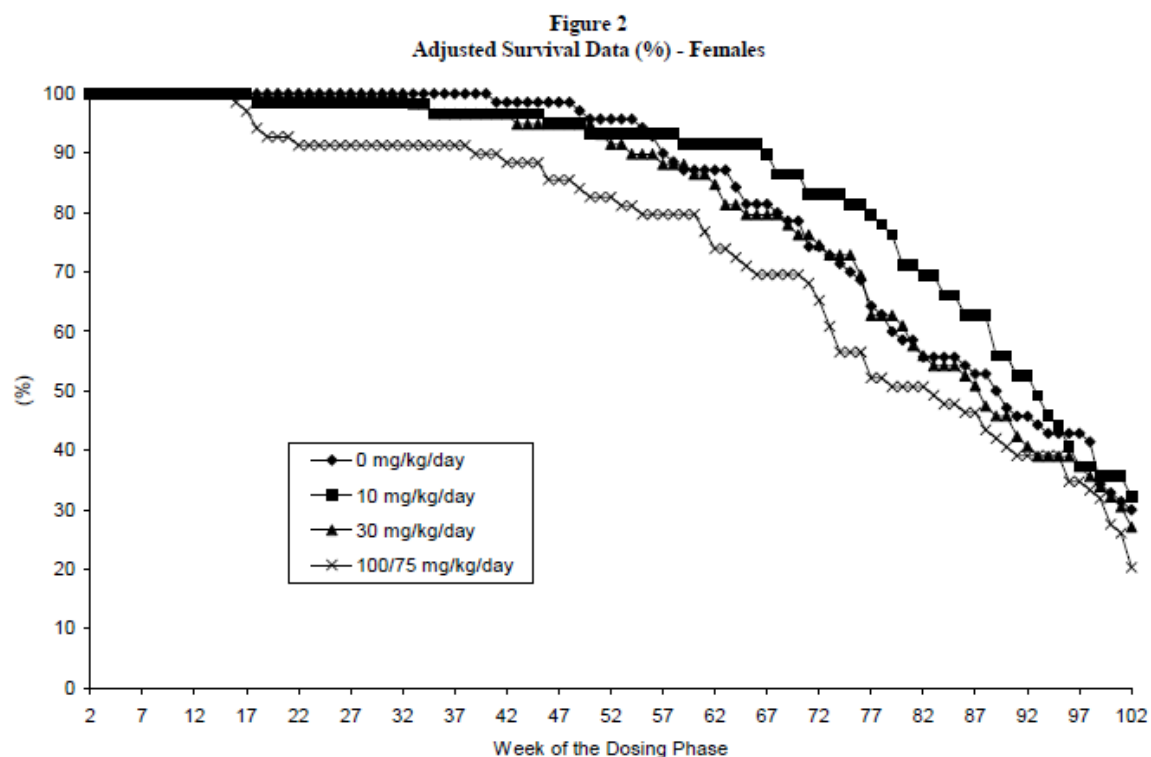
Note that the Applicant's Analysis of Survival with Adjusted Survival Rates were the same, except they did not use data for the high dose males at study termination, using instead survival of the high dose males at week 91.

Table 99: Adjusted Survival Rates

Text Table 2
Adjusted Survival Rates (%) for Carcinogenicity Animals

Sex	Males				Females			
Dose Level (mg/kg/day)	0	10	30	75	0	10	30	100/75
Week 26	99	98	98	96	100	98	100	91
Week 52	94	95	95	80	96	93	92	83
Week 78	59	65	80	44	63	78	63	52
Week 91	41	47	56	24	46	53	42	39
Week 97	30	37	47	0				
Week 102					30	32	27	20

The most common cause of death during the study was pituitary neoplasia in males and pituitary or mammary neoplasia in females. Fatal pituitary neoplasms occurred at a similar incidence across control and dosed groups in males. The incidence of fatal pituitary gland neoplasms and mammary neoplasms was slightly lower in females at 100/75 mg/kg/day. All other unscheduled deaths in control and dosed groups were attributed to a variety of causes that commonly occur in rats over the duration of a 2-year study. Unscheduled mortalities included 1 female in the 100/75 mg/kg/day dose group and 3 other rats in groups sacrificed due to tail injuries.

Figure 9: Plot of Adjusted Survival Data, Males**Figure 10: Plot of Adjusted Survival Rate, Females**

Clinical Sign

Animals were checked twice daily (a.m. and p.m.) for abnormalities, and signs of pain or distress, with detailed observations done weekly. Palpable mass was recorded once during the predose phase, during the dosing phase beginning at Week 27 of the dosing phase, once every other week thereafter, and on the day of scheduled sacrifice.

time of onset

location

size

appearance

progression

Respiratory abnormalities observed with increased incidence in animals given CP-690550 compared with controls presenting as audible, irregular, and labored respiration. Bacterial infections (heart or kidney inflammation) that occurred in a few males at 30 or 75 mg/kg/day were most likely secondary to the expected immunomodulatory effect of CP-690550. This occurred with increased incidence over time in CP-690550 treated animals, compared with controls.

Table 100: Incidence of Bacteria Infections

Text Table 11
Incidence of Bacterial Infection at One or More Sites

Sex		Male				Female			
Group		1	2	3	4	1	2	3	4
Dose (mg/kg/day)		0	10	30	75	0	10	30	100/75
Number Examined		70	60	60	70	70	60	60	70
Bacterial Organisms Present		0	1	4	17	0	0	4	9

However, respiratory abnormalities were transient and typically observed for 1 or 2 consecutive days for any individual animal. For the unscheduled deaths, these observations correlated somewhat to CP-690550-related microscopic findings in the lung (alveolar proteinosis with increased alveolar macrophage infiltrates).

Females administered 100 mg/kg/day had an increased incidence of red-stained bedding, indicating urinary tract infections with identified *Clostridium piliforme*, the infectious agent of Tyzzer's disease. Reducing the dose in the high dose female group reduced the incidence of Tyzzer's disease.

Table 101: Clinical Observations

Group	1		2		3		4	
Dose (mg/kg/day)	0		10		30		75	100/75*
	M	F	M	F	M	F	M	F
Respiratory abnormalities	4	2	10	7	3	8	12	12
audible	2	2	4	1	2	1	7	5
irregular	2	0	6	7	1	5	7	9
labored	1	2	5	1	1	2	3	4
Red-stained bedding	2	1	1	1	7	3	5	15
Palpable masses	20	41	25	29	22	24	17	22
Hypoactive	4	9	12	8	3	8	12	10
Pale Eyes	2	10	8	6	4	11	15	15
Rough haircoat	7	9	9	7	8	11	16	13

An increased number of foot sores and scabs relative to controls were observed during the first year of dosing on the hind paws of males given 75 mg/kg/day and females given 100/75 mg/kg/day. These observations were also considered secondary to immunosuppression. Foot findings responded to treatment with topical antiseptic and resolved during the second year of dosing.

There was no CP-690550-related increase in palpable tumor formation.

Other clinical signs, including increased salivation (females), malocclusions, pale eyes, and rough haircoat, which had higher frequency in treated groups, were not considered direct CP-690550-related effects because they did not exhibit dose dependency, were observed infrequently, were transient, were in only one sex, and/or were considered a common background finding for rats.

Body Weights

Recorded once during the predose phase, weekly for Weeks 1-26 of the dosing phase, and once every 4 weeks thereafter

A dose-dependent decrease in mean body weight and body weight gain was observed for males and females given CP-690550 compared with controls.

Body weight was significantly lower for all CP-690550-treated male groups compared with control males by week 6 of the dosing phase. By the end of dosing at Week 94 of

the dosing phase, the mean body weight for Group 2, 3, and 4 males was 93, 86, and 79%, respectively, of the control mean body weight.

Mean body weight for Group 4 females was significantly lower than control females beginning week 82 of the dosing phase. By the end of dosing at Week 102 of the dosing phase, the mean body weight for Group 2, 3 and 4 females was 95, 91, and 83%, respectively, of the control female mean body weight.

Body weight gain was also significantly lower in all CP-690550-treated male groups and the high dose female group.

Table 102: Body Weights and Weight Gain at Selected Time Points

Gender	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose (mg/kg/day)	0	10	30	75	0	10	30	100/75*
n	70	60	60	70	70	60	60	70
Mean Body Weight, g, (% of control)								
week 1	240	239	240	239	184	184	185	185
3	364	353 (97)	350 (96)	352 (97)	227	228 (100)	233 (103)	238 (105)
6	417	448 (107)	441 (106)	449 (108)	263	264 (100)	270 (103)	273 (104)
26	718	661 (92)	637 (89)	643 (90)	339	348 (103)	358 (106)	349 (103)
50	824	756 (92)	723 (88)	701 (85)	403	412 (102)	421 (104)	400 (99)
n	67	57	57	60	68	57	56	58
74	876	789 (90)	747 (85)	700 (80)	442	450 (102)	441 (100)	421 (95)
n	52	41	51	40	51	49	43	42
94	832	775 (93)	712 (86)	659 (79)	483	462 (96)	454 (94)	417 (86)
n	29	27	32	16	31	29	23	27
98	841	746 (89)	691 (82)	x	490	467 (95)	454 (93)	409 (83)
n	21	22	28		30	22	22	24
102	x	x	x	x	482	457 (95)	439 (91)	398 (82)
n					22	21	18	18
Mean Body Weight Gain (g)								
weeks 1-13	362	325	314	322	120	119	127	126
13-26	115	99	83	81	35	45	46	40

		(86)	(72)	(70)		(128)	(131)	(114)
26-50	107	98 (92)	87 (81)	60 (56)	63	66 (105)	63 (100)	53 (84)
50-74	58	48 (83)	22 (38)	7 (12)	46	41 (89)	24 (52)	27 (59)
74-102	x	x	x	x	30	17 (57)	-2 (<1)	-15 (<1)
1-94	592	536 (90)	472 (80)	420 (71)	-	-	-	-
1-102	-	-	-	-	300	279 (93)	252 (84)	214 (71)

x All surviving males of the high dose group (n=16) were sacrificed during week 94, with the remaining male groups sacrificed during week 98.
 - difference between the first and last weeks weights were determined week 94 for males and week 102 for females

Figure 11: Body Weight Growth Curve, Males

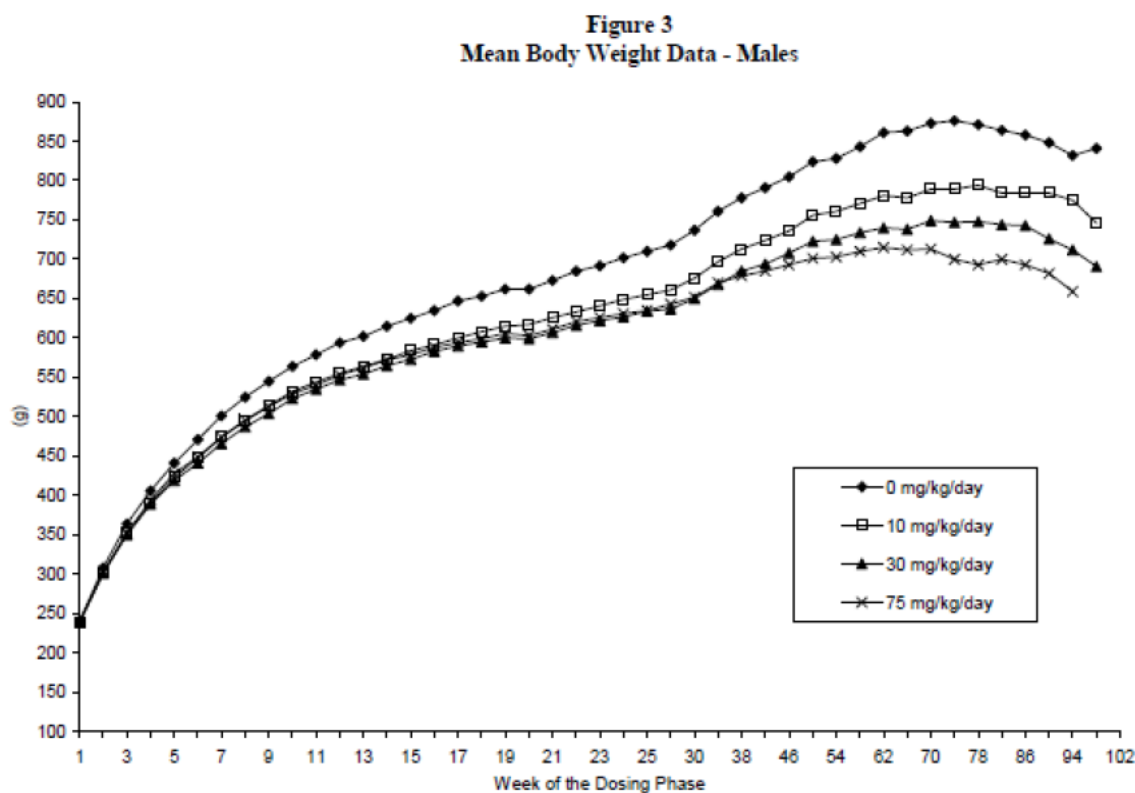
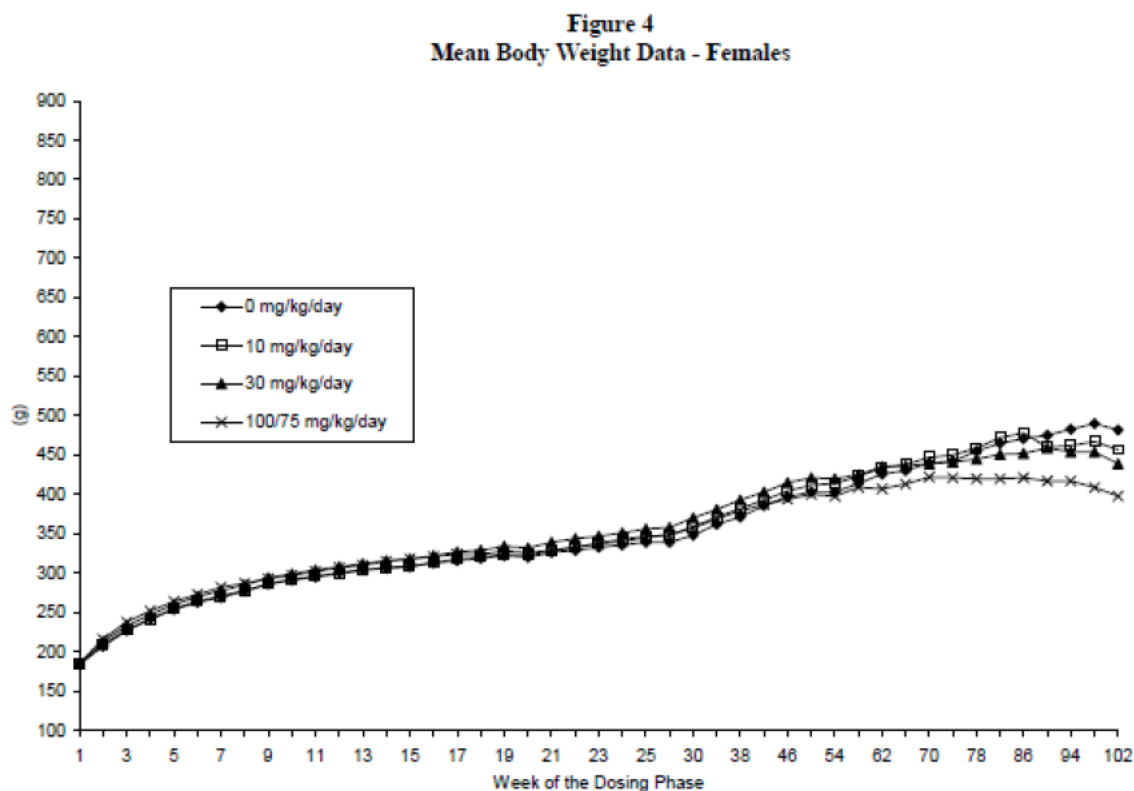


Figure 12: Body Weight Growth Curve, Females**Feed Consumption**

Measured weekly for Weeks 1-25 of the dosing phase and once every 4 weeks thereafter

In males, food consumption was reduced in all CP-690550 dose groups compared with control males, beginning at week 5, but this was not dose dependent. For females food consumption in CP-690550 dose groups was similar to controls, until week 93. The increase in food consumption of the control at week 93 resulted in reduced percentage of food consumption of the treatment groups, but the treatment food consumption was not different from previous weeks. The increase in controls was due to death toward the end of the study of animals with lower food consumption, raising the mean value compared to previous weeks.

Table 103: Food Consumption

Gender	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose (mg/kg/day)	0	10	30	75	0	10	30	100/75*
n	70	60	60	70	70	60	60	70

Mean Food Consumption g/animal/day, g, (% of control)								
week 1	183	181 (98)	183 (100)	190 (104)	124	126 (102)	129 (104)	137 (110)
3 (% of control)	208	197 (95)	199 (96)	206 (99)	136	132 (97)	140 (103)	146 (107)
6 (% of control)	222	208 (94)	202 (91)	206 (93)	138	136 (98)	138 (100)	137 (99)
25 (% of control)	209	191 (91)	184 (88)	185 (88)	127	130 (102)	131 (103)	132 (104)
49 (% of control)	214	194 (91)	190 (89)	190 (89)	131	131 (100)	132 (101)	138 (105)
73 (% of control)	210	194 (92)	185 (88)	180 (86)	129	131 (102)	132 (102)	135 (105)
93 (% of control)	192	181 (94)	186 (97)	179 (93)	152	139 (91)	144 (95)	136 (89)
97 (% of control)	201	187 (93)	184 (92)	x	141	138 (98)	138 (98)	132 (94)
101 (% of control)	x	x	x	x	132	132 (100)	135 (102)	124 (94)
x All surviving males of the high dose group (n=16) were sacrificed during week 94, with the remaining male groups sacrificed during week 98.								

Ophthalmology

Performed once during the predose phase and on Day 360 (Week 52) of the dosing phase by a veterinarian. The eyes were dilated with a mydriatic agent prior to examination using an indirect ophthalmoscope.

There were no effects of CP-690550 on ophthalmic findings.

Clinical Pathology

Blood samples were collected from the last 10 animals/sex/group via a jugular vein. Animals were fasted overnight for collections. Samples for hematology were collected on Day 132 (females)/133 (males), Day 178 (females)/179 (males; Week 26), and Day 360 (females)/361 (males; Week 52) of the dosing phase. Samples for clinical chemistry were collected on Day 132 (females)/133 (males) of the dosing phase.

CP-690550 administration reduced white blood cell counts due primarily to moderately to markedly lower absolute lymphocyte counts for males and females at all dose levels (refer to the Table below). These expected pharmacologic effects were generally dose-related and relatively consistent at all three testing intervals for all 3 sampling times, 19, 26 and 52 weeks.

Lowering the dose level for high-dose females on day 133 of the dosing phase from 100 to 75 mg/kg/day had little effect on mean absolute lymphocyte counts for these animals (1820, 2000, and 1720/ μ L on days 132, 178, and 360, respectively).

CP-690550 also reduced the number of large unstained cells in both males and females. The significance of this is unknown without further study to determine in more detail the cellular type.

Changes indicated by the applicant as minimal or mild included red blood cell numbers, hemoglobin and hematocrit, alkaline phosphatase, inorganic phosphate, total protein, and albumin. However, none of these changes (refer to the Table below) were sufficiently large to be toxicologically significant at the time of measurement. There were no correlations with histopathological findings.

Table 104: Summary of Hematology Findings (day 179, ~week 26)

Gender	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose (mg/kg/day)	0	10	30	75	0	10	30	100/75*
n	10	10	10	10	10	10	10	10
Day 179, Week 26								
RBC (10^6 /uL) (% of control)	9.14	8.87 (97)	8.97 (98)	8.52 (93)	8.18	8.17 (100)	7.88 (96)	7.45 (91)
Hemoglobin (g/dL) (% of control)	16.0	15.5 (97)	15.7 (98)	15.4 (96)	15.6	15.3 (98)	14.9 (96)	14.5 (93)
Hematocrit (%) (% of control)	49.9	48.6 (97)	49.2 (98)	47.5 (95)	47.6	46.6 (98)	45.3 (95)	44.0 (92)
WBC (10^3 /uL) (% of control)	10.4	7.07 (68)	6.03 (58)	4.41 (42)	7.58	4.49 (59)	3.37 (44)	3.29 (43)
Lymph (10^3 /uL), (% of control)	8.45	5.10 (60)	4.31 (51)	2.59 (31)	6.29	3.60 (57)	2.36 (38)	2.00 (32)
Eos (10^3 /uL) (% of control)	0.13	0.12 (92)	0.09 (69)	0.06 (46)	0.13	0.07 (54)	0.05 (38)	0.06 (46)
Large unstained cells , (10^3 /uL) (% of control)	0.09	0.04 (44)	0.04 (44)	0.03 (33)	0.07	0.04 (57)	0.02 (28)	0.03 (43)
Day 133, Week 19								
Protein (% of control)	7.4	7.9 (107)	7.7 (104)	7.9 (107)	7.8	7.5 (96)	7.6 (97)	7.9 (101)
Albumin (% of control)	4.3	4.5 (105)	4.6 (107)	4.7 (109)	5.1	4.7 (92)	4.8 (94)	4.9 (96)
ALP (% of control)	71	94 (132)	75 (106)	81 (114)	35	41 (117)	53 (151)	63 (180)

Pi (% of control)	6.8	7.0 (102)	6.5 (96)	6.4 (94)	5.7	6.2 (109)	6.2 (109)	7.1 (124)
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Viral Screen - Sentinel Animals

Blood samples (approximately 1 mL) were collected via a jugular vein from the first five surviving sentinel animals/sex once during the predose phase and on Day 358 (females)/359 (males) of the dosing phase and from the first five surviving male sentinel animals and the remaining female sentinel animals on Day 604 (females)/605 (males) of the dosing phase. The viral screens were not specified.

Blood samples taken from sentinel animals during the predose phase and during Week 52 and 87 of the dosing phase were negative for all viral screening performed.

Gross Pathology

After at least 94 weeks (Group 4 males), 98 weeks (Groups 1 through 3 males), and 102 weeks (all female groups) of dosing, after fasting overnight, all surviving animals were anesthetized with sodium pentobarbital, weighed, and exsanguinated, and examined.

Besides visible signs of the more common neoplasms of the pituitary and mammary gland, macroscopic observations were noted in the testis, lung, uterus, and stomach.

The testis findings consisted of discoloration and/or large size, and these tended to correlate to the interstitial (Leydig) cell adenomas in the testis of males at the high dose (Table below).

In the lung of males and females there was also a greater incidence of lung discoloration tan, white, or gray foci (Table below) which correlated with histopathological observations of increased incidence and severity of alveolar proteinosis.

In the testis, discoloration and larger than normal size were associated with findings of interstitial (Leydig) cell adenoma identified through histopathology. In the lungs gross observations for discoloration (tan, white, or gray foci) correlated with histopathological findings of alveolar proteinosis

Table 105: Incidence of CP-690550-Related Testis and Lung Discoloration

Sex		Male				Female			
Group	1	2	3	4	1	2	3	4	
Dose (mg/kg/day)	0	10	30	75	0	10	30	100/75	
Number Examined	70	60	60	70	70	60	60	70	
Testis									
Discolored and/or large	1	5	7	6	-	-	-	-	
Lung									
Discoloration	4	6	11	10	6	13	25	32	
- = Not applicable.									

In the uterus, there was a decreased incidence of cysts in the uterus in the mid and high doses compared to the control (8, 6, 1, and 1 in females at 0, 10, 30, or 100/75 mg/kg/day, respectively), and this also correlated histopathologically to a decreased incidence of cystic endometrial hyperplasia. This was not considered toxicologically significant.

In the glandular aspect of the stomach, there was an increased incidence in discoloration of mucosal surface in males at the high dose. Most of these observations were in unscheduled deaths and were attributed to the postmortem retention of blood, with discoloration of the mucosa. This is based on the higher incidence of this observation in males found dead, than in terminal animals, and absence of other gross findings such as erosions and ulcers. It's possible that this is due to secondary pharmacologic actions related to interaction with VEGFR1 activity in combination with the effects of aging in these animals.

Table 106: Stomach, mucosa discoloration

Gender	Males, Scheduled Deaths				Males, Unscheduled Deaths			
Group	1	2	3	4	1	2	3	4
Dose (mg/kg/day)	0	10	30	75	0	10	30	75
n	10	10	10	10	10	10	10	10
Stomach								
Discolored	2	3	2	5	2	5	5	14

Histopathology

Peer Review: by the applicant's pathologist after the initial CRO pathology examination

Codes Prefacing Neoplastic Findings

B	Primary, benign neoplasm
C	Multicentric neoplasm
F	Infiltrating neoplasm
I	Locally invasive neoplasm
M	Primary, malignant neoplasm
N	Metastatic neoplasm
X	Other neoplasm

Neoplastic Findings

CP-690550-related neoplastic findings were present in the testis, thymus, mesenteric lymph node, body cavity (brown fat), and vasculature (Table below).

The Applicant's statistical analysis is presented in the Table below. Common and rare tumors were tested at 0.01 and 0.05 significance levels, respectively. All p-values less than 0.05 are tabled in the Results section of this report, even if considered not statistically significant due to "common" (greater than 1%) tumor historical control background rates. The draft review by Dr. Matthew Jackson in the Division of Biometrics (forwarded to me Jan 26 2012) arrived at generally similar conclusions regarding the data.

Table A. Summary of Peto test results with p-values of less than 0.05

Organ	Lesion	Sex	Dose Group	p-value	Historical control
Adrenal, Medulla	B-Phaeochromocytoma	F	Mid	0.0143	common
Body, Whole/Cav	B-Angioma	M	Low	0.0203*	rare
	Angioma/Hemangiosarcoma	M	Low	0.0497*	rare
	M-Malignant Hibernoma	F	Trend	0.0192*	rare
		F	High	0.0464*	rare
		F	Mid	0.0193*	rare
Cervix	B-Polyp, Endometrial Stromal	F	Trend	0.0157*	rare
Liver	Hepatocellular Tumors	M	Trend	0.0466	common
Pancreas	Islet Cell Tumors	M	Mid	0.0361	common
Pituitary	B-Adenoma	M	Trend	0.0017†	common
		M	High	0.0056†	common
Skin/Subcutis	B-Lipoma	M	Trend	0.0376*	rare
Testis	B-Interstitial Cell Tumor	M	Trend	0.0001†	common
		M	High	0.0010†	common
Thymus	B-Thymoma	F	Trend	0.0091†	rare
		F	High	0.0410*	rare
	Thymomas	F	Trend	0.0106*	rare
		F	High	0.0410*	rare

Lesion prefix: B = Benign, M = Malignant. Lesions without a prefix are a combination of individual findings.

Sex: F = Female, M = Male.

* Significant based on FDA guidance only if this tumor type is considered rare.

† Significant based on FDA guidance regardless whether this tumor type is considered rare or common.

Neoplasms in Rats by Location

Gender	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose (mg/kg/day)	0	10	30	75	0	10	30	100/75*
N	70	60	60	70	70	60	60	70
Adrenal Cortex								
adenoma	2	1	1	1	0	2	0	1
carcinoma	1	0	0	0	1	0	1	0
combined	3	1	1	1	1	2	1	1
Adrenal Medulla								
pheochromocytoma	7	9	3	6	2	5	7	5
malignant pheochromocytoma	2	1	2	0	0	1	0	1
combined	9	10	5	6	2	5	7	5
Body whole/cavity								
angioma	0	5	3	2	1	3	1	3
hemangiosarcoma	2	1	0	1	2	2	2	1
combined*	2	6	3	3	3	5	3	4
histiocytic sarcoma	2	1	0	0	1	1	1	1
leukemia, granulocytic	0	0	0	1	0	0	0	0
leukemia, large granular cell	1	0	0	0	0	0	0	0
combined	1	0	0	1	0	0	0	0
lymphosarcoma	0	2	1	1	1	1	1	1
malignant hibernoma	1	0	1	2	0	2	5	4
malignant mesothelioma	3	2	0	0	0	1	0	0
malignant plasmacytoma	0	0	0	0	0	0	0	1
Bone								
osteosarcoma	1	0	0	1	0	0	0	0
Brain								
granular cell tumor	0	1	0	0	0	0	0	0
malignant granular cell tumor	0	0	1	0	0	0	0	
combined	0	1	1	0	0	0	0	0
malignant	0	2	0	0	1	0	1	1

astrocytoma								
malignant oligodendroglioma	0	0	1	0	0	0	0	0
combined	0	2	1	0	1	0	1	1
Cavity, Abdominal								
Fibrosarcoma	0	1	0	0	0	0	0	0
malignant schwannoma	0	1	0	0	0	0	0	0
osteosarcoma	0	1	0	0	0	0	0	0
Cavity, Oral								
squamous cell carcinoma	0	0	0	2	0	0	0	0
Duodenum								
leiomyosarcoma	0	0	0	0	0	0	1	0
Eye								
squamous cell carcinoma	0	0	0	0	1	1	1	1
Zymbal's gland								
carcinoma	0	1	1	1	0	1	0	1
Heart								
endocardial Schwannoma	1	1	0	1	0	0	0	0
Kidney								
transitional cell carcinoma	0	1	0	1	0	0	0	0
tubule cell carcinoma	0	2	0	1	0	1	0	0
combined	0	3	0	1	0	1	0	0
Lipoma	0	0	0	2	0	0	0	1
Liver								
hepatocellular adenoma	0	1	2	0	0	0	0	0
hepatocellular carcinoma	1	0	4	2	0	0	1	0
cholangioma	0	0	0	0	0	1	0	1
Lung								

bronchiolar-alveolar adenoma	0	0	0	2	0	0	0	0
Nerve, Sciatic								
malignant Schwannoma	1	0	0	0	0	0	0	0
Ovary								
granulosa/theca cell tumor	-	-	-	-	1	0	0	0
luteoma	-	-	-	-	1	0	0	0
malignant granulosa/theca	-	-	-	-	2	1	0	0
Pancreas								
islet cell adenoma	2	1	4	1	0	2	0	2
islet cell carcinoma	1	4	5	0	0	1	1	0
combined	3	5	9	1	0	3	1	2
acinar cell adenoma	0	1	1	1	0	0	0	0
acinar cell carcinoma	0	0	1	0	0	0	0	0
combined	0	1	2	1	0	0	0	0
Parathyroid								
adenoma	0	1	1	0	1	0	0	1
Pituitary								
adenoma	29	24	33	33	56	46	41	47
Seminal Vesicle								
adenoma	0	1	0	0				
Skin/Subcutis								
basal cell adenoma	0	0	0	1	0	0	0	0
basal cell carcinoma					0	1	0	0
squamous cell papilloma	2	1	0	0	0	0	0	0
squamous cell carcinoma	0	0	0	1	0	0	0	0
combined	2	1	0	1				
fibroma	0	2	1	2	2	0	0	2
fibrosarcoma	3	1	3	3	0	0	1	0
combined	3	3	4	5	2	0	1	2
keratoacanthoma	8	8	2	4	0	1	0	0
lipoma	1	0	1	3	0	0	0	0

amelanotic melanoma	0	0	1	0	0	0	0	0
sebaceous cell adenoma	0	1	0	0	0	0	0	0
sebaceous cell carcinoma	0	0	1	0	0	0	0	0
combined	0	1	1	0	0	0	0	0
Spinal Cord								
malignant astrocytoma	0	0	0	1	0	0	0	0
Stomach, nonglanular								
squamous cell carcinoma	0	0	1	0	0	0	0	0
* for females in group 4, the dose of 100 mg/kg/day was administered from day 1 to day 132, then reduced to 75 mg/kg/day on day 133 and continued at this dose (approximately 1.5 years) until the study termination.								

Testis. Dose-related increased adenoma of interstitial cells of the testis, either unilaterally or bilaterally, occurred in the mid and high dose groups, 6.7% and 20%, respectively. An increase in proliferative change, hyperplasia, also occurred in these dose groups. The severity of hyperplasia was minimal in controls and ranged from minimal to moderate in animals at 10 or 30 mg/kg/day and minimal to slight in animals at 75 mg/kg/day.

Testis. There was a dose-dependent increase in hyperplasia and adenomas of interstitial cells (Leydig) of the testis as indicated in the table below. The severity of hyperplasia was minimal in controls and ranged from minimal to moderate in the treatment group but severity was not dose-dependent.

Table 107: Incidences of Testis Hyperplasia and Maligancies

Group	1	2	3	4
Dose (mg/kg/day)	0	10	30	75
Number Examined	70	60	60	70
Hyperplasia, Interstitial Cell	4	4	11	25
Interstitial Cell Adenoma	1	2	4	14
Hyperplasia and/or Adenoma	5	6	15	32

Hibernoma: A significant increase in brown fat neoplasms (malignant hibernoma) occurred in females at >30 mg/kg/day. The incidence of brown fat neoplasms (malignant hibernoma) was 0, 2 (3.3%), 5 (8.3%), and 4 (5.7%) in females at 0, 10, 30 or 100/75 mg/kg, respectively. These were fatal tumors that originated in the thoracic or abdominal cavity, and metastatic/invasive foci were often present. Malignant hibernoma was observed grossly but varied in location (thoracic or abdominal cavity) and organ involvement (thymus, aorta, lung, liver, or kidney) as determined by macroscopic observation at necropsy.

Brown adipose tissue is not a standard tissue in toxicology studies and was not evaluated in previous animal studies with CP-690550. Therefore an exploratory study was undertaken in female rats to assess the effects of CP-690550 on brown adipose tissue after repeated oral doses in female rats (Refer to Appendix 5 studies titled "14-Day Oral Investigative Study of the Effects of CP-690550 on Brown Adipose Tissue in Female Sprague-Dawley Rats" and "An Investigative Study with Rat Brown Adipocytes Treated with Ovine Prolactin and CP-690550." This study demonstrated tissue/cellular responsiveness to CP-690550.)

Hibernoma: In females, but not males, there was a dose-dependent increase in malignant hibernomas (brown fat neoplasms) 0, 2 (3.3%), 5 (8.3%), and 4 (5.7%) in females at 0, 10, 30 or 100/75 mg/kg, respectively. They occurred in both the thoracic and abdominal cavities associated with the thymus, aorta, lung, liver, or kidney. Since this tissue is not usually examined in standard histopathology assessments in general toxicology studies, it is not known if hibernomas had occurred in earlier studies. However, if observed grossly, it would have examined.

The applicant conducted two exploratory studies to assess the effects of CP-690550 on brown adipose tissue after repeated oral doses in female rats (Refer to Appendix 5 studies titled "14-Day Oral Investigative Study of the Effects of CP-690550 on Brown Adipose Tissue in Female Sprague-Dawley Rats" and "An Investigative Study with Rat Brown Adipocytes Treated with Ovine Prolactin and CP-690550." These studies demonstrated tissue/cellular responsiveness to CP-690550, which may be mediated through prolactin stimulation of JAK2/STAT5 pathway which is inhibited by CP-690550.

Lipoma: There was a statistically significant increase for lipoma in the skin/subcutis of males with an incidence of 4.3% in males at 75 mg/kg/day.

Thymoma: Thymomas occurred in females at the high dose 100/75 mg/kg/day.

Table 108: Incidences of Malignancies in the Thymus

Sex		Male				Female			
Group		1	2	3	4	1	2	3	4
Dose (mg/kg/day)		0	10	30	75	0	10	30	100/75
Number Examined		69	59	58	66	67	60	57	63
Thymus									
B-Thymoma		1	0	0	1	0	1	1	4
M-Thymoma		0	0	1	0	0	0	1	0
Thymoma, Benign or Malignant		1	0	1	1	0	1	2	4

Angioma: Benign angiomas in the mesenteric lymph nodes were increased for males at 10 mg/kg/day compared to control males, but was not dose-dependent. There were no dose-related effects for hemangiosarcomas.

Table 109: Incidences of Angiomas and Hemangiosarcomas

Sex		Male				Female			
Group		1	2	3	4	1	2	3	4
Dose (mg/kg/day)		0	10	30	75	0	10	30	100/75
Number Examined		70	60	60	70	70	60	60	70
Mesenteric Lymph Node									
B-Angioma		0	5	3	2	1	3	1	3
M-Hemangiosarcoma		0	0	0	0	1	1	0	0
Number Examined		70	60	60	70	70	60	60	70
All Sites Combined (Body/Whole)									
B-Angioma		0	5	3	2	1	3	1	3
M-Hemangiosarcoma		2	1	0	1	2	2	2	1
Angioma or Hemangiosarcoma		2	6	3	3	3	5	3	4
Number Examined		70	60	60	70	70	60	60	70

Islet Cell Adenoma: Pancreatic islet cell adenoma and carcinoma (combined, 6.7%) were increased relative to controls in males at 30 mg/kg/day. There were no CP-690550-related increases in combined proliferative (hyperplasia, adenoma, and carcinoma) findings for islet cells in females.

Uterine and Cervical Tumors: There was an increase in endometrial stromal polyps of the cervix at the high dose. However, combining uterine and cervical endometrial stromal tumors resulted in no differences between control and CP-690550 groups.

Table 110: Other Organs, Incidences of Malignancies

Sex		Male				Female			
Group		1	2	3	4	1	2	3	4
Dose (mg/kg/day)		0	10	30	75	0	10	30	100/75
Number Examined		70	60	60	70	70	60	60	70
Liver									
B-Adenoma, Hepatocellular		0	1	2	0	0	0	0	0
M-Carcinoma, Hepatocellular		1	0	4	2	0	0	1	0
Adenoma or Carcinoma		1	1	6	2	0	0	1	0
Number Examined		70	60	60	70	70	60	60	70
Pancreas									
Hyperplasia, Islet Cell, Focal	3 (1.3) ^a	1 (3.0)	2 (2.0)	2 (2.0)	2 (1.0)	0	1 (1.0)	0	0
B-Adenoma, Islet Cell	2	1	4	1	0	2	0	2	2
M-Carcinoma, Islet Cell	1	4	5	0	0	1	1	0	0
Adenoma or Carcinoma, Islet Cell	3	5	9	1	0	3	1	2	2
Hyperplasia, Adenoma or Carcinoma, Islet Cell	6	6	11	3	2	3	2	2	2
Number Examined		70	60	60	70	70	60	60	70
Pituitary									
Adenoma	29	24	33	33	56	46	41	47	47
Number Examined		70	60	59	69	70	60	60	69
Skin									
Lipoma	1	0	1	3	0	0	0	0	0
Number Examined		70	60	60	70	70	60	60	69
Adrenal Medulla									
Benign Pheochromocytoma	7	9	3	6	2	5	7	5	5
Malignant Pheochromocytoma	2	1	2	0	0	1	0	1	1
Pheochromocytoma, Combined	9	10	5	6	2	5	7	5	5
Number Examined		-	-	-	-	70	60	60	70
Cervix									
Polyp, Endometrial Stromal	-	-	-	-	0	0	2	3	3
Uterus									
Polyp, Endometrial Stromal	-	-	-	-	3	1	1	0	0
Uterus/Cervix									
Polyp, Endometrial Stromal	-	-	-	-	3	1	3	3	3

- = Not applicable.

a Average severity for hyperplasia based on number of animals with finding.

Non-Neoplastic Findings

The non-neoplastic findings included decreased cellularity (lymphocytes) in the spleen, thymus, mesenteric lymph node, inguinal lymph node, and intestinal lymphoid tissue (Peyer's patch); decreased cellularity in the bone marrow (sternum only); and alveolar proteinosis with increased alveolar macrophage infiltrates in the lung. The reduction in lymphocytes corresponds with the expected pharmacodynamics of the CP-690550 and although this can be toxicologically significant (e.g. increased bacterial infections as demonstrated in this study), excessive pharmacodynamic effects can be monitored clinically.

Spleen: For doses >10 mg/kg/day, both males and females has reductions in lymphocytes within the spleen characterized by a decrease in the size and number of periarteriolar lymphoid sheaths (decreased cellularity) and decreased cellularity of mononuclear cells (primarily lymphocytes) throughout the red pulp. There were no changes indicative of proliferation. In the high dose groups, there was a decreased incidence or severity of extramedullary hematopoiesis and pigment (representing hemosiderin/lipofuscin).

Spleen: .There were no signs of proliferative activity. A reduced lymphocyte population was qualitatively observed at mid and high dose group (30 and 100 mg/kg/day). The applicant described these as characterized by a decrease in the size and number of periarteriolar lymphoid sheaths (decreased cellularity) and decreased cellularity of mononuclear cells (primarily lymphocytes) throughout the red pulp. Also noted was a reduction in incidence or severity of extramedullary hematopoiesis and pigment (hemosiderin/lipofuscin).

Table 111: Nonneoplastic CP-690550-Related Effects in the Spleen

Sex		Male				Female			
Group		1	2	3	4	1	2	3	4
Dose (mg/kg/day)		0	10	30	75	0	10	30	100/75
Number Examined		70	60	60	70	70	60	60	70
Cellularity Decreased, Lymphocyte									
Minimal		1	18	32	39	5	19	33	26
Slight		1	4	8	16	0	9	8	30
Moderate		0	0	0	2	0	1	2	9
Total Affected (%)		3	37	67	81	7	48	72	93
Dilatation, Increased, Sinusoids									
Minimal		0	0	0	1	0	0	0	0
Slight		0	0	0	1	0	2	6	12
Total Affected (%)		0	0	0	3	0	3	10	17
Pigment, Increased									
Minimal		45	36	38	30	29	29	35	42
Slight		7	2	1	2	26	19	10	8
Moderate		0	0	0	0	5	3	1	0
Total Affected (%)		74	63	65	46	86	85	77	71
Hematopoiesis, Extramedullary, Increased									
Minimal		29	23	16	12	16	11	11	6
Slight		3	1	1	1	9	7	7	3
Moderate		2	2	0	0	2	1	2	0
Total Affected (%)		49	43	28	19	39	32	33	13

Thymus: In the high dose male and female groups, CP-690550-related decreased cellularity of lymphocytes occurred in the thymus. Animals with marked severity had essentially no lymphocytes remaining in the thymus lobules, while those with the most severe change had only small lobules remaining that consisted of a prominent sheet of thymus epithelial cells.

Thymus: There was a dose-related increase in severity of the lymphocyte population reduction in the thymus. The applicant noted that animals with marked severity had essentially no lymphocytes remaining in the thymus lobules, while those with the most severe change had only small lobules remaining that consisted of a prominent sheet of thymus epithelial cells.

Table 112: Nonneoplastic CP-690550-Related Effects in the Thymus

Sex		Male				Female			
Group		1	2	3	4	1	2	3	4
Dose (mg/kg/day)		0	10	30	75	0	10	30	100/75
Number Examined		69	59	58	66	67	60	57	63
Cellularity Decreased, Lymphocyte									
Minimal		1	0	0	3	5	4	5	1
Slight		10	9	6	1	22	28	17	7
Moderate		45	33	38	29	38	22	22	22
Marked		10	15	13	23	0	4	9	23
Severe		0	0	1	6	0	0	1	7
Total Affected (%)		96	97	100	94	97	97	95	95

Mesenteric Lymph Node, Inguinal Lymph Node, and Peyer's Patch: The population of lymphocytes was reduced in the mesenteric and inguinal lymph nodes and Peyer's patch. Both the incidence and severity of the reduction were dose-dependent. The applicant described this effect as characterized by a decrease in the size and number of lymphoid follicles and generally, an absence of distinct germinal centers. There was a loss of small lymphocytes and increased "prominence" in stromal cells. It is unclear what is meant by prominence, increased number of stromal cells or more readily visible due to reduced numbers of lymphocytes. The lack of noted proliferative changes, hyperplasia, would suggest the latter interpretation. This was also noted in the paracortical region.

In the mesenteric lymph node, there was an increase in the incidence of sinusoidal hemorrhage in males (males: 1, 6, 7, 6; females 3, 3, 2, 2 for dose groups 0, 10, 40 and 75 respectively; with 14 to 25/group). There was insufficient information provided to decide if lymph node hemorrhage was a CP-690550-related toxicological effect, nor an obvious explanation why it would be higher in males and not females, or not present in other lymph nodes. It was not observed in the 6-month rat toxicology study, or in the 9-month monkey toxicology study. It's possible that this is due to secondary pharmacologic actions related to interaction with VEGFR1 activity in combination with the effects of aging in these animals.

Table 113: Nonneoplastic CP-690550-Related Effects Lymph Nodes and Peyer's Patch

	Sex	Male				Female			
		1	2	3	4	1	2	3	4
	Group								
	Dose (mg/kg/day)	0	10	30	75	0	10	30	100/75
	Number Examined	70	60	60	70	70	60	60	68
Mesenteric lymph node									
	Minimal	3	18	26	29	7	7	20	31
	Slight	0	2	10	12	0	3	9	22
	Moderate	0	1	1	0	0	1	1	3
	Total Affected (%)	4	35	62	59	10	18	50	82
	Number Examined	63	50	44	48	65	54	53	49
Inguinal lymph node									
	Minimal	8	17	22	23	9	19	18	22
	Slight	0	1	0	7	0	0	4	10
	Moderate	0	0	1	0	0	0	0	1
	Total Affected (%)	13	36	52	63	14	35	42	67
	Number Examined	67	58	56	60	67	60	56	52
Peyer's patch									
	Minimal	5	14	24	27	6	14	25	26
	Slight	0	1	0	1	0	0	2	7
	Moderate	0	0	0	0	0	0	0	2
	Total Affected (%)	7	26	43	47	9	23	48	67

Bone Marrow (Sternum and Femur). In both sternum and femur bone marrow, the applicant indicated cellularity was highly variable within and across all treatment groups including controls. However, decreased cellularity could be ascertained as an effect of CP-690550 in the sternum marrow, but not the femur marrow. Fat cells were more

prominent in the central regions of the marrow. .Importantly, the applicant stated that decreased cellularity was not associated with degeneration, necrosis, inflammation, atypical hyperplasia, or neoplastic changes in the marrow adipocytes or hematopoietic cells. Myeloid-erythroid composition analysis was not conducted as noted in the study deviations.

Table 114: Nonneoplastic CP-690550-Related Effects in Bone Marrow

Sex		Male				Female			
Group		1	2	3	4	1	2	3	4
Dose (mg/kg/day)		0	10	30	75	0	10	30	100/75
Number Examined		70	60	60	70	70	60	60	70
Sternum, Cellularity Decreased									
Minimal		19	24	25	30	19	18	19	26
Slight		1	0	3	1	0	1	0	1
Total Affected (%)		29	40	47	44	27	32	32	39
Femur, Cellularity Decreased									
Minimal		4	6	5	6	2	1	3	4
Total Affected (%)		6	10	8	9	3	2	5	6

Lung: There was an increased incidence alveolar macrophage infiltrates (minimal to slight) and incidence and severity of alveolar proteinosis (minimal to severe) in the lungs of males and females. Alveolar proteinosis occurred in female controls at approximately the same incidence as the low dose of CP-690550, no incidences in control or low dose males. Females appeared to be more sensitive to the occurrence of this pathology. The applicant noted there was no evidence for an increase in the number or hypertrophy of Type II cells.

The Applicant described the infiltrates as consisting of diffusely scattered cells associated with the areas of alveolar proteinosis. Alveolar proteinosis was characterized by the accumulation of an acellular, gray, amorphous to slightly granular material in alveolar spaces. The alveolar accumulation of this material exhibited a distribution pattern characterized by multiple foci to a more severe, diffuse occurrence in one or both sections of lung. Many of the lungs from dosed animals were noted grossly as discolored, and a disruption of alveolar septae with inflammation was a component of the more severe accumulation of this material.

Table 115: Nonneoplastic CP-690550-Related Effects in the Lung

Sex		Male				Female			
Group		1	2	3	4	1	2	3	4
Dose (mg/kg/day)		0	10	30	75	0	10	30	100/75
Number Examined		70	60	60	70	70	60	60	70
Infiltrate, Macrophage, Alveolus									
Minimal		23	18	25	42	22	35	50	60
Slight		1	1	8	8	11	7	9	6
Total Affected (%)		34	32	55	71	47	70	98	94
Alveolar Proteinosis									
Minimal		0	0	4	8	6	2	6	2
Slight		0	0	1	4	5	12	11	10
Moderate		0	0	8	15	1	10	14	16
Marked		0	0	2	5	0	1	12	26
Severe		0	0	0	1	0	0	1	5
Total Affected (%)		0	0	25	47	17	42	73	84

Table 116: Macroscopic Lung Findings

Males								
	Scheduled Deaths				Unscheduled Deaths			
Group	1	2	3	4	1	2	3	4
Dose (mg/kg/day)	0	10	30	75	0	10	30	100/75
n	20	21	25	15	50	39	35	55
Lung								
Discolored	0	1	10	2	4	5	1	8
Females								
	Scheduled Deaths				Unscheduled Deaths			
n	21	19	16	14	49	41	44	56
Lung								
Discolored	2	5	12	10	4	8	13	22

Decreased Incidence of Mononuclear Cell Infiltrates: Decreased incidences of mononuclear cell infiltrates (primarily lymphocytes) occurred in the kidney, liver, and prostate in males and kidney, liver, and pancreas of females at >10 mg/kg/day. There were no proliferative findings in these organs. These findings were not considered toxicologically significant.

Table 117: Nonneoplastic CP-690550-Related Effects on Mononuclear Cell Infiltrates

Sex		Male				Female			
Group		1	2	3	4	1	2	3	4
Dose (mg/kg/day)		0	10	30	75	0	10	30	100/75
Number Examined		70	60	60	70	70	60	60	70
Liver									
Minimal		47	28	24	13	45	21	19	13
Slight		2	2	0	0	9	1	3	2
Moderate		0	1	0	0	0	0	0	1
Total Affected (%)		70	52	40	19	77	37	37	23
Kidney									
Minimal		40	32	30	32	15	4	7	7
Slight		15	13	11	7	1	0	1	1
Moderate		1	0	0	0	0	0	0	0
Marked		0	0	0	0	0	0	1	0
Total Affected (%)		80	75	68	56	23	7	15	11
Prostate									
Minimal		36	23	21	17	-	-	-	-
Slight		7	2	4	5	-	-	-	-
Total Affected (%)		61	42	42	31	-	-	-	-

- = Not applicable.

Bacterial Infection: There was a dose-related increase in incident of bacterial infections (coccoid or filamentous) during the study. The infections were often in multiple sites and associated with abscess, necrosis, and extensive inflammation, and in some animals resulted in moribund sacrifice. This was also the reason for dose reduction in the high dose female group early in the study.

Table 118: Bacterial Infections

Sex		Male				Female			
Group		1	2	3	4	1	2	3	4
Dose (mg/kg/day)		0	10	30	75	0	10	30	100/75
Number Examined		70	60	60	70	70	60	60	70
Bacterial Organisms, Present		0	1	4	17	0	0	4	9

Liver, heart, adrenal cortex: There were decreased incidences and/or severity of several in the bile duct hyperplasia in the liver in males and females at >10 mg/kg/day, cardiomyopathy) in males and females at 75 or 100/75 mg/kg/day, and adrenal cortex cystic degeneration in males and females at >10 mg/kg/day.

Uterus: The incidence of cystic endometrial hyperplasia of the uterus was reduced in the mid and high dose groups. The applicant also noted that the incidences of involution/decreased corpora lutea and stromal cell hyperplasia of the ovary and mineralization and transitional cell hyperplasia in the kidney pelvis were also reduced in females at >10 mg/kg/day (data not shown in text table). These effects while common background findings, are also likely CP-690550 related through its actions to inhibit prolactin signaling through JAK/STAT pathway.

Joints: Joint inflammation occurred mostly in control animals. For the stifle joint (an pre-planned tissue to examine) in the animals that died before the study ended, there was one high dose male with chronic active inflammation of the synovium of the stifle and one control female had vessel inflammation. The other animals had neoplasm (2 high dose males and 1 high dose female with hematopoietic neoplasms. For the stifle joint in animals sacrificed at the end of 2 years, there was only 1 control male with animal with a finding of malignant schwannoma.

For other joints (non-stifle) for all animals, there were 13 males (112 control and 1 mid dose male that were examined based on gross observations of swollen or enlarge joint. Only one control male had chronic-active inflammation (the joint was not specified), the other were classified as nondiagnostic or no abnormalities.

Mammary gland: There were dose-related reductions in the incidence of mammary gland duct dilatation/galactocoele and fibroadenoma and to a lesser degree in carcinomas. These are likely related to CP-690550's pharmacologic effect of inhibiting prolactin's JAK/STAT signalling pathway, demonstrated in leydig cells and brown adipose tissue in exploratory studies submitted with this report (Reports 11GR016 and 11GR015).

Table 119: Nonneoplastic CP-690550-Related Changes in the Liver Heart, Adrenal Gland and Female Mammary Gland

Sex		Male				Female			
Group		1	2	3	4	1	2	3	4
Dose (mg/kg/day)		0	10	30	75	0	10	30	100/75
Number Examined		70	60	60	70	70	60	60	70
Liver									
Bile Duct Hyperplasia									
Minimal		31	15	19	13	12	4	5	8
Slight		9	4	1	0	6	1	1	0
Moderate		3	1	0	0	0	0	0	0
Marked		0	1	2	0	0	0	0	0
Total Affected (%)		61	35	37	19	26	8	10	11
Heart									
Murine Cardiomyopathy									
Minimal		23	18	16	21	23	15	15	19
Slight		27	23	23	23	14	9	10	7
Moderate		17	13	13	6	0	2	0	1
Total Affected (%)		96	90	87	71	53	43	42	39
Adrenal Cortex									
Cystic Degeneration									
Minimal		20	10	15	13	7	9	8	7
Slight		10	6	1	3	25	16	11	9
Moderate		2	0	0	0	26	13	11	11
Marked		0	0	0	0	7	6	5	9
Severe		0	0	0	0	1	0	2	0
Total Affected (%)		46	27	27	23	94	73	62	51
Number Examined		-	-	-	-	70	60	60	68
Female Mammary Gland									
Duct Dilatation/Galactocele		-	-	-	-	55	43	32	33
Fibroadenoma		-	-	-	-	25	17	11	12
Carcinoma		-	-	-	-	18	19	16	11

- = Not applicable.

Toxicokinetics

Blood samples were collected via a jugular vein on day 129 (females)/130 (males) from the first three animals/sex in Group 4 predose and approximately 1 and 8 hours postdose. In week 26, day 177(females)/178 (males), the first three animals/sex/time point for Groups 2, 3 and 4 were bled approximately 0.5, 1, 2, 4, 8, and 24 hours postdose, and the first three animals/sex/time point for Group 1 were bled approximately 0.5, 2, and 8 hours postdose. Toxicokinetic evaluations were done by (b) (4)

(b) (4)

Systemic exposure as assessed by C_{max} and AUC_{0-24} during week 26 of the dosing phase increased with increasing dose of CP-690550. AUC_{0-24} exposure was higher in females than in males at the same doses by 200%, 240%, and 153% at 10, 30 and 75 mg/kg/day, respectively. There were no quantifiable concentrations of CP-690550 in samples analyzed from control animals.

For the high dose, 75 mg/kg/day, toxicokinetic parameter values determined in this study were similar to the values obtained at 100 mg/kg/day in the 6-month rat toxicology study (Report 77435).

Mean Toxicokinetic Parameters at week 26

Dose	10		30		75	100/75*
Gender	M	F	M	F	M	F
T _{max} (h)	0.5	0.5	0.5	0.5	2.0	2.0
C _{max} (ng/mL)	1,600	2,800	4,190	6,940	7,760	9,450
AUC ₀₋₂₄ (ng-h/mL)	3,880	7,850	12,600	30,200	44,400	68,100
* Females were dosed at 100 mg/kg/day from day 1 to day 132 (in week 19), then the dose was reduced to 75 mg/kg on day 133 and continued at this dose until the study termination. Therefore, toxicokinetic females received 75 mg/kg from day 133 to day 177, for 44 days.						

Stability and Homogeneity

Stability: The stability of samples prepared at 0.1 to 200 mg/mL had been established by the applicant for a period of at least 8 days when stored at room temperature, refrigerated, and frozen conditions. This was verified again for this study. Sets of duplicate samples (1.0 mL each) were taken from the middle of dose formulations at 1 and 50 mg/mL and stored at -10 to -30°C, or stored refrigerated or at room temperature for 8 to 15 days. Dose formulations prepared at 1.0 and 50 mg/mL were confirmed to be stable for at least 15 days under room temperature and refrigerated conditions.

Homogeneity: Duplicate samples (1.0 mL each) were taken from the top, middle, and bottom of the low-, mid-, and high- (male and female) dose formulations prepared for week 1, day 51 (daily aliquot; mid dose only), day 70 (daily aliquots; all concentrations), week 16 (all concentrations), and week 95 of the dosing phase due to a batch size change. Also, two sets of single samples (1.0 mL each) were taken from the middle of the control formulation, except for Week 95 of the dosing phase. Mean homogeneity results for all samples analyzed varied from 97.5 to 104% of the respective theoretical value, and the individual mean value of each location was within 7% of the overall mean.

Concentration Verification:

Two sets of duplicate samples (1.0 mL each) were taken from the middle of each batch of dosing formulations prepared for use during Weeks 1, 13, 26, 39, 52, 65, 78, and 91 of the dosing phase. The middle sample collected for Week 1 of the dosing phase homogeneity analysis also served as concentration verification. Mean concentration results for all samples analyzed varied from 97.0 to 105% of the respective theoretical value.

Additional Studies

RAT LEYDIG CELLS

Study title: Investigative Study with Rat Primary Leydig Cells

Study no.:	11GR016
Study report location:	Module 4.2.3.4.2 11GR016
Conducting laboratory and location:	Pfizer Worldwide Research & Development Groton, CT
Date of study initiation:	unknown, report signed Aug 5 2011
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	CP-690550-10 citrate salt, Lot GR02684, Purity not provided

The objective of these experiments was to determine whether CP-690550, a Janus Kinase (JAK) inhibitor, is capable of inhibiting the effects of ovine prolactin (PRL) on isolated primary rat Leydig cells. There is evidence PRL stimulation is one mechanism of Leydig cell tumor formation, and this mechanism is possibly not relevant to human Leydig cell tumor formation.

Key Study Findings

CP-690550 dose-dependently blocked the effects of PRL-induced increase in LHR mRNA, either partially or completely, in primary rat Leydig cells, and is therefore capable of blocking PRL signaling in these cells.

The Applicant's hypothesis is that "CP-690550 blocks the PRL signal reducing STAT5A/B phosphorylation, lowering the amount of LH receptor mRNA, lowering the cell surface LH receptor protein, thereby reducing Leydig cell responsiveness to LH and reducing testosterone secretion. In response, there is a compensatory increase in circulating LH (due to a reduction in testosterone). Over a prolonged period of stimulation, there is increased division of Leydig cells, forming first foci of hyperplasia, and then adenomas. The applicant argues that Leydig cell tumors formed in this way were irrelevant for human risk assessment (reviewed in Cook et. al., 1999). This hypothesis of Leydig cell adenoma formation is not in agreement with the supposed reduction of LH receptors induced by CP-690550, reducing the ability of LH to stimulate of Leydig cell division. Also unexplained is why this mechanism is not relevant for humans.

BROWN ADIPOCYTE STUDIES**Study no.: An Investigative Study with Rat Brown Adipocytes Treated with Ovine Prolactin and CP-690550 (and Amendment 1 corrections to the final report)**

Study no.:	11GR015
Study report location:	Module 4.2.3.4.2 11GR015
Conducting laboratory and location:	Pfizer Worldwide Research & Development Groton, CT
Date of study initiation:	unknown, report signed July 11 2011
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	CP-690550-10 citrate salt, Lot GR02684, Purity not provided There was no Certificate of Analysis provided.

In the 2-year rat carcinogenicity study (Report 6348-463), CP-690550 resulted in a statistically significant incidence of malignant hibernomas in female rats in the mid and high doses, 30 and 75 mg/kg/day, compared to controls.

This study examined whether CP-690550 inhibits PRL-induced JAK/STAT signaling in rat brown adipocytes differentiated from the stromal vascular fraction brown adipose tissue as a mechanism involved in the occurrence of malignant hibernomas. Previous studies demonstrated that brown adipocytes from mice, including propagation of brown adipocytes from mouse hibernoma, is responsive to prolactin stimulation through the JAK2/STAT5 pathway (Yu-Lee et al, 1998; Viengchareun et al, 2008) a pathway possibly responsive also to CP-690550.

Key Study Findings

In cultured rat brown adipocytes, CP-690550 inhibited prolactin-induced increase in phosphorylated STAT5 and basal phosphorylated STAT-3 in a concentration-dependant manner at CP-690550 concentrations relevant to systemic exposures in the rat carcinogenicity study.

Without further study, its premature to extrapolate these results to the development of hibernomas in the carcinogenicity study, but if elevated PRL is associated with a reduction in brown adipose tissue weight as occurs during lactation demonstrated by Chan and Swaminathan (1990), then these data suggest that CP-690550, by suppressing PRL-induced changes in STAT5, and could allow for proliferative growth.

Study no.: 14-Day Oral Investigative Study of the Effects of CP-690550 on Brown Adipose Tissue in Female Sprague-Dawley Rats

Study number: 10GR431
Study report location: Module 4.2.3.4.2
Conducting laboratory and location: Pfizer Worldwide Research & Development
Groton, CT
Date of study initiation: Jan 13 2011
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: CP-690550-10 (citrate salt), Lot GR02684, purity not provided

The objective of this study was to assess the effects of CP-690550 on brown adipose tissue after repeated oral doses in female rats. Doses in the current study (10, 30, and 75 mg/kg/day) were based on the doses used in the 2-year rat carcinogenicity study.

Key Study Findings

CP-690550 oral administration to female rats at 10, 30, and 75 mg/kg/day for 14 days inhibited of the JAK/STAT signaling pathway evident by decreased (pSTAT5A/B, pSTAT3) (≥ 10 mg/kg/day) and UCP-1 protein (≥ 30 mg/kg/day) in association with increased brown adipose tissue weight (75 mg/kg/day) and cell proliferation (≥ 30 mg/kg/day). The reviewer agrees that since these changes occurred at doses and exposures associated with higher incidence of hibernomas in a 2-year rat carcinogenicity study, hibernoma formation may have resulted from disruption of the JAK/STAT pathway in brown adipose tissue, but whether this is the actual mechanism or not does not discount the occurrence associated with CP-690550 treatment in the carcinogenicity study..

Study title: 14-Day Oral Investigative Study of CP-690550 on Plasma Norepinephrine in Female Sprague Dawley Rats

Study no.: 11GR383
Study report location: Mod 4.2.3.4.2
submitted in SD-9, Feb 16 2012
Conducting laboratory and location: Pfizer Worldwide Research & Development
Drug Safety Research & Development
Eastern Point Road
Groton, CT 06340
Date of study initiation: Nov 1, 2011
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: CP-690550-10 (citrate salt), Lot

GR05402, Purity not provided

Composition: 61.7% active moiety, CP-690550.

The study was conducted to determine if sympathetic stimulation occurs following oral CP-690550 administration, since in the 2 year rat carcinogenicity assay hibernomas occurred in female rats and sympathetic stimulation may have contributed to brown adipose tissue proliferation. The sympathetic drugs phentolamine and varenicline, increased the incidence of hibernomas in rats (Poulet et al, 2004; Brees et al, 2008).

Possible evidence for sympathetic stimulation following CP-690550 administration in rats was previously observed in cardiovascular safety pharmacology studies (Report 11GR001) where a decrease in blood pressure was accompanied by an increase in heart rate.

Key Study Findings

- Oral administration of CP-690550 to female rats at 10, 30, and 75 mg/kg/day for 14 days resulted in increases of plasma for norepinephrin (NE) levels at ≥ 30 mg/kg/day on the first day, and at ≥ 10 mg/kg/day on day 14.
- AUC and Cmax were not calculated for NE over a comparable period as CP-690550 measurement and there were no statistical assessment for correlations between NE and CP-690550 levels. Only for day 14 were individual values presented for CP-690550 and NE at same timepoints.
- Without further bioanalytical and statistical analysis, the contribution of systemic norepinephrine as a stimulus for hibernoma development remains just a

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STUDIES IN MICE

Study title: 6-Month Oral Gavage Carcinogenicity Study with CP-690550 in Model 001178-T (hemizygous), CB6F1/Jic-TgrasH2@Tac Mice and Model 001178-W(homozygous wild type), CB6F1/Jic-TgrasH2@Tac Mice for Toxicokinetic Exposures

Study no.: 08GR481
Study report location: Module 4.2.3.4
Conducting laboratory and location: (b) (4)
Date of study initiation: Jan 13 2009
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: CP-690550-10, Lot E010008412, Purity 100.1% (by HPLC) counterion content 37.0%, total impurities (b) (4)
CAC concurrence: Yes, Fax to Applicant Nov 7 2008
Yes, for Carc study results March 6, 2012

Key Study Findings

There was no evidence for CP-690550-related oncogenic potential in rasH2 (hemizygous) mice that received CP-690550 via oral gavage for 6 months at dose levels of 25, 75, and 200 mg/kg/day.

Clinical signs (hypoactivity, recumbency) and nonneoplastic microscopic changes (subphyseal hypocellularity in the femur, red pulp cellular depletion in the spleen) were observed at 75 and/or 200 mg/kg/day. No incidence of tumors or tumor combinations was statistically significantly different from vehicle control in any of the CP-690550 treated groups. The review (Jan 31 2012) by Dr. Matthew Jackson in the Division of Biometrics arrived at similar conclusions regarding the data.

CP-690550-related, nonneoplastic microscopic findings were present in the femoral bone marrow (focal subphyseal hypocellularity characterized by an increased prominence of adipocytes) of males at 75 and 200 mg/kg/day and females at 200 mg/kg/day and in the spleen (cellular depletion, red pulp) of males at 75 and 200 mg/kg/day and females at 200 mg/kg/day.

Toxicokinetic analysis during week 20 of the 26-week study, demonstrated increasing C_{max} and AUC_{0-24} with increasing CP-690550 dose and no gender differences. At the NOAEL for CP-690550-induced malignancy, 200 mg/kg/day, the gender-averaged parameters were T_{max} 0.75 hr, C_{max} 5765 ng/mL, and AUC_{0-24} 17250 ng-h/mL. Comparison of drug exposure for the mouse with the estimated human AUC_{0-24} of 550

ng-h/mL for the maximal dose of 10 mg BID (20 mg/day), resulted in an approximate 32-fold exposure margin.

Adequacy of Carcinogenicity Study

The study was adequately conducted and interpreted.

Appropriateness of Test Models

The transgenic rasH2 mouse model was determined appropriate by the ECAC as revealing the carcinogenic potential of some compounds during a shorter period of study than the classical 2 year bioassay. This model is insufficient to determine carcinogenicity due to immunosuppressive actions, which is the demonstrated mechanism of action of CP-690550.

Evaluation of Tumor Findings

The evaluation of tumor by the Applicant was adequate. The review by Dr. Matthew Jackson in the Division of Biometrics arrived at similar conclusions regarding the data.

Methods

Doses:	0, 25, 75, 200 mg/kg/day
Frequency of dosing:	Dose concentrations were corrected for potency based on a correction factor of 1.621, due to citrate counterion content of 37% once daily for 26 weeks for groups 1-4 and 20 weeks for TK groups 6-9
Dose volume:	10 mL/kg
Route of administration:	oral gavage
Formulation/Vehicle:	0.5% methylcellulose (w/v), 4000 cps, in reverse osmosis deionized water
Positive control	N-Nitroso-N_Methlyurea (MNU), Lot XU1086, administered on day 1, a single dose of 75 mg/kg, IP, in vehicle of acidified saline, at a volume of 10 mL/kg (7.5 mg/mL)
Basis of dose selection:	Preliminary 7-day escalation dose study (Study 07KL023) and a 4-week toxicological study (07GR160) demonstrated that 200 mg/kg/day was the maximum dose that could be administered without causing death due to non-oncogenic effects. A dose of 500 mg/kg/day resulted in death, decreased activity, hunched posture, and altered respiration. At 250 mg/kg/day, transient ataxia, hypoactivity, squinted eyes, irregular respiration, tremors,

and recumbency occurred.

Species/Strain: Mouse:
for toxicity study: Model 001178-T
(hemizygous), CB6F1/Jic-TgrasH2@Tac
for toxicokinetics: Model 001178-W
(homozygous wild type), CB6F1/Jic-
TgrasH2@Tac

Number/Sex/Group: Main study 25/sex/group;
positive control 15/sex/group
TK: 15/sex/group

Age: 7 weeks of age
body weight males 18.5 to 26.1 g
females 13.9 to 21.5 g

Animal housing: Individually housed in stainless steel cages

Paradigm for dietary restriction: No dietary restriction

Dual control employed: No

Interim sacrifice: None

Satellite groups: TK groups 6-9

Deviation from study protocol: There were deviations to the study protocol as described below, but these did not affect the study conclusions concerning the potential carcinogenic effects of CP-690550. They might have contributed to the non-neoplastic findings, but their absence is acceptable for this type of study focusing on carcinogenic effects.

Protocol Amendment 4 removed footnote b from the Tissue Preservation list for the femur with marrow (articular surface of the distal end to include stifle joint) which indicated "b Collect and preserve for possible future microscopic examination distal end to include stifle joint"

It is not clear how this affected the study interpretation, since bone marrow was included in the histopathological analysis with the following result "Test article-related nonneoplastic microscopic findings were present in the bone marrow and spleen of males and females." However, the articular joint itself was not mentioned and therefore probably not evaluated microscopically if gross lesions were not identified. There was no determination of erythroid/myeloid cell ratios in the bone marrow.

Table 120: Study Design

Group ^a	No. of Animals		Dose Level (mg/kg/day; corrected)	Dose Concentration (mg/mL; corrected)
	Male	Female		
Toxicity Animals				
1 (Control)	25	25	0	0
2 (Low)	25	25	25	2.5
3 (Mid)	25	25	75	7.5
4 (High)	25	25	200	20.0
5 (Positive Control) ^c	15	15	75	7.5
Toxicokinetic Animals ^b				
6 (Control)	9	9	0	0
7 (Low)	15	15	25	2.5
8 (Mid)	15	15	75	7.5
9 (High)	15	15	200	20.0

- a Groups 1 and 6 received control article only, 0.5% methylcellulose (w/v), 4000 cps, in reverse osmosis/deionized water, which also served as the vehicle for CP-690,550 formulations. Dose concentrations were corrected for potency based on a correction factor. Dose volume for all groups was 10 mL/kg.
- b Toxicokinetic animals were included solely for the purpose of blood sample collections.
- c Group 5 animals were dosed with one intraperitoneal dose of N-nitroso-N-methylurea (MNU) in acidified saline (physiological saline, 150 mM sodium chloride and 15 mM sodium citrate, adjusted to pH 4.5 with 1 N hydrochloric acid), on Day 1 of study at a dose level of 75 mg/kg/mouse and a dose volume of 10 mL/kg.

Observations and Results

Mortality checked twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress.

Survival for the high dose 200 mg/kg/day Group 4 males was statistically significantly less than vehicle control. [Dr. Matthew Jackson's statistical analysis from the Office of Biometrics confirmed there was a significant dose related effect in mortality for males ($p=0.0022$, trend analysis), due to a significant increase in high dose mortality (Chi squared test, $p=0.013$)]. The Applicant's survival plots for male and female mice are presented below.

There were 7 deaths (3 males and 4 females). All male deaths (#A11703 on day 118, #A11704 on day 119, #A11702 on day 134) occurred at the high dose, 200 mg/kg/day. One female death occurred at each dose including the vehicle control (control #A11791 on day 97, low dose #A11821 on day 98, and mid dose #A11849 on day 145, and high dose #A11866 on day 70). There were no obvious clinical signs prior to deaths and any findings such as hypoactivity and recumbancy were common findings of others in their dose group. Gross and histopathology did not reveal a cause of death.

The only death for which a cause was determined occurred in the mid-dose, 75 mg/kg/day, female (#A11849) and found dead on day 145 was attributed to an invasive and metastatic squamous cell carcinoma of cutaneous origin. This animal's records indicated that there was no palpable mass detected before death although the size at death is fairly substantial 22 x 10x 10 mm for a mouse. The only noted signs were swollen ventral neck (from day 134) and rough haircoat (day 141) a few days before death. There was no change in body weight or food consumption compared to other

mice in this group during the weeks prior to death. Histopathology indicated metastatic cells were found in the thoracic cavity, lung, and mandibular salivary gland. Moderate myeloid hyperplasia was observed in the femur and sternum bone marrow. There is insufficient information with this transgenic mouse to know if this is a common background finding. Thymus and parathyroid tissue were noted as inadequate or unreadable and were not examined. Other tissues were noted as unremarkable.

There was a significant effect of the positive control, MNU, group on mortality with a total of 21 deaths (10 males and 11 females). MNU-related deaths were most often attributed to the presence of lymphosarcoma (7 females, 8 males) and occurred as early as Days 78 and 100 in males and females, respectively, with other tumor.

Figure 13: Survival Plot for Males

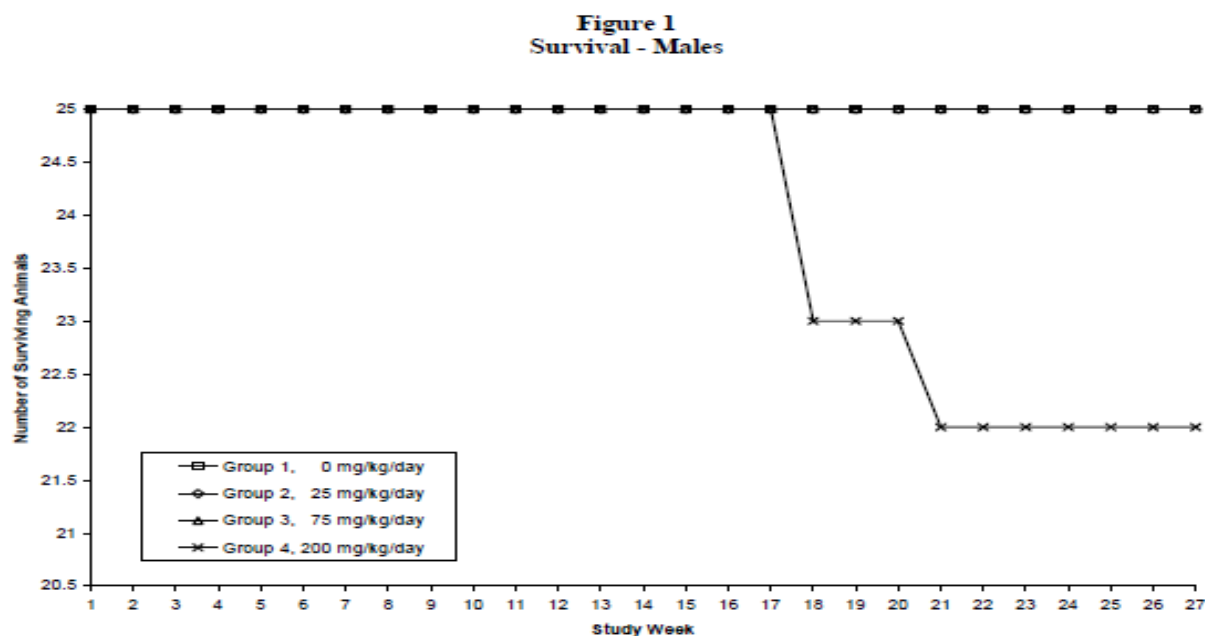
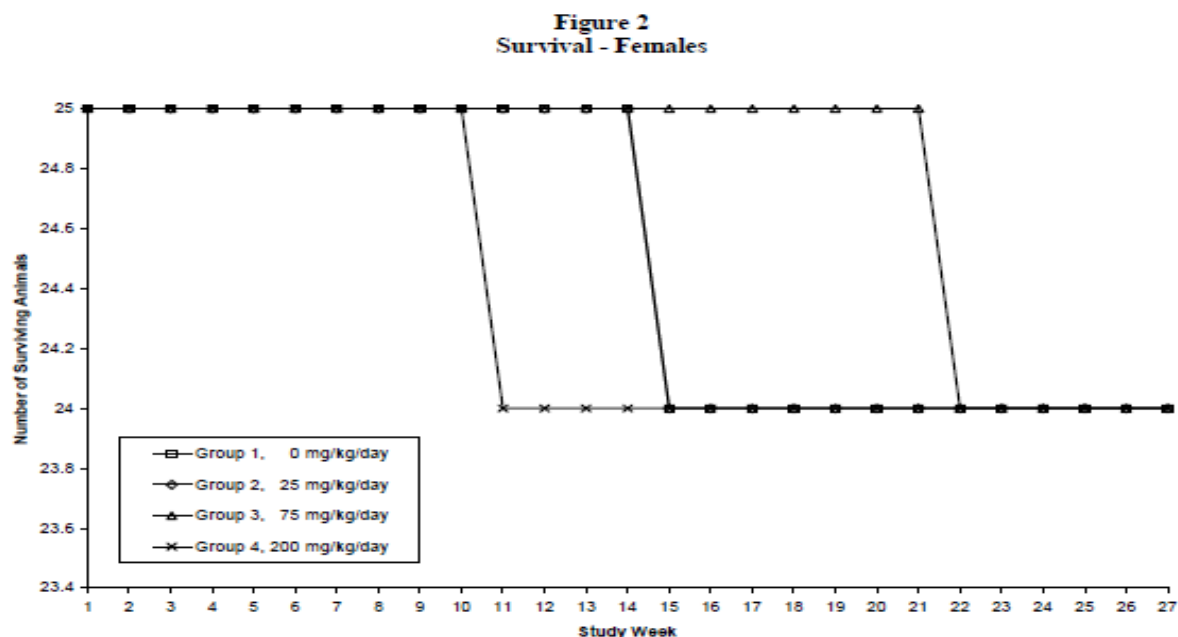


Figure 14: Survival Plot for Females

Clinical Signs checked twice daily (a.m. and p.m.) abnormalities, and signs of pain or distress

All 200 mg/kg/day animals exhibited CP-690550-related hypoactivity or recumbency on multiple days throughout the study from day 1 to the last day occurring randomly. For some individual animals, reduced activity was observed on $\geq 15\%$ of the dosing days.

Body Weights measured weekly

There was an overall effect of a CP-690550-related reduction in mean body weight in males (-4 to -7%) and females (-3 to -4%) by the end of the study.

Table 121: Body Weights

Group	Males					Females				
	1	2	3	4	5	1	2	3	4	5
Dose (mg/kg/day)	0	25	75	200	MNU	0	25	75	200	MNU
Week 1 n	24	25	25	25	15	24	25	25	25	15
(g)	22.3	21.9	22.1	22.2	21.9	18.2	17.8	18.2	17.9	18.1
Week 27 n	25	25	25	22	5	24	24	24	24	5
(g)	28.8	27.6	26.9	27.3	28.4	22.9	23.1	22.2	21.9	24.6
% of control	(100)	(96)	(93)	(95)	(99)	(100)	(101)	(97)	(96)	(107)

Gain (g)	6.5	5.7	4.8	5.2	6.6	4.7	5.3	4.1	4.0	6.4
% of control	(100)	(88)	(74)	(80)	(101)	(100)	(132)	(87)	(85)	(136)

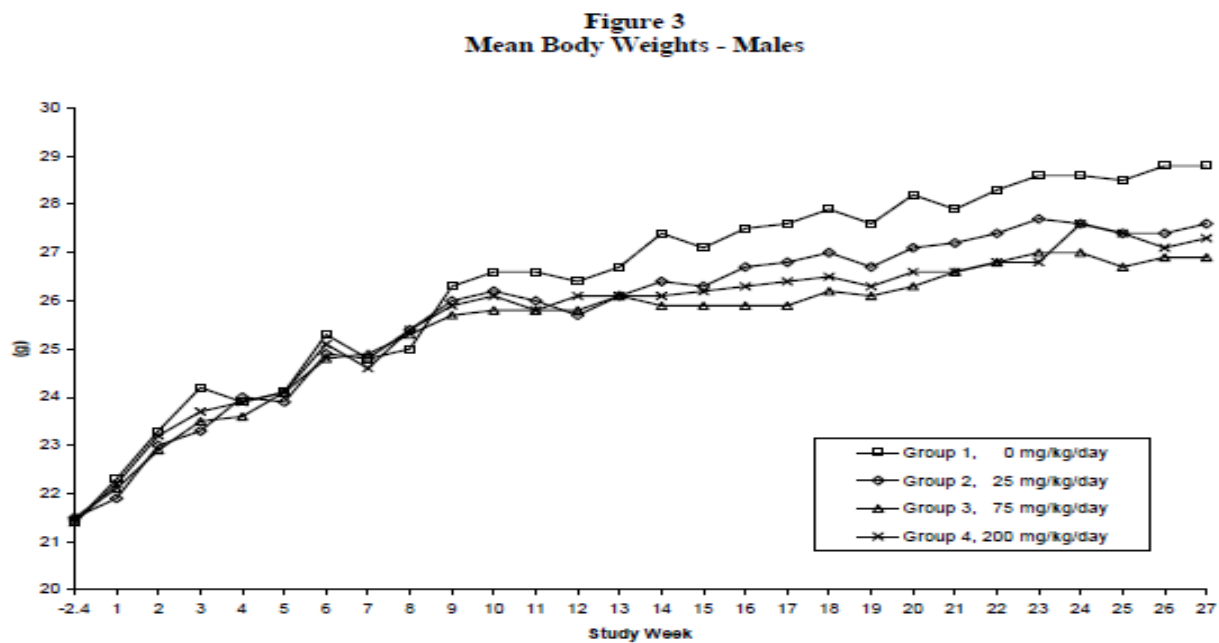
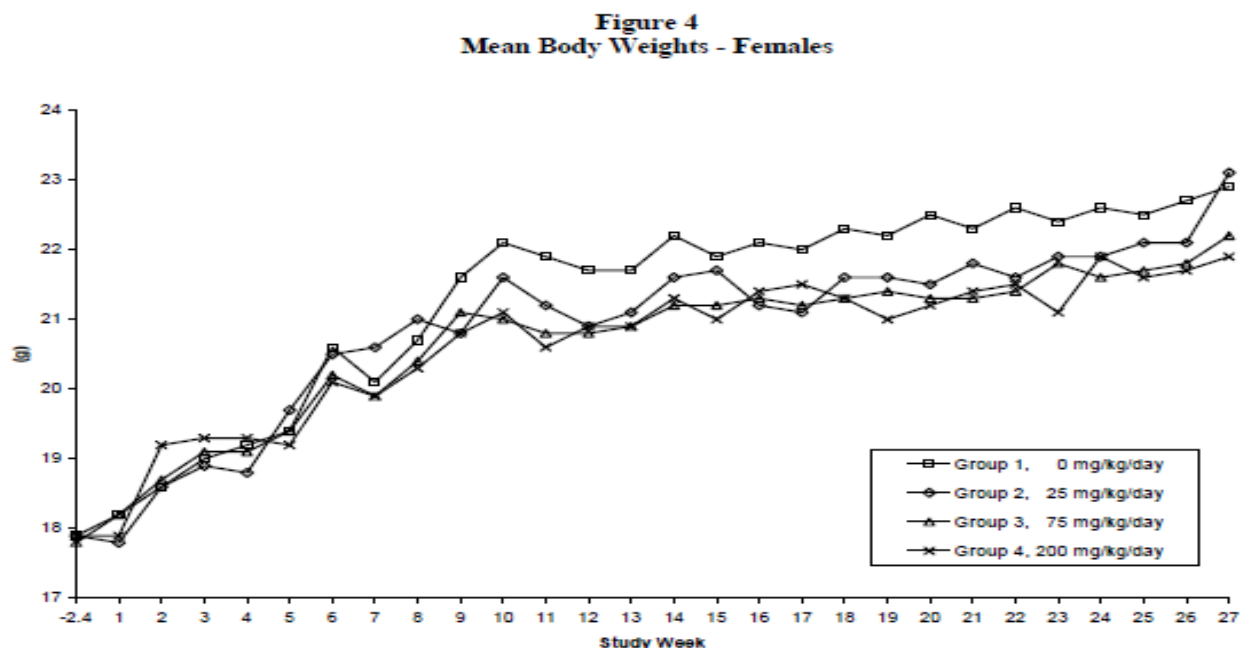
Figure 15: Growth Curve for Males

Figure 16: Growth Curve for Females

Feed Consumption measured weekly

Total mean food consumption was reduced in both males (-4 to -8%) and females (-3 to -6%) of CP-690550 treatment groups compared to vehicle treated controls.

Table 122: Percent Change in Food Consumption and Body Weight

Text Table 2
Mean Body Weight and Food Consumption - Percent Change from Control Values

Dose Level (mg/kg/day) Compared with Control (%)	25		75		200	
	Male	Female	Male	Female	Male	Female
Total Food Consumption	-4.4	-3.4	-3.8	-4.0	-8.1	-6.0
Mean Week 27 Body Weight	-4.2	+0.9	-6.6	-3.1	-5.2	-4.4

Gross Pathology

There were no test article-related macroscopic observations in males or females. Treatment-related macroscopic observations in the positive controls administered MNU were present in the forestomach (mass/raised area), skin (mass), and thymus/spleen (large/mass) of males and females. These observations were generally related to the presence of neoplasms at those sites.

Histopathology

Peer Review

Yes, initially by the CRO Pathologist, then by the Applicant's Pathologist, (refer to Protocol Amendment 3, page 3). This is not considered an independent peer review.

Neoplastic

No incidence of tumors or tumor combinations was statistically significantly different from vehicle control in any of the CP-690550 treated groups.

There was no CP-690550-related effect on the incidences of neoplasms in either sex. There was no statistically significant increase or dose-response for tumor or tumor combinations in any of the CP-690550 treated groups compared to the vehicle control group. The positive control (Group 5) had a statistically higher incidence of neoplasms than the vehicle control.

The observed neoplasms in vehicle control and CP-690550-treated groups included hemangiosarcoma (primarily in the spleen), bronchiolar-alveolar adenoma or carcinoma of the lung, and Harderian gland adenoma. The type and incidence of these neoplasms were similar to those previously reported in control rasH2 mice from other studies (Morton et al., 2002; Takaoka et al., 2003; Kanno et al., 2003), and confirmed by the Reviewer.

Thymomas occurred in the females low and mid dose groups (incidence of 1/24 or 4.17% for the 25 mg/kg/day dose, and 1/23 or 4.35% for the 75 mg/kg/day dose). This finding was not statistically significant based on trend analysis or pairwise analysis to the vehicle control. Historical control data for this finding in these transgenic mice was not provided. Except for one mid dose male, there was no hyperplasia in the CP-690550 treated groups or vehicle control. The thymomas in the mouse are therefore not related to CP-690550 treatment.

As noted in the Statistical Review, in males and females there were high number of parathyroid autolysis tissues (ranging from 32% to 56% of the tissues) which were not appropriate to analyze, therefore malignant potential on the parathyroid gland was considered inconclusive for this study.

The majority of positive control (Group 5) animals given MNU that survived to the end of the study developed squamous cell papilloma or carcinoma of the nonglandular forestomach. The combined incidence of these forestomach tumors was significant (Applicant's test: Fisher's p-value <0.0001) compared with control for both males and females. As noted above, early mortality was most often attributed to lymphosarcomas in the positive control animals (8 males and 7 females). Other common tumors in the positive control group included skin tumors (keratoacanthoma, 1 male, 7 females; squamous cell papilloma, 1 male, 2 females; or squamous cell carcinoma, 0 male, 1 female) also represented a tumor effect of MNU administration, primarily in females. The combined incidence of these skin tumors was significant (Applicant's test: Fisher's p-value <0.0001) compared with control for females.

Neoplasms in Mice of Study 08GR481

Group	1		2		3		4		5	
Dose (mg/kg/day)	0		25		75		200		MNU	
	M	F	M	F	M	F	M	F	M	F
Body whole/cavity										
angiomas	0	0	0	0	0	0	0	0	0	0
hemaangiomas	0	0	0	0	1	0	0	0	0	0
hemangiosarcoma	2	4	3	2	0	0	1	0	2	1
combined	2	4	3	2	1	0	1	0	2	1
Lymphosarcoma	0	0	0	0	0	0	0	0	8	7
Harderian Gland										
adenoma	1	0	0	2	1	1	0	1	0	
Lung										
Bronchiolar-alveolar carcinoma	0	1	0	0	1	0	0	0	0	0
adenoma	1	1	0	2	1	2	1	0	0	0
combined	1	2	0	2	2	2	1	0	0	0
Skin/Subcutis										
Squamous cell carcinoma	0	0	1	0	0	1	0	0	0	1
papilloma	0	0	0	0	0	0	0	0	1	2
combined	0	0	1	0	0	1	0	0	1	3
Keratoacanthoma	0	0	0	0	0	0	0	0	1	7
Thymus										
Malignant thymoma	0	0	0	1	0	1	0	0	0	0

Non-Neoplastic

Non-neoplastic toxicities occurred in the bone marrow, spleen, and kidney.

Bone Marrow: The results are summarized in the Applicant's table below. CP-690550-related hypocellularity occurred in femur bone marrow but not in sternal bone marrow. The applicant described the hypocellularity as a localized (focal) area of decreased cellularity of the hematopoietic marrow component, mainly in the subphyseal area. Except for these focal areas, the morphology and overall cellularity of the hematopoietic cells throughout the bone marrow of the femur and sternum were similar in control and dosed animals. The focal areas of hematopoietic hypocellularity were characterized by an increased number/prominence of the normal marrow adipocytes and was not associated with degeneration, necrosis, inflammation, or an atypical

hyperplastic/proliferative appearance in either the marrow hematopoietic cells or adipocytes.

The incidence and severity of bone marrow hypocellularity increased dose dependently. from 4 of 25 in control males, to 25 of 25 at 200 mg/kg/day . At the high dose 17 of 25 had minimal hypocellularity and 8 of 25 had slight severity. The applicant described slight hypocellularity as being characterized by larger aggregates of adipocytes adjacent to the proximal physis with adipocytes also present in the shaft and epiphyseal marrow of the femur. For females hypocellularity of minimal severity was present in 22 of 25 control animals. The change most noticeable in females was the increase in severity with dose. All 25 high dose animals had marrow hypocellularity, but there was increase in 15 of the 25 to slight (n=14) and moderate severity (n=1).

Spleen: Cellular depletion of the red pulp was dose dependent (males: 3 of 25 at 75 and 15 of 25 at 200 mg/kg/day; females at 8 of 25 at 200 mg/kg/day. The applicant described these findings as decreased density/number of the mononuclear cells in the vascular sinusoids comprising the red pulp, often with increased congestion and an overall decrease in the amount of red pulp that separates the periaarteriole lymphoid sheaths (white pulp areas) in the spleen.

Kidney: There was a slight increase in the incidence of renal tubule cell regeneration (basophilic tubules) was present in males (11 of 25) at 200 mg/kg/day compared with control (7 of 25). This finding had previously been reported as a spontaneous, background finding in the kidney of control Tg-rasH2 mice from 1- and 6-month studies (Morton et al., 2004; Kanno et al., 2003). Based on these published studies, the Applicant considers this finding incidental and not related to treatment with CP-690550. However, the Reviewer considers the presence in males at an incidence much larger than controls, indicative that at high doses of CP-690550 can exacerbate background incidences of renal tubule cell regeneration (basophilic tubules), in other words there is effect on the kidney by CP-690550, although it may not be apparent at low doses.

Table 123: Incidence of CP-690550-related findings for Bone Marrow

Incidence and severity of test article-related microscopic findings								
Sex		Male				Female		
Dose level (mg/kg/day)	0	25	75	200	0	25	75	200
No. examined/group	25	25	25	25	25	25	25	25
Bone Marrow (femur)								
Hypocellularity, Focal								
Unremarkable	21	19	7	-	1	1	1	-
Minimal	4	6	18	17	22	23	21	10
Slight	-	-	-	8	2	1	3	14
Moderate	-	-	-	-	-	-	-	1
Average Severity Grade ^a	1.0	1.0	1.0	1.3	1.1	1.0	1.1	1.6
Spleen								
Cellular Depletion, Red Pulp								
Unremarkable	25	25	22	10	25	25	25	17
Minimal	-	-	3	15	-	-	-	8
Average Severity Grade	0.0	0.0	1.0	1.0	0.0	0.0	0.0	1.0

^aAverage severity grade determined by dividing the sum of severity scores for a finding in each group by the number of animals with the finding.

Toxicokinetics Blood samples (via CO₂ anesthesia and cardiac puncture) were from 3 mice/sex/group/time point, from Groups 7, 8, and 9, during week 20 (day 136) at approximately 0.5, 1, 3, 8, and 24 hours postdose. Samples from Group 6 (vehicle control) were collected at approximately 0.5, 8, and 24 hours postdose. Serum CP-690550 concentrations were determined using LCAP/MS/MS detection.

CP-690550 systemic exposure, C_{max} and AUC₀₋₂₄, at week 20 increased with increasing dose. Mean T_{max} at week 20 occurred at 0.50 hr in females or 1.0 hr in males. Sex-related differences in CP-690550 occurred at the high dose at week 20, with males having a 2.5-fold increase in C_{max}, and 1.5-fold increase in AUC₀₋₂₄ compared to females. However, since the high dose females had a lower C_{max} value, by 0.79%, than the mid dose females C_{max} values, the sex related effect is inconsistent and possibly a random event due to large variation in the data. The toxicokinetic animals were not examined by necropsy or histopathology to determine if there was some underlying physical explanation for the reduced C_{max} (ie., absorption) in these few animals, but no general explanation was apparent from histopathology of the main study animals.

Mean Toxicokinetic Parameters at week 20

Dose	25		75		200	
Gender	M	F	M	F	M	F
T _{max} (h)	0.5	0.5	0.5	0.5	1.0	0.5
C _{max} (ng/mL)	1380	1900	3530	4120	8260	3270
AUC ₀₋₂₄ (ng-h/mL)	1990	1860	6210	8880	20800	13700

Stability and Homogeneity

Stability was previously established by the applicant at 0.1 to 200 mg/mL for a period of at least 8 days when stored at room temperature, refrigerated, and frozen conditions. The stability of samples prepared at 1.0 to 50 mg/mL was confirmed for a period of at least 15 days when stored under room temperature and refrigerated conditions.

Homogeneity of the dosing solutions was verified. The mean value of each location (top, middle, bottom) of the 2.5, 7.5, and 20 mg/mL dose preparations ranged from 99.6 to 102% of theoretical values.

Concentrations of the dosing solutions were verified. The mean concentration results for all samples analyzed of dose formulations 2.5, 7.5, and 20 mg/mL, prepared in weeks 1, 9, 17 and 20 varied from 96.6% to 104% of the respective theoretical values.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Oral fertility and embryonic development study of CP-690550-10 in male and female rats

Study no.:	05GR051
Study report location:	Mod 4.2.3.5.1
Conducting laboratory and location:	Pfizer Global Research and Development, PGRD, Groton, CT PGRD, Groton, CT
Date of study initiation:	Jan 18, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CP-690550-10, Lot 52546-119-13HS, Purity 97.7% The active moiety comprised 60.3% of the drug substance.

There was no Certificate of Analysis included with this report. Purity was indicated in Table 2.6.7.4 in Module

Key Study Findings

- Females administered the high dose of 100 mg/kg/day had a 25% reduction in pregnancy rate. There was no effect of orally administered CP-690550 on estrous cyclicity, or copulation rate.
- Dams treated with 100 mg/kg/day in association with the reduced pregnancy rate had decreases in the number of corpora lutea, implantation sites, and viable fetuses; and increases in early resorptions, preimplantation loss and postimplantation loss. The 10 mg/kg/day dose resulted in increased postimplantation loss.
- There was no effect on pregnancy rate when males were treated with 100 mg/kg/day, but mating of treated males occurred prior to an exposure duration for an entire spermatogenic cycle. There were no effects on sperm motility and sperm counts. Sperm morphology was not evaluated.
- The NOAEL for female fertility was 1 mg/kg/day. The NOAEL for parameters of male fertility (sperm motility and counts) was 100 mg/k/day, but effects on subsequent fertility in untreated female mates were not properly tested. The design of the study with regards to CP690550 exposure from oral dosing and mating was inappropriate to adequately determine the male effects of CP-690550 on mating, as recommended in ICH5(R2). Thus male mating aspects of this study will not be incorporated into regulatory safety decisions. However, other

parameters measured such spermatozoa characteristics at the end of dosing are acceptable.

Methods

Doses: 0, 1, 10, 100 mg/kg

Dose in mg/kg is based on mg of active moiety of the drug substance.

Frequency of dosing: Once daily

Dose volume: 10 mL/kg

Route of administration: Oral by gavage

Formulation/Vehicle: Suspension in 0.5% methylcellulose,

Species/Strain: Rats, Sprague-Dawley

Number/Sex/Group: 20/sex/group

Satellite groups: Toxicokinetics: main study, first 4 treated rats/group

Study design: The study consisted of two phases.

Phase 1, female fertility and early embryonic development phase, untreated males were cohoused with treated females.

Females were dosed for 14 days prior to mating, throughout cohabitation period for maximum of 2 weeks, continuing through gestation day (GD) 7.

Phase 2, male fertility, treated males were cohoused with untreated females.

Untreated males from phase 1 were dosed for a minimum of 63 days, beginning 28 days prior to cohabitation with phase 2 untreated females.

For Phase 2, this is an inadequate assessment on male fertility, since there will be substantial numbers of sperm developed without drug exposure during a complete spermatogenic cycle of development at the time mating occurs [refer to ICH5(R2) Note 12 in Section 4.1.1.]

Phase 1

Group Number	Daily Dose ^a (mg/kg)	Drug Concentration (mg/mL)	Dose Volume (mL/kg)	Animal Numbers	
				Males ^b	Females
1	0	0	10	1-20	81-100
2	1	0.1	10	21-40	101-120
3	10	1	10	41-60	121-140
4	100	10	10	61-80	141-160

^a Dose levels are expressed as mg of active moiety per kg of body weight per day.

^b Phase I males were not dosed.

Phase 2

Group Number	Daily Dose ^a (mg/kg)	Drug Concentration (mg/mL)	Dose Volume (mL/kg)	Animal Numbers	
				Males	Females ^b
1	0	0	10	1-20	161-180
2	1	0.1	10	21-40	181-200
3	10	1	10	41-60	201-220
4	100	10	10	61-80	221-240

^a Dose levels are expressed as mg of active moiety per kg of body weight per day.

^b Phase II females were not dosed.

Deviation from study protocol: There was no section indicating deviations from the study protocol.

Observations and Results

Parameters and endpoints evaluated:

Phase 1: Mortality and clinical signs (daily), female body weights (day of arrival, once weekly prior to dosing, twice weekly during treatment period, and on GD 0, 3, 7, 10, and 14), male body weights (day after arrival, once weekly during the rest of phase 1), female feed consumption (twice weekly beginning ~ 2 weeks prior to mating and on GD 3, 7, 10, and 14), male feed consumption (not measured during phase 1), vaginal smears (daily starting 2 weeks prior to treatment and continuing until evidence of mating), Cesarean sections (GD 14), # corpora lutea, # implantation sites, # resorptions, toxicokinetics (dosing day 12).

Phase 2: Mortality and clinical signs (daily), female body weights (once weekly prior to gestation, and on GD 0, 3, 7, 10, and 14), male body weights (twice weekly), female feed consumption (not measured), male feed consumption (twice weekly during treatment but not during cohabitation), vaginal smears (daily starting 2 weeks prior to treatment and continuing until evidence of mating), cesarean sections (GD 14), # corpora lutea, # implantation sites, # resorptions, toxicokinetics (dosing day 12), male reproductive organs collected (testes, epididymis, seminal vesicles, prostate), sperm

analysis (sperm motility: % motile sperm; sperm count: expressed as 10^6 sperm/g cauda weight).

Mortality

Phase 1: There were no CP-690550-related deaths; 2 control females were euthanized, #93 was moribund and noted to have an obstructed stomach, and #89 was euthanized early because of her advanced stage of pregnancy (not further explained by the Applicant).

Phase 2: One male #65 in the 100 mg/kg/day dose group was found dead on dosing day 15 and there were no clinical signs prior to death and no necropsy findings.

The death of 1 in 20 males on day 15 of at least 63 days of dosing, while a concern, is probably not solely drug related although the contribution of CP690,550 cannot be ruled out completely given the lack of necropsy details.

Clinical Signs

Phase 1: There were no CP-690550-related clinical signs in the females.

Phase 2: For CP-690550 treated males, salivation was evident in 1 high dose (100 mg/kg/day) rat and noisy respiration in 1 mid- and 1 high dose (10 and 100 mg/kg/day) rat.

Body Weight

Phase 1: At 100 mg/kg/day, there was a significant decrease in body weight gain of females from GD 0-7 (86% of the gain of control group) and GD 7-14 (51% of the gain of the control group). There were no CP-690550-related effects on female body weight at 1 mg/kg/day or at 10 mg/kg/day. The decreased weight gain from GD 7-14 was attributed to the reduced number of viable fetuses (refer to Cesarean section results, below)

Phase 2: There was no CP-690550-related effect on male body weight or weight gain.

Feed Consumption

Phase 1: For the high dose females, there was a statistically significant increase (110%) in feed consumption during the pre-mating period (days 4-15 of treatment). There were no effects on feed consumption other times or in the 1 mg/kg/day or 10 mg/kg/day dose groups. The effect during the pre-mating period was small and not toxicologically significant.

Phase 2: For males, there was a statistically significant decrease in feed consumption on mating day 25 (93% of control) in the 10 mg/kg/day and 100 mg/kg/day dose groups. This small reduction at a single timepoint was unlikely to be toxicological significant.

Toxicokinetics

Phase 1: blood samples were collected on day 12 from the first 4 treated females/dose group at ~0.5 h postdose.

Phase 2: blood samples were collected on day 16 from the first 4 treated males/dose group at ~0.5 h postdose.

Exposure to CP-690550 was observed in males and females 0.5 hour after oral administration of CP-690550-10 at doses of 100, 10 or 1 mg/kg. Concentrations of CP-690550 increased with dose.

Appendix 1. Mean Serum Concentration Data (ng/mL) of CP-690,550 in Rats After Oral Administration of CP-690,550-10 for Study Day 12 (Females) and Study Day 44 (Males, Day 16 of Dosing)

Dose (mg/kg)	Study Day	Sex	Mean Serum Concentration (ng/mL) by Time		
			0.5 h		
			Mean	SD	n
1	12	Female	262	44.4	4
10	12	Female	3000	436	4
100	12	Female	8000	1770	4

Dose (mg/kg)	Study Day	Sex	Mean Serum Concentration (ng/mL) by Time		
			0.5 h		
			Mean	SD	n
1	44	Male	76.5	17.7	4
10	44	Male	1030	260	4
100	44	Male	4920	1000	4

Necropsy

Phase 1: There were no treatment-related gross necropsy findings.

Phase 2: There were no treatment-related gross necropsy findings.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

Phase 1: There were no effects of CP-690550 on estrous cyclicity, cohabitation length or copulation rate. There were no effects on fertility at 10 and 1 mg/kg/day. At the high dose (100 mg/kg/day) the pregnancy rate was significantly reduced (a rate of 75% compared to 100% in controls). There was no difference in copulation rates.

Phase 2: There were no effects of CP-690550 on estrous cyclicity, cohabitation length or copulation rate, or pregnancy rate for untreated females mated with treated males.

Summary of estrous cycles and mating duration

Group	1	2	3	4
Dose (mg/kg/day)	0	1	10	100
Phase 1: treated females mated with untreated males				
Number of animals with sufficient cycle premating / number of animals	17/19	20/20	19/20	20/20

examined				
Number of females with insufficient cycles pre mating	2	0	1	0
Estrous cycle length (days)	4.29	4.50	4.32	4.60
Number of estrous cycles	3.59	3.40	3.47	3.20
Total occurrences of estrus	50	59	55	56
Mating				
N	19	20	20	20
Mean number of days cohabitated	2.67	3.30	2.60	2.75
Phase 2: treated males mated with untreated females				
Mating				
N	20	20	20	20
Mean number of days cohabitated	2.90	4.80	3.50	2.75

Caesarean section

Phase 1:

Ten dams in the 100 mg/kg/day group had completely resorbed litters. Referring to the Tables below, the high dose group also had significant reductions in the number of corpora lutea, implantation sites, and viable fetuses and an increase in early resorptions, resulting in an increase in pre-implantation loss (27%/litter) and post-implantation loss (87%/litter). At 10 mg/kg there was a slight increase in postimplantation loss (15%/litter). There were no effects on Cesarean section parameters at 1 mg/kg.

Phase 2:

There were no adverse CP-690550-related effects on Cesarean section findings. The number of early resorptions was significantly decreased at 100 mg/kg (control 2.2/dam, 100 mg/kg/day 0.6/dam), resulting in a decrease in postimplantation loss (control 17%, 100 mg/kg/day 3.6%). Together these findings resulted in a greater number of fetuses surviving at 100 mg/kg (14.8) than in the control group (12.4). The applicant indicated that these changes were likely the result of normal biological variation, which is plausible. Although statistically significant it may not be relevant for human fertility.

Cesarean section**Summary of Pregnancy Findings**

Group	1	2	3	4
Dose (mg/kg/day)	0	1	10	100
Phase 1: treated females				
Copulation Rate	19/20 95%	20/20, 100%	20/20 100%	20/20 100%
Pregnancy Rate	19/19 100%	18/20 90%	19/20 95%	15/20 75%
Cesarean Data (GD 14)				
N	19	18	19	15
Corpora Lutea (n)	16.9	16.2	16.6	12.2
Implantation Sites (n)	15.2	15.6	15.3	9.1
Viable Fetuses (n)	13.6	14.9	13.2	1.6
Dead Fetuses (n)	0	0	0	0
Early Resorptions	1.6	0.7	2.1	7.5
Late Resorptions	0	0	0	0
Preimplantation Loss (%)	9.0	3.7	7.5	27.3
Postimplantation Loss (%)	10.5	5.3	14.7	86.6
Phase 2: treated males				
Copulation Rate	20/20 100%	18/20 90%	20/20 100%	20/20 100%
Pregnancy Rate	19/20 95%	17/18 94%	20/20 100%	19/20 95%
Cesarean Data (GD 14)				
N	19	17	20	19
Corpora Lutea (n)	15.9	16.7	17.2	16.4
Implantation Sites (n)	14.6	15.4	16.1	15.4
Viable Fetuses (n)	12.4	14.6	15.3	14.8
Dead Fetuses (n)	0	0	0	0
Early Resorptions	2.2	0.7	0.8	0.6
Late Resorptions	0	0	0	0
Preimplantation Loss (%)	8.7	8.1	5.6	5.8
Postimplantation Loss (%)	17.0	6.1	4.7	3.6

MALES

Untreated males from phase 1 were dosed for a minimum of 63 days after which organ weights, and sperm counts and motility were assessed. This is an appropriate drug exposure period for to evaluate drug-related effects. However, mating to untreated females occurred earlier, beginning 28 after the initiation of treatment. This is roughly half of the time period for a complete cycle of spermatozoa development. Therefore at the time of mating approximately half of the spermatozoa would have gone through a

normal developmental process if the drug effects were in the first half of spermatozoa development.

Phase 2:

Necropsy: There were no treatment-related gross necropsy findings.

Male reproductive organ weights: Male reproductive organ weights were obtained for the epididymis, testes, and accessory sex glands (prostate, seminal vesicles, and coagulating glands weighed as a single unit), and the right cauda epididymis (weighed at the time of sperm analysis was conducted).

There was a significant increase (110% of control) in relative testis weight (expressed as a % of body weight) in the mid dose group, 10 mg/kg/day, but was not considered toxicologically relevant due to lack of dose-relationship (not observed in the high dose group).

Sperm analysis: The right cauda epididymis was homogenized and evaluated for sperm number after mixing with a DNA-specific fluorescent dye

There was a statistically ($p < 0.05$) a slight increase in sperm counts with dose, but this is unlikely to be toxicologically significant or clinically meaningful.

Sperm Motility: Sperm were obtained from a small (~1 cm) distal section of the right vas deferens and analyzed by an IVOS system. Two-hundred sperm were analyzed from each rat.

There was no effect of CP-690550 on sperm motility.

Summary of Sperm Parameters (Sperm Motility and Sperm Count)

Dose (mg/kg)		0	1	10	100
Sperm Motility ^a	Mean	91.20	87.55	95.30	94.58
	S.D.	17.80	24.45	2.25	1.74
	N	20	20	20	19
Sperm Counts ^b	Mean	777.05	695.23	764.76	874.95*
	S.D.	159.85	184.95	187.95	114.34
	N	20	20	20	19

^a Percent motile cells.

^b Million sperm/gram cauda weight.

* $p \leq 0.05$ Jonckheere–Terpstra trend test.

Stability and Homogeneity

Stability: CP-690550-10 was demonstrated to be stable in 0.5% methylcellulose over a concentration range of 0.1 to 200 mg CP-690550/mL for a period of 13 days when stored at room temperature.

Homogeneity: Verification of the concentration and homogeneity of the dosing formulations was performed on Study Day 2. Samples of CP-690550-10 and control formulations were collected from the top, middle, and bottom of each formulation prior to dosing and from the middle of each formulation following dosing. All samples were within specifications ($\pm 10\%$ of intended concentrations) and were considered homogenous.

Concentration: Samples for formulation concentration verification were also be collected from middle of each formulation prior to and following dose administration from formulations prepared for dosing on Weeks 7 and 11. All samples were within specifications ($\pm 10\%$ of intended concentrations) and were considered appropriately prepared.

FERTILITY IN JUVENILE RATS

Study title: Fertility Study of Tasocitinib (CP-690550) in Juvenile Rats

(Title note: the generic drug name tasocitinib was later changed to tofacitinib)

Study no.:	09GR250
Study report location:	Mod 4.2.3.5.4
Conducting laboratory and location:	Pfizer Global Research and Development, Drug Safety Research and Development, Groton, CT
Date of study initiation:	March 4, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CP-690550-10, GR02684 (sub-lot of Lot E010009450) Purity 99.9% The active moiety comprised 61.8% of the drug substance.

Key Study Findings

- Doses of CP-690550-10 were administered orally at 0, 1, 10 or 100 mg/kg/day to juvenile rat from PND 21 to 55 for females and PND 21 to 70 for males. Mating of treated females with untreated males and treated males with untreated females started on PND 84. The design of the study with regards to CP690550 exposure from oral dosing and mating was inappropriate to adequately determine the effects of CP-690550 on mating, as recommended in ICH5(R2). The results from mating will not be incorporated into regulatory safety decisions. However, other parameters measured such as estrous cyclicity and spermatozoa characteristics are acceptable.
- The systemic exposure increased with increased dose. The C_{max} and AUC₀₋₂₄ in males at 100 mg/kg at the end of dosing (PND 70) were 7480 ng/mL and 67500 ng-h/mL, respectively. The C_{max} and AUC₀₋₂₄ in females at 100 mg/kg at the end of dosing (PND 55) were 10100 ng/mL and 77200 ng-h/mL, respectively.
- There was no effect of CP-690550 treatment on signs of sexual development (mean day of preputial separation, PND 40-43 or mean day of vaginal opening, PND 33) or mating and fertility (assessed 35 day after the last dose in females and 21 days after the last dose in males) and male reproductive organ weights, and spermatozoa number and motility (assessed 60 days after the last dose).
- Dosing from weaning (PND 21) through the expected pubertal period for males, and up to the peripubertal period for females, did not affect fertility and mating parameters when evaluated at 21 days after the end of treatment for males and at 35 days after the end of treatment for females. Again, an inappropriate testing design.

Methods			
Doses:	0, 1, 10, 100 mg/kg Dose levels expressed as mg/kg refer to mg of the active moiety of the drug substance.		
Frequency of dosing:	Once daily, males for 50 days from PND 21 to PND 70 females for 35 from PND 21 to PND 55		
Dose volume:	10 mL/kg		
Route of administration:	Oral by gavage		
Formulation/Vehicle:	Suspension in 0.5% methylcellulose,		
Species/Strain:	Rats, Sprague-Dawley		
Number/Sex/Group:	20/sex/group		
Satellite groups:	Toxicokinetics: 3 CP-690550 treatment groups 24/sex/group 1; control group of 10/sex/group		
Study design:	F ₀ females were obtained with with a cross-fostered litter of 6 F ₁ males and 6 F ₁ females. None of the F ₀ females were treated. By PND 14, litters were culled to 5 males and 5 females.		
Events/ Observations	Dates ^a	Study Day ^b	Age
F ₀ Females/ F ₁ pups arrived	18 Mar 2010 (TK phase)		NA
	25 Mar 2010 (Fertility phase)	pre	
Dosing began – F ₁ generation pups	29 Mar 2010 (TK phase)	1	PND 21
	05 Apr 2010 (Fertility phase)		
TK serum collection (first dose)	29 Mar 2010	1	PND 21
Dosing ended – F ₁ female	02 May 2010 (TK phase)	35	PND 55
	09 May 2010 (Fertility phase)		
Dosing ended – F ₁ male	17 May 2010 (TK phase)	50	PND 70
	24 May 2010 (Fertility phase)		
TK serum collection for females	02 May 2010	35	PND 55
TK serum collection for males	17 May 2010	50	PND 70
Untreated females arrived	20 May 2010	NA	NA
Estrous cycle monitoring began treated and untreated females	24 May 2010	57	PND 70
Untreated males arrived	27 May 2010	NA	NA
Treated females/untreated males mating & treated males/untreated females mating began	07 Jun 2010	71	PND 84
Treated and untreated females cesarean section	22 Jun 2010 – 05 Jul 2010	NA	GD 14
Treated male necropsy	12-13 July 2010	106-107	PND 119-120
^a Actual dates varied due to increased or decreased length of parturition of F ₀ dams.			
^b Study Day 1 was the first day of dosing.			
TK = toxicokinetic, PND = postnatal day, GD = gestation day			

Animal Subsets	Group Number	Daily Dose ^a (mg/kg)	Drug Concentration (mg/mL)	Dose Volume (mL/kg)	Animal Numbers	
					Males	Females
Fertility Phase (Treated)	1	0	0	10	1-20	81-100
	2	1	0.1	10	21-40	101-120
	3	10	1.0	10	41-60	121-140
	4	100	10	10	61-80	141-160
Toxicokinetic Phase ^b (Treated)	5	0	0	10	201-210	301-310
	6	1	0.1	10	211-234	311-334
	7	10	1.0	10	235-258	335-358
	8	100	10	10	259-282	359-382
Untreated Females	9	c	c	c	-	401-420
	10	c	c	c	-	421-440
	11	c	c	c	-	441-460
	12	c	c	c	-	461-480
Untreated Males	13	c	c	c	501-520	-
	14	c	c	c	521-540	-
	15	c	c	c	541-560	-
	16	c	c	c	561-580	-

^a All dose levels are expressed as mg of active moiety per kg of body weight.

^b The TK animal numbers include the first dose (PND 21) and last dose TK animals. Therefore, after the PND 21 terminal blood collections (84 animals), only the remaining 80 animals were dosed throughout the treatment period.

^c Not dosed, used for mating with treated animals of opposite sex.

Deviation from study protocol:	There were no protocol deviations that affected the study results or conclusions.
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Observations and Results

Mortality

All animals were checked at least once daily.

There were no mortalities of the main study animals. There was one death in the TK set of animals, female #343 in the 10 mg/kg/day dose group that died on the first dosing day after blood collection. This is unrelated to CP-690550 treatment.

Clinical Signs

All animals were observed daily in their cages for clinical signs (any changes in appearance or behavior). Twice daily checks were conducted for F₀ dams and F₁ preweaning pups prior to the treatment period. They were checked three time daily during treatment. F₁ females during gestation when they were observed at least twice daily

There were no CP-690550-related clinical observations.

Body Weight

F₀ females were weighed on the day of arrival and on lactation day (LD) 10, 14, 17 and 20. F₁ pups were weighed on PND 14, 17 and 20, 21, 23, 25, 28, 31, 35, 38, 42, 45, 48, 51, 55, 58, 62, 66, 70 and twice weekly up to mating and weekly thereafter for the duration of the study. Gestating F₁ females were weighed on gestation days (GD) 0, 3, 7, 10, and 14.

Untreated females for mating with F₁ treated males were weighed on the day after arrival, once a week until gestation, and then on GD 0, 3, 7, 10, and 14. Untreated male rats ordered for mating with F₁ treated females were weighed on the day after arrival and then once a week for the duration of the study.

Male mean body weights and body weight gain of the 10 and 100 mg/kg/day dose groups was slightly lower than control starting at 2 to 3 weeks of treatment. and this persisted throughout treatment. By the end of treatment on PND 70 for males, body weights were 93.3% and 92.8% of control and body weight gain was 92.4% and 91.9% of control for the 10 and 100 mg/kg/day dose groups, respectively.

After the end of the treatment period for males (PND 70 and prior to the start of mating (PND 84), the high dose males weight gain was 78% of control reflected in a reduction in body weight by PND 84 (92% of control).

For females, there was no effect of CP-690550 on body weight or body weight gain during treatment, during mating, or during gestation. There was no effect of treated males on untreated gestating female body weights.

Body Weight and Body Weight Gain (g; selected days, values rounded by Reviewer)

Dose (mg/kg/day)	Males				Females			
	0	1	10	100	0	1	10	100
Day 1 (PND 21)	57	57	57	57	55	55	55	55
Day 8	101	100	99	101	93	91	92	94
Day 15	165	162	161	160 (97%)	137	135	137	142
Day 22	231	223	219	219 (95%)	168	165	166	175
Day 28	291	281	276	268 (92%)	193	190	189	200
Day 35 (PND 55)	358	344 (%)	337 (%)	327 (91%)	217	215 (99%)	215 (99%)	226 (104%)
Body Weight Gain (days 1 to 35)	301	287 (95%)	280 (93%)	270 (90%)	162	160 (99%)	160 (99%)	171 (105%)
Day 42	410	392	382	378 (92%)				
Day 50 PND 70	454	442 (97%)	424 (93%)	422 (93%)				

Body Weight Gain (days 1 to 50)	397	385 (97%)	368 (93%)	365 (92%)				
Matings initiated Day 84	-	-	-	-	-	-	-	-
Day 8 PND 91	536	528 (%)	504 (%)	490 (91%)				
Post mating								
Day 1, PND 98	557	557 (%)	529 (%)	517 (93%)				
Post mating Day 23, PND 120	618	644 (104%)	566 (92%)	584 (94%)				
- mean body weight not provided								

Feed Consumption

not monitored

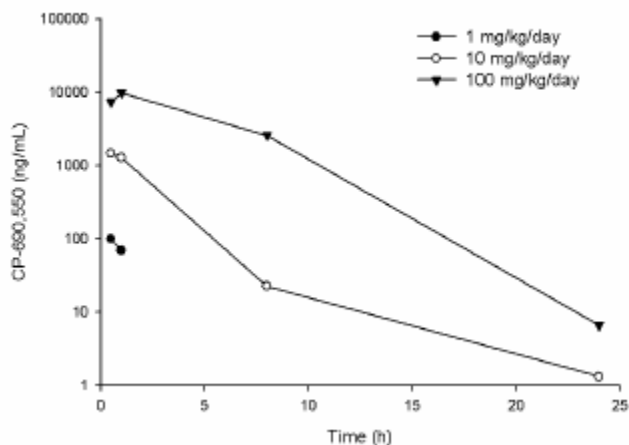
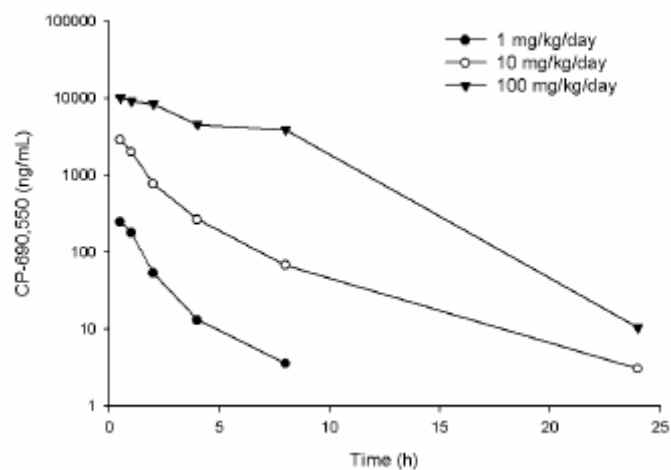
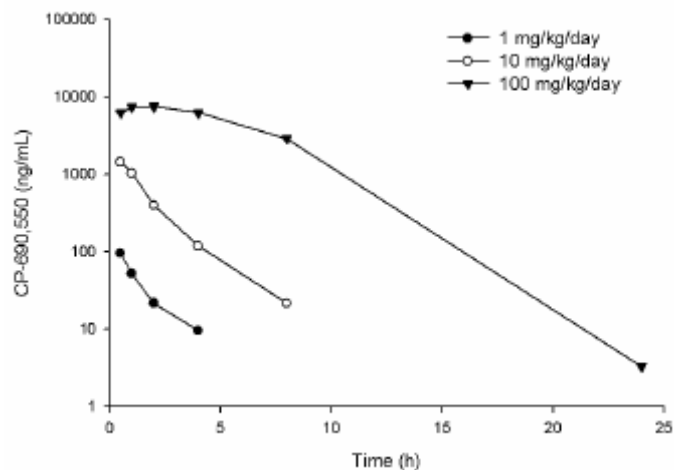
Toxicokinetics

Blood was collected after the first dose (PND 21) and the last dose (PND 70 for males, PND 55 for females) via jugular venipuncture following isoflurane anesthesia. On PND 21 samples were obtained at 0.5, 1, 8, and 24 hours post dose on PND 21. On the last dosing day, samples were obtained at 0.5, 1, 2, 4, 8, and 24 hours post dose. Samples were analyzed at (b) (4). Serum was analyzed for CP-690550 using a validated LC-MS/MS method. The lower limit of quantification was 5 ng/mL.

CP-690550 systemic exposure, AUC_{0-24} and C_{max} , increased with increasing dose, but there was no accumulation from the first to the last dosing day (35 days for females, 50 days for males). There were no substantial gender-related differences in exposure. T_{max} occurred at 0.5 h for the 1 and 10 mg/kg/day doses. T_{max} for the 100 mg/kg/day dose occurred at 1 h on day 21, and at 0.5 hr for females on PND 55 and at 2 h for males on PND 70. The figures indicate that dose exposure over a 24 hour period increased with dose. Exposure was minimal beyond the 8 hour timepoint at the high dose since the 24 hour values were near the analysis detection limit.

Toxicokinetic Parameters

Dose	1		10		100	
	M	F	M	F	M	F
C_{max} (ng/mL)						
PND 21	90.5	109	1320	1610	8640	11000
PND 55		249		2890		10100
PND 70	95.3		1440		7480	
AUC_{0-24} (ng-h/mL)						
PND 21	281	336	4890	6720	69100	71200
PND 55		412		5620		77200
PND 70	148		2660		67500	

Mean CP-690550 Concentration on Day 1 for Juvenile Rats (males and females averaged together for each dose level)**Mean CP-690550 Concentration in Female Juvenile Rats on Day 35****Mean CP-690550 Concentration in Male Juvenile Rats on Day 50**

FEMALES**Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)**Postweaning development

Treated female rats were evaluated for age at vaginal patency, beginning on PND 28. Body weights were also collected on the day of achievement of the endpoint.

CP-690550-treatment had no effect on the mean day of achievement of vaginal opening.

Estrous Cycling

Vaginal smears were collected daily treated females and the stage of estrous recorded beginning on PND 70 and continuing through completion of the cohabitation period (2 weeks).

For untreated females, vaginal smears were collected daily from all untreated female rats and the stage of estrous recorded beginning at least 2 weeks prior to cohabitation and continuing through completion of the cohabitation period or until there is evidence of mating (maximum) or until there was evidence of mating.

CP-690550-treatment had no effect on the occurrences of estrous or cycle length.

Mating

Untreated, sexually mature male and female rats of the same strain were used for mating with treated F₁ females and treated F₁ males, respectively. During the mating segment, animals were pair-housed (1 male and 1 female) until after signs of copulation were observed or when a 2 week mating phase had ended. Males and females were mated in a 1:1 ratio during a 2-week mating period starting ~12 weeks of age (~PND 84). Each morning of the cohabitation period, paired females were examined for evidence of copulation, ie, a sperm positive vaginal smear or observation of a copulatory plug. When positive signs of copulation were noted, the male and female were separated and returned to individual housing. The day that evidence of copulation is found was designated as GD 0. Females that did not exhibit signs of copulation during the 14 days of the cohabitation period were assigned to GD 0 on the last day of cohabitation.

CP-690550-treatment had no effect on mating, fertility, or number of mating days. The mating index for all animal pairs was 100%.

Caesarean section

On GD 14, all surviving maternal animals were euthanized by isoflurane anesthesia, necropsy conducted, and reproductive organs examined and uterus weighed. The number of corpora lutea for each ovary, and the location and viability status of each implantation site in the uterus was recorded. All resorptions were recorded as early resorptions. Uteri with no evidence of implantation were placed in a 10% ammonium sulfide solution for detection of early embryonic death.

CP-690550-treatment had no effect on corpora lutea, implantation sites, pre- and postimplantation loss in either treated females mated with untreated males or in the untreated females mated with treated males.

There were no CP-690550-related gross observations in this study.

The only findings for females observed on the study were ovarian cysts in a 10 mg/kg treated female (#122) and an untreated female (#407) mated with a vehicle control treated male.

MALES

Fertility Parameters

Postweaning development

Treated male rats were evaluated for age at preputial separation, beginning PND 35. Body weights were also collected on the day of achievement of the endpoint.

CP-690550-treatment had no effect on the mean day of achievement of preputial separation.

Necropsy

A gross examination of the animal was performed and the reproductive organs (testes, epididymes, seminal vesicles, and prostate) were retained. The right epididymis and right testis were frozen for later evaluation of sperm count. A section of the right distal vas deferens was removed for evaluation of sperm motility.

The only finding in males was a dilated kidney in a vehicle control treated male (#20).

Organ Weights

The accessory sex glands were weighed as a unit (prostate, seminal vesicles and coagulating glands). The weights of the left and right testes and left and right epididymides were recorded individually. The right cauda epididymis was weighed at the time of sperm count determination.

There were no CP-690550-related effects on organ weights.

Sperm Analysis

Sperm analysis was performed on all treated males.

The right cauda epididymis was homogenized and evaluated for sperm number and sperm motility. Two-hundred sperm were analyzed in 1 glass slide.

There were no CP-690550-related effects on epididymal sperm counts and percent motility of distal vas deferens sperm cells.

Stability and Homogeneity

Stability: The formulations were stored at room temperature. CP-690550-10 was demonstrated to be stable in 0.5% methylcellulose over a concentration range of 0.05 to 200 mg CP-690550/mL for a period of 8 days when stored at room temperature or refrigerated.

Homogeneity: During dosing, the formulations were continuously stirred with a magnetic stir bar and plate. Homogeneity of the CP-690550 formulations was assessed on the formulations used for the first and seventh calendar week of dosing and the low dose formulation prepared on calendar week 8 of dosing was also analyzed. Homogeneity analyses were all found to be within acceptable limits ($\pm 10\%$ of target) with one exception. The low dose concentration formulation of 0.1 mg/mL on calendar week 7 that was found to be 112, 111, and 112% of target concentration from the top, middle, and bottom strata, respectively.

Concentration: Concentration analyses were all found to be within acceptable limits ($\pm 10\%$ of target) with one exception of the low dose formulation of week 7.

The low dose formulation was administered to fertility phase females on their last dose (PND 55), low dose fertility phase males for PND 55 to 61, and low dose toxicokinetic phase males for PND 62 to 68. Since the low dose animals received a slightly greater dosage (1.1 mg/kg/day) than the intended 1.0 mg/kg/day, the Reviewer does not consider this detrimental to the study results.

9.2 Embryonic Fetal Development

Study title: Oral Dose Range-Finding Study of CP-690550 in Pregnant Rats

Study no:	04-2063-22
Study report location:	Mod 4.2.3.5.2
Conducting laboratory and location:	Safety Sciences Pfizer Global Research & Development Pfizer Inc Groton, Connecticut, 06340
Date of study initiation:	Nov 4 2004
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	CP-690550-10, Lot 52546-119-13HS, Purity 97.7% The active moiety comprised 60.3% of the drug substance.

There was no Certificate of Analysis included with this report. Purity was indicated in Table 2.6.7.4 in Module

Key Study Findings

- CP-690550 treatment from GD 6 to GD 18 resulted in complete post-implantation loss due to early resorptions of all the litters at doses of 500 and 300 mg/kg/day and increased in post-implantation loss at 100 and 30 mg/kg.
- Therefore, there were no viable fetuses available for evaluation at 500 and 300 mg/kg and the number of viable fetuses were reduced at 100 and 30 mg/kg. Mean gravid uterine weights were also reduced at all dose levels. Fetal body weights were reduced in the 100 mg/kg dose group 81%, there was no effect at 30 mg/kg/day.
- Anasarca occurred in 2 fetuses and fetal edema in 1 fetus in the 100 mg/kg/day dose.
- The fetuses with external findings were present in the litters that were most severely affected with postimplantation losses.

Methods

Doses: 0, 30, 100, 300, and 500 mg/kg/day

Dose in mg/kg is based on mg of active moiety of the drug substance.

Frequency of dosing: from Gestation Day (GD) 6-17 (12 consecutive days)

Dose volume: 10 mL/kg

Route of administration: oral gavage

Formulation/Vehicle: suspension of 0.5% methylcellulose

Species/Strain: Sprague-Dawley rats

Number/Sex/Group: 6 pregnant females/group

Satellite groups: None

Study design:

Group Number	Daily Dose* (mg/kg)	Dose Volume (mL/kg)	Drug Concentration (mg/mL)	Female Animal Numbers ^a
1	0	10	0	43050001-43050006
2	30	10	3	43050007-43050012
3	100	10	10	43050013-43050018
4	300	10	30	43050019-43050024
5	500	10	50	43050025-43050030

* All dose levels are expressed as mg active moiety per kg of body weight.

Methods

The animals were observed a minimum of twice daily for clinical signs on non-dosing study days, and a minimum of 4 times daily on dosing days. Body weights were measured on the day the animals arrived, on GD 3, and daily from GD 5-21. Food consumption was measured daily from GD 7-21. Animals that were found dead or euthanized because of moribundity underwent a gross examination of the thoracic, abdominal, and pelvic viscera to determine cause of death. The number of aborted or delivered fetuses was recorded. Uteri with no evidence of implantation were placed in a 10% ammonium sulfide solution for detection of early embryonic death. Cesarean sections were performed on all surviving animals on GD 21. Gravid uterine weights were collected and net body weight changes were calculated. The numbers of corpora lutea, implantation sites, late and early resorptions, and viable or dead fetuses were recorded for each rat. The fetuses were examined for external anomalies and developmental variations, sexed, weighed, and then discarded.

Results

Mortality

There was 1 dam death at 300 mg/kg/day and 4 dam deaths at 500 mg/kg/day. At 500 mg/kg, #28 was euthanized moribund on GD 15, and #29, #25, and #26 were found dead on GD 17, 20, and 20, respectively. At 300 mg/kg, animal #22 was euthanized moribund on GD 14.

Survivors to the scheduled necropsy, included 5 dams in the 300 mg/kg/day group and 2 dams in the 500 mg/kg/day group.

Clinical Observations

CP-690550 related clinical signs were predominantly noted at 500 and 300 mg/kg and included pale skin and/or eyes, salivation, and decreased activity.

Red vaginal discharge occurred in all the dose groups (6/6 at 500 and 300 mg/kg and 4/6 at 100 and 30 mg/kg) beginning around GD 14. This is commonly noted in the presence of fetal resorption.

Body Weights and Body Weight Change

Reductions in mean body weight, mean maternal corrected body weight, and occurred at 500 and 300 mg/kg/day. Mean body weights were reduced 61% to 93% in the 500 mg/kg/day and 66% to 91% in the 300 mg/kg/day group compared to the control group. During the first 6 days of treatment, reductions in mean body weight gain occurred at all dose levels which were partly due to post-implantation loss.

Mean gestation body weights were also reduced at 100 and 30 mg/kg, but only following the completion of dosing, on GD 20-21 (ranging from 92 to 95% of control). The corrected maternal body weight at these doses was similar to the control. The reduction in mean body weight at 100 and 30 mg/kg were attributed to the increase in post-implantation loss and not treatment.

Compared to controls, body weight gain was reduced throughout the study at all doses. This was partly due to post-implantation loss. Body weight gain was also reduced in the 30 mg/kg/day group during the dosing phase.

Food Consumption

During the dosing period (GD 6-18) reductions in mean food consumption occurred in the 300 and 500 mg/kg/day groups (79% and 31%, respectively). which continued to a variable extent until necropsy. There was no effect on mean food consumption at 100 and 30 mg/kg.

Necropsy

Treatment-related findings included small spleen and thymus in all dams at 500 mg/kg and 5/6 of the dams at 300 mg/kg. Small thymus was also noted at 100 and 30 mg/kg in 3 and 2 animals, respectively. These were not weighed in this study.

Gestation Day 21 Cesarean Section Data

Increased post-implantation loss occurred at all dose levels. There was 100% postimplantation loss in the 300 and 500 mg/kg dose groups, all due to early resorptions. Post-implantation loss was increased in the 30 and 100 mg/kg/day dose groups, also primarily due to early resorptions. Subsequently, the number of viable fetuses was reduced (76% and 57% of the control mean in the 30 and 100 mg/kg/day groups, respectively). Mean gravid uterine weights were also reduced at all dose levels (26% of control at 500 mg/kg/day, 36% at 300 mg/kg/day, 5% at 100 mg/kg/day and 1% control mean in the 500, 300, 100, and 30 mg/kg dose groups, respectively). There was no effect on the number of corpora lutea, implantations, and the percent preimplantation loss.

Fetal Body Weights

There were no fetuses available for weight collection at 500 and 300 mg/kg due to complete litter resorption. At 100 mg/kg, fetal body weights were reduced to 81% of control mean. There was no effect on fetal body weights at 30 mg/kg.

Fetal External Examination

There were no fetuses available for external evaluation at 500 and 300 mg/kg due to complete litter resorption. Anasarca and localized fetal edema in the neck region occurred in 2 and 1 fetuses in the 100 mg/kg dose group, respectively. There were no external fetal findings noted at 30 mg/kg.

Study title: Oral embryo-fetal development study of CP-690550-10 in rats

This study is a repeat of the initial study with a higher maximal dose. In the initial study (reviewed next) no maternal toxicity was obtained.

Study no:	09GR353
Study report location:	Mod. 4.2.3.5.2
Conducting laboratory and location:	Pfizer Global Research & Development, Drug Safety Research & Development, Groton, CT USA
Date of study initiation:	29 Sep 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CP-690550-10, Lot E010008412 Purity: 100.1%, The active moiety consisted of 61.7% of the drug substance.

Key Study Findings

- Mortalities occurred in the 300 mg/kg/day group (13 of 20 main study animals and another 3 of 5 TK group animals died or were sacrificed as moribund). Survivors all had total litter loss.
- Seven females in the 100 mg/kg group also had total litter loss.
- In the 300 mg/kg group, CP-690550-related clinical signs were decreased activity, piloerection, vaginal discharge, ptosis, eyes partially closed, mouth lesion, abrasion, and decreased skin turgor. At 100 mg/kg/day there were decreased incidences of vaginal discharge, nasal discharge, rapid breathing, decreased skin turgor, and piloerection.
- Reduction in body weight or weight gain occurred in the 100 and 300 mg/kg/day dose groups. This was associated with reduced food consumption, as well as pre- and post-implantation loss.
- Seven females in the 100 mg/kg group also had total litter loss.
- Even excluding animals with total litter loss, fetal mortality was dramatically increased (early and late resorptions), with an average postimplantation loss of 56.8% in the 100 mg/kg group as compared to 4.0% in the controls. Fetal body weights were significantly reduced in the 100 mg/kg group (77.6% of the control group).
- At 100 mg/kg, the treatment-related external malformation of anasarca was seen. Viscerally, the only treatment-related malformation observed was membranous ventricular septal defect.
- In the 100 mg/kg group, treatment-related skeletal malformations of absent cervical arch, bent femur, fibula, humerus, radius, scapula, tibia, and ulna, as well as sternoschisis, absent rib, misshapen femur, branched rib, fused rib, fused sternebra, and hemicentric thoracic centrum were seen. The increased incidence of the skeletal variations of 7th cervical centrum unossified, wavy rib, misaligned

sternebra, thoracic centrum incomplete ossification, metatarsal and metacarpal unossified, short rib, unossified sternebra Number 1-4, and incomplete ossification of the supraoccipital are likely associated with the reduction in fetal weight observed in this group.

- There were no CP-690550-related effects on mortality, clinical signs, body weight, food consumption, gross necropsy observations, cesarean section parameters, or fetal body weight in the 30 mg/kg group.
- The maternal NOAEL was 30 mg/kg/day
- The developmental NOAEL is also 30 mg/kg/day due to embryofetal toxicity evident by increased fetal anomalies at 100 mg/kg/day, 7 completely resorbed litters, increased postimplantation loss, decreased numbers of live fetuses, reduced fetal body weights. There were no litters to evaluate at 300 mg/kg/day.
- The corresponding toxicokinetic values at the NOAEL dose of 30 mg/kg/day were C_{max} 6360 ng/mL and AUC_{0-24} a 29400 ng-h/mL.

Methods

Doses:	0, 30, 100, and 300 mg/kg
Frequency of dosing:	Dose in mg/kg is based on mg of active moiety of the drug substance. once daily from gestation day 6 (GD 6) through GD 17.
Dose volume:	10 mL/kg
Route of administration:	orally by gavage
Formulation/Vehicle:	suspension in 0.5% methylcellulose (MC)
Species/Strain:	Sprague-Dawley rats (CrI:CD®[SD]) 10-12 weeks of age 240.1-298.3 g on GD 6
Number/Sex/Group:	20/dose
Satellite groups:	Toxicokinetic (TK) satellite phase females were randomly assigned to 3 groups of 5 animals per group for each treatment group and 1 control group of 3 animals.
Study design:	

Group Number	Daily Dose* (mg/kg)	Drug Concentration (mg/mL)	Dose Volume (mL/kg)	Animal Numbers	
				Main Study	TK Satellite
1	0	0	10	1-20	-
2	30	3	10	21-40	-
3	100	10	10	41-60	-
4	300	30	10	61-80	-
5	0	0	10	-	81-83
6	30	3	10	-	84-88
7	100	10	10	-	89-93
8	300	30	10	-	94-98

TK = Toxicokinetics.

*All dose levels are expressed as mg of active moiety per kg of body weight.

Events/ Observations	Dates	Study Day ^a	Gestation Day
Experimental start date (females arrive)	01 Oct 2009	pre	1-3
Dosing began	02 Oct 2009	pre	1
TK blood collection	04-07 Oct 2009	1	6
TK pregnancy status determination	15 Oct 2009	12	17
Dosing ended	16 Oct 2009	13	18
End of In-life (cesarean section)	15-18 Oct 2009	12	17
	19-22 Oct 2009	16	21

^a Days prior to treatment are designated by pretreatment (pre). The remaining days are study days. Study Day 1 is the first day of dosing.

Deviation from study protocol: There were no deviations that affected the study results or conclusions.

Observations and Results

Mortality

Animals were checked at least twice daily

At the high dose, 300 mg/kg, 13 of 20 main study animals died, and another 3 of 5 TK group animals died or were sacrificed as moribund. Although 1 animal was found dead on GD 7 (treatment day 2), the other deaths occurred between GD 15-18. There were no deaths in the lower dose groups, 100 and 30 mg/kg groups. There was one death in the control group on GD 9 which was attributed to dosing error, since the pathology indicated abnormal contents in the esophagus.

Clinical Signs

Observations were made at least once daily

In the 300 mg/kg group clinical signs of decreased activity were seen in 16 females, piloerection and vaginal discharge in 18 females, ptosis in 14 females, and eyes partially closed, mouth lesion, abrasion, and decreased skin turgor in individual females.

At 100 mg/kg clinical signs of vaginal discharge were noted in 4 females, as well as nasal discharge, rapid breathing, decreased skin turgor, and piloerection in individual females.

There were no clinical signs noted in either the 30 mg/kg group or the control group.

Body Weight

Body weights were measured on the day the animals arrive, GD 3, and daily from GD 5-21.

At 300 mg/kg/day body weight and body weight gain was decreased compared to controls for most measurement periods. In the 100 mg/kg/day group, reduction in body weight occurred only on GD 20 and 21, although decreased body weight gain occurred across the gestation intervals of 18-21 and 6-21. No effect on body weight or body weight gain was noted in the 30 mg/kg/day group.

Summary of Body Weight Changes (g, rounded values by Reviewer, selected times)

Group	1	2	3	4
Dose (mg/kg/day)	0	30	100	300
Body Weight				
day 6	270	268	269	272
day 7	274	271	269	258 (94%)
day 10	291	286	290	281
day 13	309	302	308	295
day 15	323	316	323	275
day 18	358	356	352	268 (75%)
Body Weight Change				
6 - 18	88	88	83 (94%)	-2.5 (-103%)
18 - 21	53	48 (90%)	11 (21%)	12 (23%)
6 - 21	141	126 (89%)	94 (67%)	9 (6%)

Gravid uterine weights were statistically significantly decreased in both the 100 and 300 mg/kg groups as well as the corrected maternal body weight at 300 mg/kg.

Table 4: Summary of Mean Adjusted Body Weight Change and Uterine Weight (g)

Sex: Female					
		0 mg/kg F	30 mg/kg F	100 mg/kg F	300 mg/kg F
Uterus weight (g)	Mean	101.48 n	104.98	43.61 **	6.02 **
	S.d.	17.94	9.72	31.39	1.24
	N	19	20	19	7
Carcass Weight (g)	Mean	309.71 n	298.42	321.32	273.33 **
	S.d.	17.09	21.75	26.46	27.68
	N	19	20	19	7
Net weight change (g) From Gestation day 6	Mean	39.84 n	30.49	51.92	3.21 **
	S.d.	13.22	13.97	28.29	21.06
	N	19	20	19	7

n=DUNNETT; ** = p < 0.01

Feed Consumption Food consumption was measured daily from GD 6-21.

In the 300 mg/kg/day group mean maternal food consumption was reduced throughout gestation, resulting in an overall reduction of 69% compared to the control mean. At 100 mg/kg/day, mean food consumption was reduced only for the days 15-16. In this group, food consumption was increased during GD 9-GD 12. There was no CP-690550-related effect on food consumption at 30 mg/kg/day.

Summary of Food Consumption (g/animal/day)

Group	1	2	3	4
Dose (mg/kg/day)	0	30	100	300
Gestation Interval				
GD6 - GD 18	22.9	22.8	23.0	16.0 (70%)
GD 6 - GD 18	25.8	24.9	22.8	17.8 (69%)
GD 18 - GD 21	23.5	23.2	22.9	16.3 (69%)

Toxicokinetics Blood samples were collected on GD 17 via jugular venipuncture at ~ 0.5, 1, 3, 8, and 24 hours postdose and from TK control animals at ~ 0.5, 8, and 24 hours postdose.

All dams were exposed to drug during the treatment period. Systemic exposure (C_{max} and AUC_{0-24}) on GD 17 increased with increasing dose.

Table 1. Mean Toxicokinetic Parameters for CP-690,550 in Pregnant Female Rats after Oral Administration of CP-690,550 on Gestation Day 17

Dose (mg/kg/day)	Study Day	Gender	C_{max} (ng/mL)			T_{max} (h)			$AUC_{(0-24)}$ (ng•h/mL)		
			Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n
30 ¹	17	Female	6360	1510	5	0.60	0.22	5	29,400	3060	5
100 ¹	17	Female	9390	1130	5	1.2	1.0	5	73,800	16,600	5
300 ²	17	Female	14,400	NA	NA	0.50	NA	NA	108,000	NA	NA

NA = Not Applicable

Stability and Homogeneity

Stability: CP-690550-10 was stable in 0.5% methylcellulose over a concentration range of 1 to 50 mg CP-690550/mL for a period of 15 days when stored at room temperature or refrigerated conditions (b) (4) Study 6348-463, 15 day stability results).

Homogeneity: Homogeneity of the on the first set of CP-690550 formulations was assessed and found to be 100%-102% of the target concentration.

Formulation Concentration: Analysis was performed from the predose samples of CP-690550 and control vehicle formulations of CP-690550 concentrations from the first and second set of prepared formulations were 100%-102% of the target concentration. No CP-690550 was detected in control formulations.

Necropsy

The Pathology report was not signed.

On GD 21, all surviving maternal animals were euthanized by carbon dioxide inhalation. The abdominal, thoracic, and pelvic viscera were examined grossly, uterus and ovaries removed, uterus weight was collected, corpora lutea for each ovary counted,

location and viability status of each uterine implantation site was recorded, and placentas were examined grossly. Uteri with no evidence of implantation were placed in a 10% ammonium sulfide solution for detection of early embryonic death. From the 2nd day of necropsies, hearts were retained from animals #10, 29, 50, 68, 69, and 70 as well as liver sections from animal #30. On the remaining days of scheduled necropsy, heart and liver sections (2) were collected for all animals. In addition, tissues with gross observations (and corresponding samples from control animals) were retained. All retained tissues were preserved in 10% neutral buffered formalin. All 4 hearts collected from the 300 mg/kg group as well as a comparable number of hearts from control animals were processed, sectioned, stained with hematoxylin and eosin and examined microscopically. Giemsa and Warthin-Starry stains for infectious agents were performed using sections of the 8 hearts.

Approximately one-half of the viable fetuses from each litter were examined internally by dissection in the fresh state.

The other one-half of the litter was eviscerated and fixed in 95% ethanol for subsequent examination of skeletons.

Two dams in the 300 mg/kg/day dose group had enlarged right atrium and ventricle, and one also had enlarged left ventricle and tan foci. No abnormalities of the heart were observed at necropsy in other animals. The 4 hearts examined in this dose group had minimal or mild myocardial degeneration and/or fibrosis located most frequently beneath the left ventricular endocardium in the left ventricular free wall and septum near the apex of the heart. No infectious agents (bacterial or fungal) were identified using Giemsa and Warthin-Starry silver stains. The microscopic myocardial degeneration was considered a treatment-related finding. The pathologist noted the myocardial degeneration seen in here was compatible with ischemic or toxic injury.

Other findings included for the 300 mg/kg group, were abnormal color of the lung, liver, and kidney in 1 dam; stomach dilation with foci in 3 dams, and a uterus with abnormal content (in 9 dams) while in the 100 mg/kg group uterus with abnormal content (1 dam) and kidneys with abnormal consistency (1 dam) and color (1 dam) were noted.

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

All surviving 300 mg/kg females at GD 21 had total litter loss due to early resorptions. Seven females in the 100 mg/kg group also had total litter loss due to early resorptions and postimplantation loss, yielding reduced number of live fetuses. Excluding animals with total litter loss in the 100 mg/kg group, the increase in postimplantation loss was still dramatically increased in this group (56.8% as compared to 4.0% in the control). There was no effect on any cesarean section parameter in the 30 mg/kg group.

Table 8: Summary of Mean Cesarean Section Values

		0 mg/kg F	30 mg/kg F	100 mg/kg F	300 mg/kg F
Pregnant, used for calculation	N	19	20	20	7
Corpora Lutea	Total	290	291	298	85
No. per animal	Mean	15.3s	14.6	14.9	12.1*
	S.d.	2.4	1.7	2.6	2.9
Implantation Sites	Total	260	268	259	83
No. per animal	Mean	13.7s	13.4	12.9	11.9
	S.d.	1.9	1.7	2.7	2.5
Preimplantation Loss	Total	30	23	39	2
No. per animal	Mean	1.6s	1.1	1.9	0.3
	S.d.	1.8	1.1	2.8	0.5
% per animal	Mean	9.6s	7.7	12.0	1.9
	S.d.	9.9	7.4	15.0	3.2
Fetuses	Total	249	258	78	0
No. per animal	Mean	13.1s	12.9	3.9**	0.0**
	S.d.	2.1	1.7	4.5	0.0
Alive	%	100.0	100.0	100.0	0.0
Dead	%	0.0	0.0	0.0	0.0
Live Fetuses	Total	249	258	78	0
No. per animal	Mean	13.1s	12.9	3.9**	0.0**
	S.d.	2.1	1.7	4.5	0.0
Malformed Fetuses (External)	Total	0	0	3	0
No. per animal	Mean	0.0s	0.0	0.1	0.0
	S.d.	0.0	0.0	0.4	0.0
Dead Fetuses	Total	0	0	0	0
No. per animal	Mean	0.0s	0.0	0.0	0.0
	S.d.	0.0	0.0	0.0	0.0
% per animal	Mean	0.0s	0.0	0.0	0.0
	S.d.	0.0	0.0	0.0	0.0
Early Resorption	Total	10	9	152	83
No. per animal	Mean	0.5s	0.5	7.6**	11.9**
	S.d.	1.4	0.6	4.1	2.5
% per animal	Mean	3.6s	3.2	61.6**	100.0**
	S.d.	9.8	4.3	34.4	0.0
Late Resorption	Total	1	1	29	0
No. per animal	Mean	0.1s	0.1	1.4**	0.0
	S.d.	0.2	0.2	2.4	0.0
% per animal	Mean	0.4s	0.4	10.3**	0.0
	S.d.	1.8	1.9	17.5	0.0
Not Applicable for Pfizer DART Studies	Total	0	0	0	0
No. per animal	Mean	0.0s	0.0	0.0	0.0
	S.d.	0.0	0.0	0.0	0.0
Postimplantation Loss	Total	11	10	181	83
No. per animal	Mean	0.6s	0.5	9.1**	11.9**
	S.d.	1.4	0.7	3.9	2.5
% per animal	Mean	4.0s	3.6	71.9**	100.0**
	S.d.	9.8	5.1	30.5	0.0

s=DUNN; * = p < 0.05; ** = p < 0.01

Preimplantation Loss = Corpora Lutea - Implantation Sites

Postimplantation Loss = Early/Late resorptions + Dead Fetuses

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Table 8: Summary of Mean Cesarean Section Values

		0 mg/kg F	30 mg/kg F	100 mg/kg F	300 mg/kg F
Pregnant, used for calculation	N	19	20	20	7
Affected Implants	Total	11	10	184	83
No. per animal	Mean	0.6s	0.5	9.2**	11.9**
	S.d.	1.4	0.7	3.8	2.5
% per animal	Mean	4.0s	3.6	72.9**	100.0**
	S.d.	9.8	5.1	29.5	0.0

s=DUNN; ** = p < 0.01

Affected Implants = Early/Late resorptions + Dead Fetuses + Malformed Fetuses (External)

Fetal Body Weights

Fetal body weights were significantly reduced in the 100 mg/kg/day group (78% of the control group). There was no effect on fetal body weights in the 30 mg/kg/day group. There were no surviving fetuses in the 300 mg/kg/day group.

Table 9: Summary of Mean Fetal Body Weights (g)

		0 mg/kg F	30 mg/kg F	100 mg/kg F
Litters, used for calculation	N	19	20	13
Fetus Weight	Mean	5.8 _n	5.8	4.5**
	S.d.	0.3	0.3	0.5

n=DUNNETT; ** = p < 0.01

No viable fetuses available in 300 mg/kg group

Offspring (Malformations, Variations, etc.)

There were no fetuses to evaluate in the 300 mg/kg/day group due to total litter loss.

External Examination Findings

There were no fetuses to evaluate in the 300 mg/kg/day group.

At 100 mg/kg/day, fetal malformations of anasarca were found in 2 fetuses from 2 litters. One of these fetuses (from Litter #53) was the only surviving fetus in a litter with 9 early resorptions and 6 late resorptions. Another malformation, cranium meningoencephalocele occurred in 1 fetus (0.6% fetuses). Historical control data for cranium meningoencephalocele indicated an incidence rate of 0.0%-0.4% fetuses (records from Feb. 2004 - Feb. 2009 (HCD)), which this single finding exceeded.

Table 10: Summary of Fetal External Evaluations

			0 mg/kg F	30 mg/kg F	100 mg/kg F
Fetuses Examined		N	249	258	78
Litters evaluated		N	19	20	13
Total CS External Observation	Litters Affected	N	0	0	3
		%	0	0	23.1
	Fetuses Affected	N	0	0	3
	% per Litter	Mean	0.0	0.0	8.9
General Anasarca (M)	Litters Affected	N	0	0	2
		%	0	0	15.4
	Fetuses Affected	N	0	0	2
	% per Litter	Mean	0.0	0.0	8.3
Cranium Meningoencephalocele (M)	Litters Affected	N	0	0	1
		%	0	0	7.7
	Fetuses Affected	N	0	0	1
	% per Litter	Mean	0.0	0.0	0.6

f=FISHER-EXACT, s=DUNN, * = p < 0.05, M = Malformation

Visceral Examination Findings

In the 100 mg/kg/day group membranous ventricular septal defect was present in 6 fetuses from 3 litters. This malformation was also present in the 30 mg/kg/day group and the control group (5 fetuses in 4 litters and 4 fetuses in 3 litters, respectively). Since there were so few evaluable fetuses in the 100 mg/kg group (35 fetuses examined) this malformation is considered to be likely related to treatment in this group. Other treatment-related findings at 100 mg/kg/day dose included pale heart in 2 fetuses from 2 litters and pale kidney in 2 fetuses from a single litter, and hemorrhagic adrenal gland in 3 fetuses from 2 litters.

The visceral malformation of retroesophageal aortic arch occurred in a single fetus in the 30 mg/kg group. This individual observation is likely spontaneous and unrelated to treatment since it occurred in only 1 fetus and was not seen at the higher dose level of 100 mg/kg. All other visceral malformations, general anomalies and visceral variations were seen only in the control group and not associated with treatment with CP-690550-10. Control group only malformations included: right sided aortic arch, situs inversus, absent kidney, absent ovary and oviduct, malpositioned branch of the subclavian artery, and absent ureter and uterine horn; control group general anomalies included pale spleen; and control variations were absent innominate, absent lung lobes, and retroesophageal right subclavian artery.

Table 11: Summary of Fetal Visceral Evaluations

			0 mg/kg F	30 mg/kg F	100 mg/kg F
Fetuses Examined		N	119	124	35
Litters evaluated		N	19	20	12
Total CS Visceral Observation	Litters Affected	N	7 f	4	5
		%	36.8	20.0	41.7
	Fetuses Affected	N	9	5	9
	% per Litter	Mean	7.9 s	3.8	22.2
Adrenal gland <i>Hemorrhagic (V)</i>	Litters Affected	N	0 f	0	2
		%	0	0	16.7
	Fetuses Affected	N	0	0	3
	% per Litter	Mean	0.0 s	0.0	12.5 *
Aortic arch	Litters Affected	N	1 f	1	0
		%	5.3	5.0	0
	Fetuses Affected	N	1	1	0
	% per Litter	Mean	0.9 s	0.8	0.0
<i>Retroesophageal (M)</i>	Litters Affected	N	0 f	1	0
		%	0	5.0	0
	Fetuses Affected	N	0	1	0
	% per Litter	Mean	0.0 s	0.8	0.0
<i>Right-sided (M)</i>	Litters Affected	N	1 f	0	0
		%	5.3	0	0
	Fetuses Affected	N	1	0	0
	% per Litter	Mean	0.9 s	0.0	0.0
General <i>Situs inversus (M)</i>	Litters Affected	N	1 f	0	0
		%	5.3	0	0
	Fetuses Affected	N	1	0	0
	% per Litter	Mean	0.7 s	0.0	0.0
Heart	Litters Affected	N	3 f	4	5
		%	15.8	20.0	41.7
	Fetuses Affected	N	4	5	8
	% per Litter	Mean	3.4 s	3.8	18.1
<i>Membranous ventricular septum defect (M)</i>	Litters Affected	N	3 f	4	3
		%	15.8	20.0	25.0
	Fetuses Affected	N	4	5	6
	% per Litter	Mean	3.4 s	3.8	9.7
<i>Pale (G)</i>	Litters Affected	N	0 f	0	2
		%	0	0	16.7
	Fetuses Affected	N	0	0	2
	% per Litter	Mean	0.0 s	0.0	8.3
Innominate <i>Absent (V)</i>	Litters Affected	N	3 f	0	0
		%	15.8	0	0
	Fetuses Affected	N	3	0	0
	% per Litter	Mean	3.2 s	0.0	0.0

f=FISHER-EXACT, s=DUNN, V = Variation, * = p < 0.05, M = Malformation, G = General

Table 11: Summary of Fetal Visceral Evaluations

			0 mg/kg F	30 mg/kg F	100 mg/kg F
Fetuses Examined	N		119	124	35
Litters evaluated	N		19	20	12
Kidney	Litters Affected	N	1 f	0	1
		%	5.3	0	8.3
	Fetuses Affected	N	1	0	2
	% per Litter	Mean	0.7 s	0.0	8.3
Absent (M)	Litters Affected	N	1 f	0	0
		%	5.3	0	0
	Fetuses Affected	N	1	0	0
	% per Litter	Mean	0.7 s	0.0	0.0
Pale (G)	Litters Affected	N	0 f	0	1
		%	0	0	8.3
	Fetuses Affected	N	0	0	2
	% per Litter	Mean	0.0 s	0.0	8.3
Lung Absent lobes (V)	Litters Affected	N	1 f	0	0
		%	5.3	0	0
	Fetuses Affected	N	1	0	0
	% per Litter	Mean	0.7 s	0.0	0.0
Ovary Absent (M)	Litters Affected	N	1 f	0	0
		%	5.3	0	0
	Fetuses Affected	N	1	0	0
	% per Litter	Mean	0.7 s	0.0	0.0
Oviduct Absent (M)	Litters Affected	N	1 f	0	0
		%	5.3	0	0
	Fetuses Affected	N	1	0	0
	% per Litter	Mean	0.7 s	0.0	0.0
Spleen Pale (G)	Litters Affected	N	1 f	0	0
		%	5.3	0	0
	Fetuses Affected	N	1	0	0
	% per Litter	Mean	0.9 s	0.0	0.0
Subclavian artery	Litters Affected	N	2 f	0	0
		%	10.5	0	0
	Fetuses Affected	N	2	0	0
	% per Litter	Mean	2.2 s	0.0	0.0
Malpositioned branch (M)	Litters Affected	N	1 f	0	0
		%	5.3	0	0
	Fetuses Affected	N	1	0	0
	% per Litter	Mean	0.9 s	0.0	0.0
Retrosophageal right (V)	Litters Affected	N	1 f	0	0
		%	5.3	0	0
	Fetuses Affected	N	1	0	0
	% per Litter	Mean	1.3 s	0.0	0.0

f=FISHER-EXACT, s=DUNN, M = Malformation, G = General, V = Variation

Table 11: Summary of Fetal Visceral Evaluations

			0 mg/kg F	30 mg/kg F	100 mg/kg F
Fetuses Examined	N		119	124	35
Litters evaluated	N		19	20	12
Ureter Absent (M)	Litters Affected	N	1 f	0	0
		%	5.3	0	0
	Fetuses Affected	N	1	0	0
	% per Litter	Mean	0.7 s	0.0	0.0
Uterus Absent uterine horn (M)	Litters Affected	N	1 f	0	0
		%	5.3	0	0
	Fetuses Affected	N	1	0	0
	% per Litter	Mean	0.7 s	0.0	0.0

M = Malformation, f=FISHER-EXACT, s=DUNN

Skeletal Examination Findings

Malformations: In the 100 mg/kg/day group the statistically significant skeletal malformations included absent cervical arch (3 fetuses /3 litters), bent femur (8 fetuses/5 litters), bent fibula (9 fetuses/4 litters), bent humerus (8 fetuses/6 litters), bent radius (9 fetuses/4 litters), bent scapula (13 fetuses/6 litters), sternoschisis (6 fetuse/5 litters), bent tibia (6 fetuses/4 litters), and bent ulna (10 fetuses/5 litters) were seen.

The non-statistically significant skeletal malformation of absent rib in 2 fetuses/2 litters was also seen at 100 mg/kg along with the malformations of misshapen femur, branched rib, fused rib, fused sternebra, and hemicentric thoracic centrum seen only in individual fetuses. All of these anomalies are considered to be treatment-related because they were observed in the highest dose tested and were either not observed in the recent historical control data or were seen at levels greater than the recent historical control data.

Variations: The statistically significant skeletal variations of 7th cervical centrum unossified (17 fetuses/12 litters), wavy rib (15 fetuses/9 litters), misaligned sternebra (3 fetuses/3 litters), thoracic centrum incomplete ossification (5 fetuses/4 litters), and metatarsal unossified (20 fetuses/11 litters) were observed in the 100 mg/kg/day group, were seen at incidence levels exceeding the recent historical controls and are likely associated with the reduction in fetal weight observed in this group.

Non-statistically significant variations of metacarpal unossified (2 fetuses/2 litters), short rib (2 fetuses/2 litters), and unossified sternebra Numbers 1-4 (2 fetuses/2 /litters), incomplete ossification of the supraoccipital (1 fetus) were also seen at incidence levels exceeding the recent historical control data and are likely associated with the reduction in fetal weight observed in this group.

All other variations observed were considered unrelated to treatment since they were either seen in only 1 or 2 fetuses in the low dose (30 mg/kg/day) but not the higher (100 mg/kg/day) dose – skull extra ossification site (2 fetuses), and 27 presacral vertebra (1 fetus); or were not seen in a treatment-related manner [7th cervical rib (1, 4, and 1 fetuses in the control, 30, and 100 mg/kg groups, respectively), short supernumerary rib (10, 16, and 5 fetuses in the control, 30, and 100 mg/kg groups, respectively), and sternebra Number 5 and/or Number 6 unossified (2, 0, and 1 fetuses in the control, 30, and 100 mg/kg groups, respectively)].

Table 12: Summary of Fetal Skeletal Evaluations

			0 mg/kg F	30 mg/kg F	100 mg/kg F
Fetuses Examined	N		130	134	43
Litters evaluated	N		19	20	13
Total CS Skeletal Observation	Litters Affected	N	12 f	13	13 *
		%	63.2	65.0	100.0
	Fetuses Affected	N	18	25	37
	% per Litter	Mean	13.9 s	20.0	92.5 **
Cervical arch Absent (M)	Litters Affected	N	0 f	0	3
		%	0	0	23.1
	Fetuses Affected	N	0	0	3
	% per Litter	Mean	0.0 s	0.0	5.2 *
Cervical centrum 7th cervical centrum unossified (V)	Litters Affected	N	3 f	3	12 **
		%	15.8	15.0	92.3
	Fetuses Affected	N	4	3	17
	% per Litter	Mean	3.3 s	2.4	54.6 **
Femur	Litters Affected	N	0 f	0	6 **
		%	0	0	46.2
	Fetuses Affected	N	0	0	9
	% per Litter	Mean	0.0 s	0.0	30.7 **
Bent (M)	Litters Affected	N	0 f	0	5 **
		%	0	0	38.5
	Fetuses Affected	N	0	0	8
	% per Litter	Mean	0.0 s	0.0	23.0 **

f=FISHER-EXACT, * = p < 0.05, s=DUNN, ** = p < 0.01, M = Malformation, V = Variation

Table 12: Summary of Fetal Skeletal Evaluations

			0 mg/kg F	30 mg/kg F	100 mg/kg F
Fetuses Examined	N		130	134	43
Litters evaluated	N		19	20	13
Misshapen (M)	Litters Affected	N	0 f	0	1
		%	0	0	7.7
	Fetuses Affected	N	0	0	1
	% per Litter	Mean	0.0 s	0.0	7.7
Fibula Bent (M)	Litters Affected	N	0 f	0	4 *
		%	0	0	30.8
	Fetuses Affected	N	0	0	9
	% per Litter	Mean	0.0 s	0.0	23.1 **
Humerus Bent (M)	Litters Affected	N	0 f	0	6 **
		%	0	0	46.2
	Fetuses Affected	N	0	0	8
	% per Litter	Mean	0.0 s	0.0	16.6 **
Metacarpal Unossified (V)	Litters Affected	N	0 f	0	2
		%	0	0	15.4
	Fetuses Affected	N	0	0	2
	% per Litter	Mean	0.0 s	0.0	11.5
Radius Bent (M)	Litters Affected	N	0 f	0	4 *
		%	0	0	30.8
	Fetuses Affected	N	0	0	9
	% per Litter	Mean	0.0 s	0.0	25.4 **

M = Malformation, f=FISHER-EXACT, s=DUNN, * = p < 0.05, ** = p < 0.01, V = Variation

Table 12: Summary of Fetal Skeletal Evaluations

			0 mg/kg F	30 mg/kg F	100 mg/kg F
Fetuses Examined	N		130	134	43
Litters evaluated	N		19	20	13
Rib	Litters Affected	N	9 f	13	11
		%	47.4	65.0	84.6
	Fetuses Affected	N	12	20	22
	% per Litter	Mean	9.1 s	16.0	55.8 **
Absent (M)	Litters Affected	N	0 f	0	2
		%	0	0	15.4
	Fetuses Affected	N	0	0	2
	% per Litter	Mean	0.0 s	0.0	5.4
Branched (M)	Litters Affected	N	0 f	0	1
		%	0	0	7.7
	Fetuses Affected	N	0	0	1
	% per Litter	Mean	0.0 s	0.0	1.5
7th Cervical (V)	Litters Affected	N	1 f	4	1
		%	5.3	20.0	7.7
	Fetuses Affected	N	1	4	1
	% per Litter	Mean	0.6 s	3.2	3.8
Fused (M)	Litters Affected	N	0 f	0	1
		%	0	0	7.7
	Fetuses Affected	N	0	0	1
	% per Litter	Mean	0.0 s	0.0	3.8

f=FISHER-EXACT, s=DUNN, ** = p < 0.01, M = Malformation, V = Variation

Table 12: Summary of Fetal Skeletal Evaluations

			0 mg/kg F	30 mg/kg F	100 mg/kg F
Fetuses Examined	N		130	134	43
Litters evaluated	N		19	20	13
Short (V)	Litters Affected	N	0 f	1	2
		%	0	5.0	15.4
	Fetuses Affected	N	0	1	2
	% per Litter	Mean	0.0 s	0.7	2.6
Short supernumerary (V)	Litters Affected	N	7 f	10	5
		%	36.8	50.0	38.5
	Fetuses Affected	N	10	16	5
	% per Litter	Mean	7.9 s	12.8	10.6
Wavy (V)	Litters Affected	N	1 f	0	9 **
		%	5.3	0	69.2
	Fetuses Affected	N	1	0	15
	% per Litter	Mean	0.7 s	0.0	43.6 **
Scapula Bent (M)	Litters Affected	N	0 f	0	6 **
		%	0	0	46.2
	Fetuses Affected	N	0	0	13
	% per Litter	Mean	0.0 s	0.0	30.7 **
Skull Extra ossification site (V)	Litters Affected	N	0 f	1	0
		%	0	5.0	0
	Fetuses Affected	N	0	2	0
	% per Litter	Mean	0.0 s	1.7	0.0

V = Variation, f=FISHER-EXACT, s=DUNN, ** = p < 0.01, M = Malformation

Table 12: Summary of Fetal Skeletal Evaluations

			0 mg/kg F	30 mg/kg F	100 mg/kg F
Fetuses Examined	N		130	134	43
Litters evaluated	N		19	20	13
Sternebra	Litters Affected	N	2 f	0	8 **
		%	10.5	0	61.5
	Fetuses Affected	N	2	0	11
	% per Litter	Mean	1.5 s	0.0	37.5 **
Fused (M)	Litters Affected	N	0 f	0	1
		%	0	0	7.7
	Fetuses Affected	N	0	0	1
	% per Litter	Mean	0.0 s	0.0	3.8
Misaligned (V)	Litters Affected	N	0 f	0	3
		%	0	0	23.1
	Fetuses Affected	N	0	0	3
	% per Litter	Mean	0.0 s	0.0	7.9 *
Sternoschisis (M)	Litters Affected	N	0 f	0	5 **
		%	0	0	38.5
	Fetuses Affected	N	0	0	6
	% per Litter	Mean	0.0 s	0.0	18.0 **
Unossified #5 and/or #6 (V)	Litters Affected	N	2 f	0	1
		%	10.5	0	7.7
	Fetuses Affected	N	2	0	1
	% per Litter	Mean	1.5 s	0.0	3.8

f=FISHER-EXACT, ** = p < 0.01, s=DUNN, M = Malformation, V = Variation, * = p < 0.05

Table 12: Summary of Fetal Skeletal Evaluations

			0 mg/kg F	30 mg/kg F	100 mg/kg F
Fetuses Examined	N		130	134	43
Litters evaluated	N		19	20	13
Unossified #1-4 (V)	Litters Affected	N	0 f	0	2
		%	0	0	15.4
	Fetuses Affected	N	0	0	2
	% per Litter	Mean	0.0 s	0.0	11.5
Supraoccipital Incomplete ossification (V)	Litters Affected	N	0 f	0	1
		%	0	0	7.7
	Fetuses Affected	N	0	0	1
	% per Litter	Mean	0.0 s	0.0	1.1
Thoracic centrum	Litters Affected	N	0 f	2	5 **
		%	0	10.0	38.5
	Fetuses Affected	N	0	2	6
	% per Litter	Mean	0.0 s	1.3	13.2 **
Hemicentric (M)	Litters Affected	N	0 f	0	1
		%	0	0	7.7
	Fetuses Affected	N	0	0	1
	% per Litter	Mean	0.0 s	0.0	3.8
Incomplete ossification (V)	Litters Affected	N	0 f	2	4 *
		%	0	10.0	30.8
	Fetuses Affected	N	0	2	5
	% per Litter	Mean	0.0 s	1.3	9.3 *

V = Variation, f=FISHER-EXACT, s=DUNN, ** = p < 0.01, M = Malformation, * = p < 0.05

Table 12: Summary of Fetal Skeletal Evaluations

			0 mg/kg F	30 mg/kg F	100 mg/kg F
Fetuses Examined	N		130	134	43
Litters evaluated	N		19	20	13
Tibia <i>Bent (M)</i>	Litters Affected	N	0 f	0	4 *
		%	0	0	30.8
	Fetuses Affected	N	0	0	6
	% per Litter	Mean	0.0 s	0.0	20.8 **
Ulna <i>Bent (M)</i>	Litters Affected	N	0 f	0	5 **
		%	0	0	38.5
	Fetuses Affected	N	0	0	10
	% per Litter	Mean	0.0 s	0.0	26.9 **
Vertebra <i>27 Presacral (V)</i>	Litters Affected	N	0 f	1	0
		%	0	5.0	0
	Fetuses Affected	N	0	1	0
	% per Litter	Mean	0.0 s	0.7	0.0
Metatarsal <i>Unossified (V)</i>	Litters Affected	N	1 f	1	11 **
		%	5.3	5.0	84.6
	Fetuses Affected	N	1	1	20
	% per Litter	Mean	0.9 s	0.8	47.4 **

M = Malformation, f=FISHER-EXACT, * = $p < 0.05$, s=DUNN, ** = $p < 0.01$, V = Variation

Study title: Oral embryo-fetal development study of CP-690550-10 in rats

Study no:	05-2063-24
Study report location:	Mod. 4.2.3.5.2
Conducting laboratory and location:	Pfizer Global Research & Development Groton, CT 06340
Date of study initiation:	Jan 10 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CP-690550-10, Lot # 52546-119-13HS, Purity 97.7% The active moiety comprised 60.3% of the drug substance.

There was no Certificate of Analysis included with this report. Purity was indicated in Table 2.6.7.4 in Module

Key Study Findings

- There were no CP-690550-related effects on mean numbers of corpora lutea, implantation sites, viable fetuses, dead fetuses, early resorptions, late resorptions, pre-implantation loss, post-implantation loss, gravid uterine weight, or fetal sex ratio.
- There were no CP-690550-related effects on fetal body weights, external observations, skeletal observations, or visceral observations.
- The NOAEL for both maternal and developmental toxicity was 30 mg/kg, the highest dose tested. **However, the study was considered inconclusive because the highest dose was not maternally toxic.**

Note: The Applicant was informed to repeat the study at higher maternal doses, which they submitted to the NDA, refer to the review of report 09GR353.

Methods

Doses: 0, 1, 10, 30 mg/kg

Dose in mg/kg is based on mg of active moiety of the drug substance.

Based on the findings of the non-GLP preliminary embryo-fetal development study in rats (04-2063-22), the Applicant determined that 30 mg/kg was the appropriate high dose for the main study. This was reviewed earlier in Section 9.2.

Frequency of dosing: once daily, from gestation day 6 (GD6) through GD17
 Dose volume: 10 mL/kg
 Route of administration: oral
 Formulation/Vehicle: 0.5% methylcellulose
 Species/Strain: rats/Crl:CD® Sprague-Dawley
 Number/Sex/Group: 20 pregnant females/group
 Satellite groups: Toxicokinetics: 5/group
 Study design: Rats were dosed for 12 consecutive days (GD 6-17)

Group Number	Daily Dose* (mg/kg)	Drug Concentration (mg/mL)	Dose Volume (mL/kg)	Animal Numbers	
				Main Study	TK Satellite
1	0	0	10	1-20	81-85
2	1	0.1	10	21-40	86-90
3	10	1	10	41-60	91-95
4	30	3	10	61-80	96-100

*All dose levels were expressed as mg of active moiety per kg of body weight.

Deviation from study protocol:

Parameters and endpoints evaluated: clinical signs (twice daily during pretreatment and non-dosing days, at least 4x daily during treatment), body weight (on arrival, on GD 3, daily from GD 5 through GD 21), maternal corrected body weight (GD 21), feed consumption (daily from GD 7 through GD 21), gravid uterine weights, number of corpora lutea, number of implantation sites, number of late and early resorptions, number of dead fetuses, fetal sex ratio, number of viable fetuses, fetal and placental weights, and external, visceral and skeletal morphology.

Observations and Results

Mortality:

There were no treatment-related mortalities in dams. One animal (#98) in the 30 mg/kg TK group was found dead on GD 18 and a jugular hemorrhage was noted at gross necropsy. Therefore, death was attributed to the blood collection procedure.

Clinical signs:

There were no treatment-related clinical signs.

Body weight:

There were no statistically significant treatment-related effects on body weight. However, mean body weight gain was slightly reduced during the first three days of dosing (GD 6-9) at 30 and 10 mg/kg (85% and 84%, respectively). In addition, mean maternal corrected body weight (maternal body weight–gravid uterine weight) was slightly reduced at 30 mg/kg (87% of control mean). There were no treatment-related effects on mean body weight or maternal corrected body weight at 1 mg/kg.

Feed consumption:

There were no treatment-related effects on feed consumption.

Toxicokinetics:

Rats were exposed to CP-690550 and systemic exposure increased with increasing dose. A 30x increase in dose (1 vs 30 mg/kg/day) of CP-690550-10 resulted in a 26.5X increase in mean C_{max} and 46.5X AUC_{0-24} , respectively.

Table 1. Mean Toxicokinetic Parameters of CP-690,550 in Rats After Oral Administration of CP-690,550-10 on Gestational Day 17 (Dosing Day 12 of Administration of CP-690,550-10)

Dose (mg/kg)	Day	Sex	C_{max} (ng/mL)			T_{max} (h)			AUC^a (ng·h/mL)		
			Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n
1	GD17	Female	185	18.7	5	0.500	0	5	516	117	5
10	GD17	Female	2690	465	5	0.500	0	5	8400	1420	5
30	GD17	Female	4900	449	5	0.600	0.224	5	24000	2590	4

^a AUC interval is 0-24h

Stability and Homogeneity

Stability: CP-690550 was stable in 0.5% methylcellulose over a concentration range of 0.1 to 200 mg CP-690550/mL for a period of 3 days when stored at room temperature.

(b) (4) Development Project No.: 894-003-1).

Homogeneity and Formulation Concentrations: Pre- and postdose samples of the test article and control vehicle formulations were frozen at □ -15-20°C and submitted to Analytical & Formulations for shipment to (b) (4) for determinations of CP-690550 concentrations. Homogeneity samples were collected from formulations containing the test article only. All samples were collected on Study

Day 2. All admixtures were within an acceptable range ($\pm 10\%$) of the intended concentrations and were considered homogenous

Necropsy

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

There were no treatment-related effects on mean numbers of corpora lutea, implantation sites, viable fetuses, dead fetuses, early resorptions, late resorptions, pre-implantation loss, post-implantation loss, gravid uterine weight, or fetal sex ratio. The only treatment-related maternal observation was small thymus in 2 animals (#63, #65) in the 30 mg/kg dose group. There were no maternal necropsy observations in the 10 and 1 mg/kg dose groups.

Offspring (Malformations, Variations, etc.)

There were no treatment-related effects on fetal body weights, external observations, skeletal observations, or visceral observations.

Conclusions: Based on the results of this test, the NOAEL for both maternal and developmental toxicity was 30 mg/kg, the highest dose tested.

Study title: Oral Dose Range-Finding Study of CP-690550 in Pregnant New Zealand White Rabbits

Study no:	04-2063-23
Study report location:	Mod 4.2.3.5.2
Conducting laboratory and location:	Safety Sciences Pfizer Global Research & Development Pfizer Inc Groton, Connecticut 06340
Date of study initiation:	Nov 18 2004
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	CP-690550-10, Lot # 52546-119-13HS, Purity 97.7% The active moiety comprised 60.3% of the drug substance.

There was no Certificate of Analysis included with this report. Purity was indicated in Table 2.6.7.4 in Module

Key Study Findings

- The dose of 30 mg/kg/day resulted in reduced fetal body weights.
- The dose of 100 mg/kg/day resulted in abortion in 1 of 6 does, an increase in post-implantation loss evident by early and late resorptions, and reduced mean gravid uterine weights and fetal body weights.
- The dose of 300 mg/kg/day resulted in mortality of 3 of 6 does, reduced body weight gain during the first 3 days of dosing, reduced food consumption, reduced mean gravid uterine weights, complete post-implantation loss, small thymus in 3 of 6 des and stomach irritation.
- Based on these results the embryo-fetal developmental toxicology study in the rabbit used 10, 30 and 100 mg/kg/day doses of CP-690550-10.

Methods

Doses:	0, 10, 30, 100, and 300 mg/kg
	Dose in mg/kg is based on mg of active moiety of the drug substance.
Frequency of dosing:	once daily from GD 7 to GD 19
Dose volume:	2 mL/kg
Route of administration:	oral
Formulation/Vehicle:	suspension in 0.5% methylcellulose
Species/Strain:	rabbits/New Zealand White
Number/Sex/Group:	6 pregnant females/group
Satellite groups:	none
Study design:	Rabbits were dosed from GD 7 to GD 19. They were sacrificed on GD 29
Deviation from study protocol:	Not mentioned

Observations and Results**Results****Mortality**

Animals were checked at least 4 times daily

In the 100 mg/kg/day group, there was 1 doe (#22) that aborted on GD23 and was euthanized. In the 300 mg/kg/day, there were 3 mortalities, 2 does (#27 and #30) were euthanized on GD15, also one doe (#25) died on GD21. In the 3 does at 300 mg/kg/day that were euthanized or found dead, a small thymus was noted and in 2 there were signs of stomach irritation. No further information was provided.

Clinical Signs

Animals were checked at least 4 times daily

Clinical signs were observed at 100 and 300 mg/kg/day. At 300 mg/kg/day does 27 and 30 were both recumbent prior to euthanasia. Doe 27 had tremors. Doe 25 (300 mg/kg/day) had clinical signs of red fluid in/under cage, fur stained (urogenital region) and loose stools noted prior to death on GD 21. In the 100 mg/kg/day dose group, fetal or placenta tissue was noted for Doe 22 prior to abortion. Other clinical signs at 100 mg/kg/day included red fluid in/under cage (Does 19 and 21), loose stools (Doe 19), and vaginal discharge (Doe 21), all of which occurred during the post-dosing period.

Body Weights and Body Weight Change

Body weight was monitored daily for the majority of the study (GD 6-20), and on GD 23, 26 and 29.

There were no treatment-related effects on mean body weight at any dose level. A transient decrease in mean body weight gain was noted during the first 3 days of dosing

(GD 7-10) at 300 mg/kg/day, which recovered during the GD 10-13 interval. A reduction in mean body weight gain was noted at 300 mg/kg/day (-0.01 g - 0.13 g) for all of the GD intervals throughout the remainder of the study. There was no effect at other dose levels. Corrected maternal body weights were comparable to the control for all dose groups.

Food Consumption

Food consumption was monitored daily from GD 7-29.

Mean food consumption was reduced at 300 mg/kg/day when compared to the control mean throughout the dosing period GD 8-20 (81% of control mean), but was similar to control during the post- dosing period (GD 21-29). There was no effect on food consumption at other doses.

Necropsy

Necropsy and Cesarean sections was performed on GD29. Gravid uterine weights were obtained and a corrected maternal body weight was calculated. Abdominal, thoracic, and pelvic viscera and placenta were examined grossly. The numbers of corpora lutea, implantation sites, late and early resorptions, and viable or dead fetuses were recorded for each doe. Each viable fetus was weighed and examined for external anomalies. Uteri with no evidence of implantation were processed for detection of early embryonic death.

There were no gross necropsy findings in any doe at the scheduled necropsy. Small thymus was noted in the 3 does that were either euthanized or found dead in the 300 mg/kg/day dose group (Does 25, 27, and 30). In addition, 2 of these does (27 and 30) had dark red areas and 1 (Doe 30) had pitted areas on the mucosal lining of the stomach.

Gestation Day 29 Cesarean Section Data

Complete post-implantation loss and an increase in post-implantation loss occurred at 300 and 100 mg/kg/day, respectively. The number of early resorptions was increased at 300 mg/kg/day and both the early and late resorptions were increased at 100 mg/kg/day. There were no viable fetuses available for evaluation in the 300 mg/kg/day dose group and the number of viable fetuses at 100 mg/kg/day was reduced.

Gravid uterine weights were reduced at 300 and 100 mg/kg/day and comparable to control at 30 and 10 mg/kg/day. There was no effect on corpora lutea, implantations, or pre-implantation loss at 300 and 100 mg/kg/day and there was no effect on any reproductive parameter at 30 and 10 mg/kg/day.

Fetal Body Weights

There were no fetuses available for fetal weight collection in the 300 mg/kg/day dose group due to complete litter resorption. There was a treatment-related reduction in

mean fetal body weights in the 30 and 100 mg/kg/day dose groups (92% and 87% control mean, respectively). There was no effect on fetal body weights at 10 mg/kg/day.

Dose (mg/kg/day)	0	10	30	100	300
Mean fetal weight on GD 29	43.73	43.83	40.03	38.06	-

- not applicable due to complete litter resorption

Fetal External Gross Examination

There were no fetuses available for external examination at 300 mg/kg/day due to complete litter resorption. There were no treatment-related external observations in this study. One fetus, Doe #7, Fetus #3, in the 10 mg/kg/day dose group had spina bifida, which was likely a spontaneous event in this low dose group, unrelated to CP-690550.

SUMMARY OF FETAL GROSS EVALUATIONS					
	0 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
TRUNK					
M-SPINA BIFIDA					
FETUSES AFFECTED (MEAN#/LIT)	0/41 (0.0)	1/49 (2.1)	0/54 (0.0)	0/22 (0.0)	
LITTER INCIDENCE (%)	0/ 5 (0.0)	1/ 6 (16.7)	0/ 6 (0.0)	0/ 3 (0.0)	

Study title: Oral embryo-fetal development study of CP-690550-10 in rabbits

Study no: 05-2063-25
 Study report location: Mod 4.2.3.5.2
 Conducting laboratory and location: Safety Sciences
 Pfizer Global Research & Development
 Pfizer Inc
 Groton, Connecticut 06340
 Date of study initiation: Jan 30 2005, April 14 2005
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: CP-690550-10, Lot # 52546-119-13HS,
 Purity 97.7%
 The active moiety comprised 60.3% of the drug substance.

There was no Certificate of Analysis included with this report. Purity was indicated in Table 2.6.7.4 in Module

Key Study Findings

- No adverse maternal toxicity was noted; therefore the maternal NOAEL for systemic toxicity was 100 mg/kg/day.

- Adverse findings included
 - increased post-implantation loss at 30 and 100 mg/kg/day,
 - at 30 and 100 mg/kg/day, increased fetal developmental malformations (cardiovascular, cranio-facial skeletal defects, midline and tail defects)
 - at 100 mg/kg/day, increased fetal developmental variations (absent gallbladders, skeletal defect)
 - reduction in fetal body weight at 100 mg/kg/day
- The NOAEL for developmental toxicity was 10 mg/kg/day

Methods

Doses: 0, 10, 30, and 100 mg/kg

Dose in mg/kg is based on mg of active moiety of the drug substance.

Frequency of dosing: Once daily from GD7 to GD19

Dose volume: 2 mL/kg

Route of administration: Oral

Formulation/Vehicle: Suspension in 0.5% methylcellulose

Species/Strain: Rabbits/New Zealand White

Number/Sex/Group: 20 pregnant females/group

Satellite groups: Toxicokinetics: 5/dose group

Study design:

Study design:

Group Number	Daily Dose* (mg/kg)	Drug Concentration (mg/mL)	Dose Volume (mL/kg)	Animal Numbers		
				Initial Main Study ^a	Repeat Main Study	TK Satellite
1	0	0	2	1-20	101-120	81-85
2	10	5	2	21-40	121-140	86-90
3	30	15	2	41-60	141-160	91-95
4	100	50	2	61-80	161-180	96-100

*All dose levels were expressed as mg of active moiety per kg of body weight.

^aDue to the disarticulation of the skeletons from a processing error during the initial main study and the subsequent lack of readable skeletons, this study was repeated with the exception of the TK portion.

Group Number	Daily	Drug	Dose	Animal Numbers ^a		
	Dose* (mg/kg)	Concentration (mg/mL)	Volume (mL/kg)	Initial Main Study ^b	Repeat Main Study	TK Satellite
1	0	0	2	1-20	101-120	81-85
2	10	5	2	21-40	121-140	86-90
3	30	15	2	41-60	141-160	91-95
4	100	50	2	61-80	161-180	96-100

*All dose levels were expressed as mg of active moiety per kg of body weight.

^a Animal numbers in the repeat main study were expressed as listed by the abbreviated equivalent of 101-180 or the Xybion numbering scheme (5680101-568180). TK satellite animal numbers were expressed as the abbreviated equivalent of 81-100 or the Xybion numbering scheme (5220081-522100). In addition, the tables for the repeat study are labeled as A5206325.

^b Due to the disarticulation of the skeletons from a processing error during the initial main study and the subsequent lack of readable skeletons required by regulatory guidance, this study was repeated with the exception of the TK portion. The summary and individual tables (excluding skeletal examination) for the data collected during the initial main study (Xybion PTS number 0521) for animal numbers 05210001 – 05210080 (expressed as 1-80) were added into the final report as Appendix E. No skeletal data was collected and the disarticulated skeletons were stored and archived along with the skeletons from the repeat study at the issuance of the final report.

Deviation from study protocol: There were no deviations that affected the study results or conclusions.

Due to the disarticulation of the skeletons from a processing error during the main study, resulting in the lack of skeletons for evaluation required by regulatory guidance, this study was repeated with the exception of the TK portion.

The repeat of the study was the appropriate procedure. The TK obtained from the initial study should be sufficiently representative of the second study since drug formulations were appropriately prepared.

The applicant has conducted a preliminary (non-GLP) embryo-fetal development study in rabbits (04-2063-23) to determine the high dose for the subsequent main study (05-2063-25). In the preliminary study CP-690550-10 was administered orally at 0, 10, 30, 100, and 300 mg/kg/day during Days 7 to 19 of gestation. Treatment with CP-690550-10 caused mortality at 300 mg/kg/day (3/6) and abortion in one doe at 100 mg/kg/day. Complete post-implantation loss and an increase in post-implantation loss occurred at 300 and 100 mg/kg/day, respectively. There were no viable fetuses available for evaluation in the 300 mg/kg/day dose group and the number of viable fetuses at 100 mg/kg/day was reduced. Fetal body weights were reduced at 100 and 30 mg/kg/day. There were no treatment-related external fetal findings at any dose level tested. Based

on these findings 100 mg/kg/day was determined to be the appropriate high dose for the main study.

Parameters and endpoints evaluated: clinical signs (twice daily during pretreatment and non-dosing days, at least 4x daily during treatment), body weight (on arrival, on GD 4, daily from GD 6 through GD 20, and on GD 23, 26, and 29), feed consumption (daily from GD 8 through GD 29), gravid uterine weights, number of corpora lutea, number of implantation sites, number of late and early resorptions, number of dead fetuses, fetal sex ratio, number of viable fetuses, fetal and placental weights, and external, visceral and skeletal morphology.

Observations and Results

The Applicant noted that the data from both studies are similar to each other, however, there was a greater effect in the repeat study. Therefore, the data from the repeat study will be presented and discussed in the body of the report. The data from the initial study were submitted and presented in an Appendix.

Mortality:

Two does aborted and were euthanized in the 100 mg/kg/day group (#161, 174) on GD 23 and GD 27, respectively. Placental or fetal tissue was observed in the cage and/or red vulvular discharge. A third doe at 100 mg/kg/day (#164) was euthanized on GD 15 in poor condition with red fluid under the cage and urogenital staining. This doe showed inappetence and body weight loss from the beginning of the study and was the only doe that had changes in body weight and feed consumption. No mortality or abortions were observed at 30 or 10 mg/kg/day.

Clinical signs:

Loose stool was observed in one doe at 10 mg/kg/day (#124) on GD 20 and in two does at 30 mg/kg/day (#152, 154) on GD 21. In the 100 mg/kg group, two does (#161, 174) had fetal or placental tissue in the cage and/or red vulvular discharge. A third doe at 100 mg/kg/day (#164) had red fluid under the cage and urogenital staining.

Body weight:

There were no treatment-related effects on mean body weight in any dose group. Treatment-related statistically significant reductions in mean body weight gain occurred late in gestation at 100 and 30 mg/kg/day. At 100 mg/kg/day, mean body weight gain was reduced 0.25x, 0.29x, and 0.43 x control mean from GD 20-23, GD 23-26 and GD 20-29, respectively. In the 30 mg/kg/day, mean body weight gain was reduced from GD 20-23 and GD 20-29 (0.50x and 0.70x control mean, respectively). There were no effects on body weight gain at 10 mg/kg/day.

Increases in mean body weight gain were noted in all treated groups from GD 13-16 (1.83x, 2.00x, and 1.83x control in the 100, 30, and 10 mg/kg/day groups, respectively). This resulted in an increase in body weight gain for the dosing period inclusive, GD 7-20

(1.60x, 1.48x, and 1.43x control in the 100, 30, and 10 mg/kg groups, respectively) and an increase in maternal corrected body weight at 100 and 30 mg/kg/day.

Feed consumption

There were no treatment-related effects on feed consumption.

Necropsy

There were no treatment-related maternal gross necropsy observations.

Toxicokinetics

Samples were collected predose, and at 0.5, 1, 2, 4, and 8 hours postdose.

Rabbits were exposed to circulating concentrations of CP-690550 and systemic exposure increased with increasing dose. An approximate 10x increase in dose (10-100 mg/kg/day) of CP-690550-10 resulted in a 13.5x and 21.8x increase in C_{max} and AUC_{0-24} , respectively.

Table 1. Mean Toxicokinetic Parameters of CP-690,550 in Rabbits on Gestational Day 19 After Oral Administration of CP-690,550-10 (Dosing Day 13 of Administration of CP-690,550-10)

Dose (mg/kg)	Day	Sex	C_{max}			T_{max}			AUC^a		
			(ng/mL)			(h)			(ng·h/mL)		
			Mean	S.D.	n	Mean	S.D.	n	Mean	S.D.	n
10	GD19	Female	610	211	4	0.875	0.750	4	1470	264	4
30	GD19	Female	2490	876	5	1.20	0.447	5	6350	1380	5
100	GD19	Female	8220	2200	5	1.20	0.758	5	32100	7910	5

^a AUC interval is 0-24h

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

A treatment-related increases in post-implantation loss occurred at 100 and 30 mg/kg/day (8.7x and 3.8x control, respectively) due primarily to an increase in early resorptions (8.0x and 3.0x control at 100 and 30 mg/kg, respectively). An increase in late resorptions was also noted at 100 and 30 mg/kg/day. Although this increase was not statistically significant, the number of late resorptions increased with increasing dose, and therefore, it was considered treatment-related. Both the number of viable fetuses and the gravid uterine weight were reduced at 100 and 30 mg/kg/day (viable fetuses: 0.69x and 0.85x control mean and gravid uterine weight: 0.73x and 0.88x control mean at 100 and 30 mg/kg, respectively). The number of corpora lutea, implantation sites, dead fetuses, pre-implantation loss, and sex ratio were unaffected by treatment at 100 and 30 mg/kg/day. There was no effect on any reproductive parameter at 10 mg/kg/day.

Fetal body weights

A treatment-related reduction in mean fetal body weight occurred at 100 mg/kg/day (0.90x control mean). There was no effect on fetal body weight at 30 or 10 mg/kg/day.

Dose (mg/kg/day)	0	10	30	100
Mean fetal weight on GD 29	44.02	41.86	43.85	39.48

Offspring (Malformations, Variations, etc.)

External A treatment-related increase in external malformations occurred at 100 and 30 mg/kg/day. Two and 1 fetus(es) at 100 and 30 mg/kg/day, respectively, had midline defects including thoracogastroschisis (#178-5) and omphalocele (#172-1) at 100 mg/kg/day and another omphalocele (#142-3) at 30 mg/kg/day. Treatment-related tail malformations occurred in one fetus each at 100 and 30 mg/kg/day dose. Although the number of affected fetuses did not increase with increasing dose, there was an increase in the severity of the tail malformation from a short tail at 30 mg/kg/day (#143-3) to acaudia or absent tail at 100 mg/kg/day (#167-1). Two additional fetuses had cranio-facial malformations. One fetus (#143-1) had microstomia with an absent eye bulge (confirmed as microphthalmia and hemorrhagic eye viscally). A second fetus (#156-3) had cleft lip and cleft palate with absent eye bulges (confirmed as microphthalmia viscally) as well as hydrocephaly (noted viscally). Although there was not a dose-response relationship, these types of findings have been reported in developmental studies with other immunosuppressants (Polifika and Friedman, 2002; Le Ray, et al, 2004), and therefore, were considered treatment-related. The reduced number of viable fetuses available for evaluation at 100 mg/kg/day compared to 30 mg/kg/day (79 vs. 131, respectively) may have prevented a dose response. There were no treatment-related external observations at 10 mg/kg/day.

SUMMARY OF FETAL GROSS EVALUATIONS								
	0 mg/kg	10 mg/kg	P=	30 mg/kg	P=	100 mg/kg	P=	
EYE								
ABSENT EYE BULGE								
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)	0/167 (0.0)	TND	2/131 (1.6)	TND	0/79 (0.0)	0.401	
LITTER INCIDENCE (%)	0/19 (0.0)	0/20 (0.0)	TND	2/19 (10.5)	TND	0/14 (0.0)	0.316	
MOUTH								
M-CLEFT LIP								
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)	0/167 (0.0)	TND	1/131 (0.6)	TND	0/79 (0.0)	0.450	
LITTER INCIDENCE (%)	0/19 (0.0)	0/20 (0.0)	TND	1/19 (5.3)	TND	0/14 (0.0)	0.458	
M-CLEFT PALATE								
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)	0/167 (0.0)	TND	1/131 (0.6)	TND	0/79 (0.0)	0.450	
LITTER INCIDENCE (%)	0/19 (0.0)	0/20 (0.0)	TND	1/19 (5.3)	TND	0/14 (0.0)	0.458	
M-MICROSTOMIA								
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)	0/167 (0.0)	TND	1/131 (1.1)	TND	0/79 (0.0)	0.450	
LITTER INCIDENCE (%)	0/19 (0.0)	0/20 (0.0)	TND	1/19 (5.3)	TND	0/14 (0.0)	0.458	
TAIL								
M-ACAUDATE								
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)	0/167 (0.0)	TND	0/131 (0.0)	TND	1/79 (1.8)	0.385	
LITTER INCIDENCE (%)	0/19 (0.0)	0/20 (0.0)	TND	0/19 (0.0)	TND	1/14 (7.1)	0.194	
M-SHORT TAIL								
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)	0/167 (0.0)	TND	1/131 (1.1)	TND	0/79 (0.0)	0.450	
LITTER INCIDENCE (%)	0/19 (0.0)	0/20 (0.0)	TND	1/19 (5.3)	TND	0/14 (0.0)	0.458	
TRUNK								
M-OMPHALOCELE								
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)	0/167 (0.0)	TND	1/131 (0.7)	TND	1/79 (3.6)	0.336	
LITTER INCIDENCE (%)	0/19 (0.0)	0/20 (0.0)	TND	1/19 (5.3)	TND	1/14 (7.1)	0.140	
M-THORACOGASTROSCHISIS								
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)	0/167 (0.0)	TND	0/131 (0.0)	TND	1/79 (1.2)	0.385	
LITTER INCIDENCE (%)	0/19 (0.0)	0/20 (0.0)	TND	0/19 (0.0)	TND	1/14 (7.1)	0.194	

Visceral A treatment-related increase in visceral malformations occurred in the 100 mg/kg/day dose group. The malformations were related to cardiovascular development and included 8 fetuses in 4 litters with membranous ventricular septal defects (VSD). Three of these fetuses had a VSD as the only cardiovascular observation (#163-5, #167-2, and #173-1); 4 fetuses had dilated aortic arch with a VSD (#163-2, #167-4, #171-4, #163-6, the latter also had dilated pulmonary trunk); and 1 fetus had transposition of the great vessels with a VSD (#171-2). One fetus in the 10 mg/kg/day dose group had a VSD (#124- 8), which was not considered treatment-related as it occurred at the same frequency as the control group and there were none present at 30 mg/kg/day. At 30 mg/kg/day, one fetus (#145-1) had cardiomegaly that was not associated with VSD. Although there was not a dose relationship and the finding was only seen in a single fetus, a relationship to treatment can not be dismissed due to the increase in treatment-related cardiovascular malformations at 100 mg/kg/day. There was also a treatment-related increase in visceral variations at 100 mg/kg/day. Absent gallbladder was noted in 2 fetuses (#171-4, # 178-5) at 100 mg/kg/day and was statistically significant for litter incidence (14.3%). This finding was not observed at any other dose level or in the control and the percent per litter was outside of the historical control database (HCDB) range for both fetus (maximum 0.7%) and litter (maximum

5.6%); therefore, it was considered treatment-related. There was an increase in supernumerary liver lobes at 100 mg/kg/day. This liver variation was noted in 4 fetuses within the same litter (#166-3, -6, 7, 8) and there was no incidence of this finding in the Pfizer HCDB nor in the Middle Atlantic Reproduction and Teratology Association or Midwest Teratology Association historical control for New Zealand White rabbits. It was unclear whether the liver finding was related to treatment or a spontaneous litter affect. There were no treatment-related effects on visceral development at 10 mg/kg/day.

SUMMARY OF FETAL VISCERAL EVALUATIONS							
	0 mg/kg	10 mg/kg	P=	30 mg/kg	P=	100 mg/kg	P=
AORTIC ARCH							
M-DILATED AORTIC ARCH							
FETUSES AFFECTED (MEAN#/LIT)	1/153 (0.8)	0/167 (0.0) TND		0/131 (0.0) TND		4/79 (6.0)	0.266
LITTER INCIDENCE (%)	1/19 (5.3)	0/20 (0.0) TND		0/19 (0.0) TND		3/14 (21.4)	0.083
BRAIN							
M-HYDROCEPHALY							
FETUSES AFFECTED (MEAN#/LIT)	0/153 (0.0)	0/167 (0.0) TND		1/131 (0.6) TND		0/79 (0.0)	0.450
LITTER INCIDENCE (%)	0/19 (0.0)	0/20 (0.0) TND		1/19 (5.3) TND		0/14 (0.0)	0.458
CAROTID ARTERY							
V-LEFT CAROTID FROM INNOMINATE							
FETUSES AFFECTED (MEAN#/LIT)	3/153 (1.8)	5/167 (2.7) TND		7/131 (4.5) TND		3/79 (4.8)	0.454
LITTER INCIDENCE (%)	3/19 (15.8)	2/20 (10.0) TND		3/19 (15.8) TND		2/14 (14.3)	0.546
EYE							
V-HEMORRHAGIC EYE							
FETUSES AFFECTED (MEAN#/LIT)	0/153 (0.0)	0/167 (0.0) TND		1/131 (1.1) TND		0/79 (0.0)	0.450
LITTER INCIDENCE (%)	0/19 (0.0)	0/20 (0.0) TND		1/19 (5.3) TND		0/14 (0.0)	0.458
M-MICROPTALMIA							
FETUSES AFFECTED (MEAN#/LIT)	0/153 (0.0)	0/167 (0.0) TND		2/131 (1.6) TND		0/79 (0.0)	0.401
LITTER INCIDENCE (%)	0/19 (0.0)	0/20 (0.0) TND		2/19 (10.5) TND		0/14 (0.0)	0.316
GALLBLADDER							
V-ABSENT GALLBLADDER							
FETUSES AFFECTED (MEAN#/LIT)	0/153 (0.0)	0/167 (0.0) TND		0/131 (0.0) TND		2/79 (3.0)	0.279
LITTER INCIDENCE (%)	0/19 (0.0)	0/20 (0.0) TND		0/19 (0.0) 0.500		2/14 (14.3)	0.036+
V-SMALL GALLBLADDER							
FETUSES AFFECTED (MEAN#/LIT)	1/153 (0.5)	2/167 (0.9) TND		0/131 (0.0) TND		0/79 (0.0)	0.630
LITTER INCIDENCE (%)	1/19 (5.3)	1/20 (5.0) TND		0/19 (0.0) TND		0/14 (0.0)	0.933
GREAT VESSELS							
M-TRANSPPOSITION OF GREAT VESSELS							
FETUSES AFFECTED (MEAN#/LIT)	0/153 (0.0)	0/167 (0.0) TND		0/131 (0.0) TND		1/79 (1.8)	0.385
LITTER INCIDENCE (%)	0/19 (0.0)	0/20 (0.0) TND		0/19 (0.0) TND		1/14 (7.1)	0.194

SUMMARY OF FETAL VISCERAL EVALUATIONS							
	0 mg/kg	10 mg/kg	P=	30 mg/kg	P=	100 mg/kg	P=
HEART							
M-CARDIOMEGALY							
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)	0/167 (0.0)	TND	1/131 (0.9)	TND	0/79 (0.0)	0.450
LITTER INCIDENCE (%)	0/19 (0.0)	0/20 (0.0)	TND	1/19 (5.3)	TND	0/14 (0.0)	0.458
M-MEMBRANOUS VENT. SEPTAL DEFECT							
FETUSES AFFECTED (MEAN%/LIT)	2/153 (1.3)	1/167 (0.4)	TND	0/131 (0.0)	TND	8/79 (11.9)	0.269
LITTER INCIDENCE (%)	2/19 (10.5)	1/20 (5.0)	TND	0/19 (0.0)	TND	4/14 (28.6)	0.156
LIVER							
V-SUPERNUMERARY LIVER LOBE							
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)	0/167 (0.0)	TND	0/131 (0.0)	TND	4/79 (3.6)	0.385
LITTER INCIDENCE (%)	0/19 (0.0)	0/20 (0.0)	TND	0/19 (0.0)	TND	1/14 (7.1)	0.194
LUNG							
V-ABSENT POST CAVAL LUNG LOBE							
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)	0/167 (0.0)	TND	4/131 (2.2)	TND	1/79 (1.2)	0.250
LITTER INCIDENCE (%)	0/19 (0.0)	0/20 (0.0)	TND	3/19 (15.8)	TND	1/14 (7.1)	0.083
OVARY							
CYST ON OVARY							
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)	1/167 (0.6)	TND	0/131 (0.0)	TND	0/79 (0.0)	0.528
LITTER INCIDENCE (%)	0/19 (0.0)	1/20 (5.0)	TND	0/19 (0.0)	TND	0/14 (0.0)	0.736
PULMONARY TRUNK							
M-DILATED PULMONARY TRUNK							
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)	0/167 (0.0)	TND	0/131 (0.0)	TND	1/79 (1.2)	0.385
LITTER INCIDENCE (%)	0/19 (0.0)	0/20 (0.0)	TND	0/19 (0.0)	TND	1/14 (7.1)	0.194
SPLEEN							
V-SMALL SPLEEN							
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)	0/167 (0.0)	TND	0/131 (0.0)	TND	1/79 (1.2)	0.385
LITTER INCIDENCE (%)	0/19 (0.0)	0/20 (0.0)	TND	0/19 (0.0)	TND	1/14 (7.1)	0.194
URETER							
V-RETROCAVAL URETER							
FETUSES AFFECTED (MEAN%/LIT)	2/153 (1.2)	0/167 (0.0)	TND	1/131 (0.9)	TND	1/79 (1.2)	0.538
LITTER INCIDENCE (%)	2/19 (10.5)	0/20 (0.0)	TND	1/19 (5.3)	TND	1/14 (7.1)	0.684

SUMMARY OF FETAL VISCERAL EVALUATIONS											
	0 mg/kg		10 mg/kg		P=	30 mg/kg		P=	100 mg/kg		P=
VRINE											
V-ACCESSORY VESSEL											
FETUSES AFFECTED (MEAN%/LIT)	1/153 (0.7)	0/167 (0.0)	TND	1/131 (0.5)	TND	0/79 (0.0)	0.558
LITTER INCIDENCE (%)	1/19 (5.3)	0/20 (0.0)	TND	1/19 (5.3)	TND	0/14 (0.0)	0.784

Skeletal There was a statistically significant treatment-related increase in skeletal malformations at 100 mg/kg/day. Fetuses with fused sternebrae were noted in the 100, 30 and 10 mg/kg/day and control groups and this finding was statistically significant for litter incidence at 100 mg/kg/day. There was also a statistically significant increase in the litter incidence of fused ribs at 100 mg/kg/day. Absent caudal centra were noted in one fetus each at 100 and 30 mg/kg/day (#167-1 and #143-3, respectively) and correlated with the tail malformation noted in these fetuses externally. In addition, one fetus at 100 mg/kg/day (#178-5) was noted with a reduced number of caudal vertebrae present skeletally. It was likely the reduction in caudal vertebrae noted skeletally was a

slighter version of the treatment-related tail defects noted externally. Fused skull bones, shortened premaxilla and small eye sockets were noted at 30 mg/kg/day and correlated with the fetuses that had cranio-facial malformation noted externally (#143-1, #156-3). Sternoschisis was noted in one fetus at 100 mg/kg/day (#178-5) and correlated to the thoracogastroschisis noted externally in the fetus. There were no treatment-related skeletal findings at 10 mg/kg/day. The treatment-related fetal developmental malformations noted in this study have previously been reported for other immunosuppressive agents including midline closure, tail and cranio-facial defects (USDA, 1993; Polifika and Friedman, 2002; Le Ray, et al., 2004), cardiovascular defects and gallbladder agenesis (USDA, 1993), and skeletal defects (USDA, 1993). Based on the literature, it is likely that the fetal effects produced by CP-690550-10 are secondary to the intended immunosuppressive activity.

SUMMARY OF FETAL SKELETAL EVALUATIONS									
	0 mg/kg		10 mg/kg		30 mg/kg		100 mg/kg		
				P=				P=	P=
CAUDAL CENTRUM									
M-ABSENT CAUDAL CENTRUM									
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)		0/167 (0.0)	TND	1/131 (1.1)	TND	1/79 (1.8)		0.336
LITTER INCIDENCE (%)	0/19 (0.0)		0/20 (0.0)	TND	1/19 (5.3)	TND	1/14 (7.1)		0.140
M-FUSED CAUDAL CENTRUM									
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)		0/167 (0.0)	TND	1/131 (1.1)	TND	0/79 (0.0)		0.450
LITTER INCIDENCE (%)	0/19 (0.0)		0/20 (0.0)	TND	1/19 (5.3)	TND	0/14 (0.0)		0.458
CAUDAL VERTEBRA									
V-REDUCED NO. CAUDAL VERT. OSSIF.									
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)		0/167 (0.0)	TND	0/131 (0.0)	TND	1/79 (1.2)		0.385
LITTER INCIDENCE (%)	0/19 (0.0)		0/20 (0.0)	TND	0/19 (0.0)	TND	1/14 (7.1)		0.194
CERVICAL CENTRUM									
V-UNOSSIFIED CERVICAL CENTRUM									
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)		0/167 (0.0)	TND	1/131 (0.6)	TND	0/79 (0.0)		0.450
LITTER INCIDENCE (%)	0/19 (0.0)		0/20 (0.0)	TND	1/19 (5.3)	TND	0/14 (0.0)		0.458
CERVICAL VERT.									
M-CERVICAL HEMI-VERTEBRA									
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)		0/167 (0.0)	TND	0/131 (0.0)	TND	1/79 (1.2)		0.385
LITTER INCIDENCE (%)	0/19 (0.0)		0/20 (0.0)	TND	0/19 (0.0)	TND	1/14 (7.1)		0.194
FOREPAW PHALANX									
V-UNOSSIFIED PHALANX									
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)		0/167 (0.0)	TND	1/131 (0.6)	TND	1/79 (1.2)		0.336
LITTER INCIDENCE (%)	0/19 (0.0)		0/20 (0.0)	TND	1/19 (5.3)	TND	1/14 (7.1)		0.140
GENERAL OBSERV.									
M-SMALL EYE SOCKET									
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)		0/167 (0.0)	TND	1/131 (0.6)	TND	0/79 (0.0)		0.450
LITTER INCIDENCE (%)	0/19 (0.0)		0/20 (0.0)	TND	1/19 (5.3)	TND	0/14 (0.0)		0.458
HYOID									
V-BENT HYOID ARCH									
FETUSES AFFECTED (MEAN%/LIT)	2/153 (1.1)		3/167 (1.7)	TND	5/131 (3.2)	TND	2/79 (2.4)		0.362
LITTER INCIDENCE (%)	2/19 (10.5)		2/20 (10.0)	TND	3/19 (15.8)	TND	2/14 (14.3)		0.371

	0 mg/kg	10 mg/kg	P=	30 mg/kg	P=	100 mg/kg	P=
LUMBAR CENTRUM							
M-FUSED LUMBAR CENTRUM							
FRTUSSE AFFECTED(MEAN%/LIT)	0/153 (0.0)	1/167 (0.6)	TND	0/131 (0.0)	TND	0/79 (0.0)	0.528
LITTER INCIDENCE (%)	0/19 (0.0)	1/20 (5.0)	TND	0/19 (0.0)	TND	0/14 (0.0)	0.736
LUMBAR VERTEBRA							
M-SUPERNUMERARY LUMBAR VERTEBRA							
FRTUSSE AFFECTED(MEAN%/LIT)	0/153 (0.0)	0/167 (0.0)	TND	0/131 (0.0)	TND	1/79 (0.9)	0.385
LITTER INCIDENCE (%)	0/19 (0.0)	0/20 (0.0)	TND	0/19 (0.0)	TND	1/14 (7.1)	0.194
METACARPAL							
V-UNOSSFIED METACARPAL							
FRTUSSE AFFECTED(MEAN%/LIT)	2/153 (1.2)	1/167 (0.5)	TND	1/131 (0.6)	TND	1/79 (1.2)	0.568
LITTER INCIDENCE (%)	2/19 (10.5)	1/20 (5.0)	TND	1/19 (5.3)	TND	1/14 (7.1)	0.726
NASAL							
M-FUSED NASAL							
FRTUSSE AFFECTED(MEAN%/LIT)	0/153 (0.0)	0/167 (0.0)	TND	1/131 (0.6)	TND	0/79 (0.0)	0.450
LITTER INCIDENCE (%)	0/19 (0.0)	0/20 (0.0)	TND	1/19 (5.3)	TND	0/14 (0.0)	0.458
PREMAXILLA							
M-SHORT PREMAXILLA							
FRTUSSE AFFECTED(MEAN%/LIT)	0/153 (0.0)	0/167 (0.0)	TND	1/131 (0.6)	TND	0/79 (0.0)	0.450
LITTER INCIDENCE (%)	0/19 (0.0)	0/20 (0.0)	TND	1/19 (5.3)	TND	0/14 (0.0)	0.458
RIB							
V-7TH CERVICAL RIB							
FRTUSSE AFFECTED(MEAN%/LIT)	2/153 (1.6)	0/167 (0.0)	TND	2/131 (1.5)	TND	0/79 (0.0)	0.613
LITTER INCIDENCE (%)	2/19 (10.5)	0/20 (0.0)	TND	2/19 (10.5)	TND	0/14 (0.0)	0.831
M-ABSENT RIB							
FRTUSSE AFFECTED(MEAN%/LIT)	0/153 (0.0)	0/167 (0.0)	TND	0/131 (0.0)	TND	1/79 (1.2)	0.385
LITTER INCIDENCE (%)	0/19 (0.0)	0/20 (0.0)	TND	0/19 (0.0)	TND	1/14 (7.1)	0.194
M-FUSED RIB							
FRTUSSE AFFECTED(MEAN%/LIT)	0/153 (0.0)	1/167 (0.6)	TND	1/131 (0.6)	TND	3/79 (4.2)	0.169
LITTER INCIDENCE (%)	0/19 (0.0)	1/20 (5.0)	TND	1/19 (5.3)	0.333	3/14 (21.4)	0.025+

SUMMARY OF FETAL SKELETAL EVALUATIONS										
	0 mg/kg		10 mg/kg		P=	30 mg/kg		P=	100 mg/kg	P=
SACRAL CENTRUM										
M-FUSED SACRAL CENTRUM										
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)		0/167 (0.0)		TND	1/131 (1.1)		TND	0/79 (0.0)	0.450
LITTER INCIDENCE (%)	0/19 (0.0)		0/20 (0.0)		TND	1/19 (5.3)		TND	0/14 (0.0)	0.458
SKULL										
M-FUSED SKULL BONE(S)										
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)		0/167 (0.0)		TND	2/131 (1.6)		TND	0/79 (0.0)	0.401
LITTER INCIDENCE (%)	0/19 (0.0)		0/20 (0.0)		TND	2/19 (10.5)		TND	0/14 (0.0)	0.316
STERNEBRA										
M-COSTAL CARTILAGE FUSED										
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)		1/167 (0.7)		TND	0/131 (0.0)		TND	0/79 (0.0)	0.528
LITTER INCIDENCE (%)	0/19 (0.0)		1/20 (5.0)		TND	0/19 (0.0)		TND	0/14 (0.0)	0.736
V-EXTRA STERNEBRA OCCIP. SITE										
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)		1/167 (0.7)		TND	0/131 (0.0)		TND	1/79 (1.2)	0.410
LITTER INCIDENCE (%)	0/19 (0.0)		1/20 (5.0)		TND	0/19 (0.0)		TND	1/14 (7.1)	0.316
M-FUSED STERNEBRA										
FETUSES AFFECTED (MEAN%/LIT)	1/153 (0.8)		2/167 (1.1)		TND	1/131 (0.9)		TND	7/79 (9.4)	0.058
LITTER INCIDENCE (%)	1/19 (5.3)		2/20 (10.0)		TND	1/19 (5.3)	0.621		6/14 (42.9)	0.008+
V-MISALIGNED STERNEBRA										
FETUSES AFFECTED (MEAN%/LIT)	1/153 (0.5)		1/167 (0.6)		TND	1/131 (0.9)		TND	1/79 (1.0)	0.460
LITTER INCIDENCE (%)	1/19 (5.3)		1/20 (5.0)		TND	1/19 (5.3)		TND	1/14 (7.1)	0.507
V-STERNEBRA(B) #5 &/OR 6 UNOCCIP.										
FETUSES AFFECTED (MEAN%/LIT)	4/153 (2.6)		21/167 (11.6)		TND	22/131 (15.2)		TND	6/79 (6.7)	0.206
LITTER INCIDENCE (%)	4/19 (21.1)		7/20 (35.0)		TND	8/19 (42.1)		TND	4/14 (28.6)	0.276
M-STERNOSCHISIS										
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)		0/167 (0.0)		TND	0/131 (0.0)		TND	1/79 (1.2)	0.385
LITTER INCIDENCE (%)	0/19 (0.0)		0/20 (0.0)		TND	0/19 (0.0)		TND	1/14 (7.1)	0.194
TALUS										
V-UNOCCIPED TALUS										
FETUSES AFFECTED (MEAN%/LIT)	2/153 (1.3)		2/167 (0.9)		TND	1/131 (0.6)		TND	1/79 (1.2)	0.596
LITTER INCIDENCE (%)	2/19 (10.5)		2/20 (10.0)		TND	1/19 (5.3)		TND	1/14 (7.1)	0.760

SUMMARY OF FETAL SKELETAL EVALUATIONS										
	0 mg/kg		10 mg/kg		P=	30 mg/kg		P=	100 mg/kg	P=
THORACIC ARCH										
M-SMALL THORACIC ARCH										
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)		0/167 (0.0)		TND	0/131 (0.0)		TND	1/79 (1.2)	0.385
LITTER INCIDENCE (%)	0/19 (0.0)		0/20 (0.0)		TND	0/19 (0.0)		TND	1/14 (7.1)	0.194
THORACIC CENTRUM										
M-HEMICENTRIC THORACIC CENTRUM										
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)		0/167 (0.0)		TND	0/131 (0.0)		TND	1/79 (1.2)	0.385
LITTER INCIDENCE (%)	0/19 (0.0)		0/20 (0.0)		TND	0/19 (0.0)		TND	1/14 (7.1)	0.194
THORACIC VERT.										
M-THORACIC HEMI-VERTEBRA										
FETUSES AFFECTED (MEAN%/LIT)	0/153 (0.0)		1/167 (0.6)		TND	1/131 (0.6)		TND	1/79 (1.2)	0.362
LITTER INCIDENCE (%)	0/19 (0.0)		1/20 (5.0)		TND	1/19 (5.3)		TND	1/14 (7.1)	0.240

Conclusion: No adverse maternal toxicity was noted; therefore the NOAEL was 100 mg/kg/day. Based on the increased post-implantation loss at 100 and 30 mg/kg/day, fetal developmental malformations at 100 and 30 mg/kg/day, fetal developmental

variations at 100 mg/kg/day, and the reduction in fetal body weight at 100 mg/kg/day, the NOAEL for developmental toxicity was 10 mg/kg/day.

9.3 Prenatal and Postnatal Development

Study title: Oral (Gavage) Developmental and Perinatal/Postnatal Reproduction Toxicity Study of CP-690550-10 in Rats, Including a Postnatal Behavioral/Functional Evaluation

Study no:	08GR095
Study report location:	Mod 4.2.3.5.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 20 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CP-690550-10, Lot E010005898, Purity 99.8%, The active moiety consists of 61.6% % of the drug substance.

Key Study Findings

- Pregnant rats were treated once daily from gestation day 6 through day 20 of lactation with CP-690550 at doses of 0, 1, 10, and 50 mg/kg/day
- The maternal NOAEL was 50 mg/kg/day. Reduced feed consumption values throughout the lactation period in the 50 mg/kg/day dosage group had no effect on maternal body weights or body weight gains and were considered secondary to the lowered energy requirements due to the smaller litter size that occurred in this dosage group.
- Reproductive NOAEL in the dams was 10 mg/kg/day. At 50 mg/kg/day, the averages for the total number of delivered pups and the number of liveborn pups were reduced.
- The NOAEL for viability and growth in the offspring was 10 mg/kg/day. At 50 mg/kg/day, pup viability was severely reduced between days 1 and 4 postpartum followed by reduced pup survivability between days 4 and 21. This resulted in termination of all F1 offspring from the 50 mg/kg/day treated dams at weaning.
- Assessments of growth, sexual development and behavior were conducted only for the 0, 1, and 10 mg/kg/day dose groups.
- Reductions in pup weight that occurred at 10 mg/kg/day prior to weaning were evident in reduced body weights of F1 male rats during the post weaning period, however body weight gains post weaning were generally similar to the vehicle control group gains.
- There was no effect of CP-690550 treatment of dams on F1 generation rats sexual maturation, learning and memory, startle response, motor activity, estrous cyclicity, mating and fertility, or viability of F2 generation fetuses.

Methods

Doses: 0, 1, 10, and 50 mg/kg/day

Dose in mg/kg is based on mg of active moiety of the drug substance.

Frequency of dosing: Once daily from GD 6 through lactation day 20 (or GD 24 if a litter was not delivered). Any dam in the process of parturition was not dosed until the following work day.

Dose volume: 10 mL/kg

Route of administration: oral by gavage

Formulation/Vehicle: 0.5% methylcellulose (MC)

Species/Strain: Rat/Crl:CD(SD)

Number/Sex/Group: 25/dose group

Satellite groups: none

Study design:

F0 Generation Treatments

Dosage Group	Dosage* (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Rats	Assigned F0 Generation Rat Numbers
I	0 (Vehicle)	0	10	25	18801 - 18825
II	1	0.1	10	25	18826 - 18850
III	10	1	10	25	18851 - 18875
IV	50	5	10	25	18876 - 18900

a. The test article was considered 61.6% active/pure for the purpose of dosage calculations.

Female rats were paired with breeder male rats, one male rat per female rat, for a maximum of five days. Observations were made for viability, adverse clinical and necropsy observations, body weights, and absolute and relative feed consumption values. Mating performance and fertility of female rats were determined. Female rats were evaluated for adverse clinical signs observed during parturition, duration of gestation (DG 0 to the day the first pup was observed), litter sizes (all pups delivered), pup viability at birth, fertility index (percentage of matings that result in pregnancies), gestation index (percentage of pregnancies that result in birth of live litters), number of offspring per litter (live and dead pups), number of implantation sites, general condition of dam and litter during the postpartum period, viability indices (percentage of pups born that survive 4 and 7 days) and lactation index (percentage of pups born that survive 21 days). After completion of the 21-day postpartum period, female rats were euthanized and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed.

On postpartum day 4 (PPD 4), a table of random units was used to select F1 generation pups to be culled, and litters were reduced to eight pups each. Whenever possible, the same number of male and female pups per litter was continued on study. **Surviving litters assigned to the 50 mg/kg/day dosage**

group (Group IV) were not weaned, but were terminated on PPD 21 because of excessive mortality that occurred during parturition and up to day 4 postpartum. At weaning on PPD 21, a table of random units was used to select 25 male and 25 female pups per group from litters in dosage Groups I through III for continued evaluation. At least one male pup and one female pup per litter, when possible, were selected.

F1 Generation Groups

Dosage Group	Maternal Dosage (mg/kg/day)	Number of Rats Per Sex	Assigned F1 Generation Rat Numbers	
			Male Rats	Female Rats
I	0 (Vehicle)	25	4201 - 4225	4301 - 4325
II	1	25	4226 - 4250	4326 - 4350
III	10	25	4251 - 4275	4351 - 4375
IV	50	a	a	a

a. The 50 mg/kg/day dosage group was terminated before completion of the in-life phase because of excessive mortality that occurred during parturition and up to day 4 postpartum. Six of 25 dams and litters in this dosage group survived to DL 21.

The F1 generation rats from Groups I through III selected for continued observation were weaned on postpartum day 21 (PPD 21). Those F1 rats from Groups I through III not selected for continued observation and all F1 generation rats from Group IV were euthanized on PPD 21 and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed (including a cross-section of the head to evaluate the brain).

Female rats were evaluated for the age of vaginal patency, beginning on PPD 28. Male rats were evaluated for the age of preputial separation, beginning on PPD 39. For behavioral assessments, one male and one female rat (when possible) from each litter was examined throughout the two testing periods. Motor activity was evaluated (in a lighted room) on PPD 22 and again on PPDs 54 to 57. Beginning at PPDs 23 to 25, passive avoidance test for learning, short-term retention and long-term retention were evaluated. Beginning at PPDs 63 to 65, rats were evaluated in a water-filled M-maze for overt coordination, swimming ability, learning and memory. Acoustic startle habituation was evaluated on PPDs 78 to 81.

Estrous cycling was evaluated by examination of vaginal cytology for 14 days before initiation of cohabitation and then until spermatozoa were observed in a smear of the vaginal contents and/or a copulatory plug was observed *in situ* during the cohabitation period. At PPD 94 to PPD 97, the F1 generation rats within each dosage group were assigned to cohabitation, one male rat per female rat, based on a random unit table, with the exclusion of sibling matings. The cohabitation period consisted of a maximum of 17 days. Female rats with spermatozoa observed in a smear of the vaginal contents and/or a copulatory plug observed *in situ* were considered to be at DG 0 and assigned to individual housing. Female rats not mated within the first 14 days of cohabitation were assigned alternate male rats from the same dosage group that had mated for an additional three days. Two female rats [4310 in the 0 (Vehicle) mg/kg/day dosage

group and 4328 in the 10 mg/kg/day dosage group] were presumed to be pregnant by palpation. One female rat (4310) delivered and was euthanized and the other female rat (4328) was euthanized on an estimated DG 21.

The schedule of study events and evaluations is indicated in the Table below:

4.1.15.1. F0 Generation Rats

Rat Arrival	20 MAY 2008
Cohabitation Period	25 MAY 2008 PM - 30 MAY 2008 AM
DG ^a 0	26 MAY 2008 - 29 MAY 2008
Dosage Period (DG 6 to DL 20 [or DG 24 for rats that did not deliver a litter])	01 JUN 2008 - 09 JUL 2008
Delivery Period (DL ^b 1)	17 JUN 2008 - 21 JUN 2008
DG 25 Euthanasia (Rats that did not deliver a litter)	20 JUN 2008 - 22 JUN 2008
DL 21 Euthanasia (Dams and litters not selected for continued observation)	07 JUL 2008 - 10 JUL 2008

4.1.15.2. F1 Generation Rats

Motor Activity (day 22 postpartum)	08 JUL 2008 - 11 JUL 2008
Motor Activity (days 54 to 57 postpartum)	09 AUG 2008 - 15 AUG 2008
Passive Avoidance	
Session 1 - days 23 to 25 postpartum	10 JUL 2008 - 14 JUL 2008
Session 2 - days 30 to 32 postpartum	17 JUL 2008 - 21 JUL 2008
Watermaze Testing	
Session 1 - days 63 to 65 postpartum	19 AUG 2008 - 22 AUG 2008
Session 2 - days 70 to 72 postpartum	26 AUG 2008 - 29 AUG 2008
Acoustic Startle (days 78 to 81 postpartum)	03 SEP 2008 - 07 SEP 2008
Cohabitation Period	
Male 1	21 SEP 2008 PM - 05 OCT 2008 AM
Male 2	05 OCT 2008 PM - 08 OCT 2008 AM
DG 0	22 SEP 2008 - 08 OCT 2008
Male Rats Euthanized - days 127 to 130 postpartum	24 OCT 2008
DG 21 Cesarean-Sectioning	13 OCT 2008 - 29 OCT 2008 ^c

- DG is an abbreviation used for day of (presumed) gestation.
- DL is an abbreviation for day of lactation.
- Includes one female rat (4328 in the 10 mg/kg/day dosage group) without a confirmed date of mating that was euthanized on an estimated DG 21.

Deviation from study protocol: There were no deviations that affected the study results or conclusions. There were numerous instances of missing observations for individual animals in the various observations and behavioral evaluations. However, the Applicant commented and the Reviewer concurs that "relative to the total number of animals evaluated and/or the number of data points collected per parameter, the deviations were not significant."

Observations and Results

F₀ Dams

Survival

One rat #18821 in the control group was found dead on GD18 period, and one rat #18878 in the 50 mg/kg/day dosage group was euthanized on GD23 due to declining clinical condition presumably associated with dystocia.

The death in the vehicle control group was probably secondary to aspiration of the vehicle. There were no clinical signs prior to death, with body weight and feed consumption values similar to values for other control group rats. Necropsy revealed mottled (red and dark red) lung lobes; all other tissues appeared normal. The litter for this rat consisted of 16 fetuses *in utero*.

The unscheduled euthanasia in the 50 mg/kg/day dosage group was presumed related to CP-690550 treatment since it occurred at the highest dosage level (late in gestation when the maximum dosage of CP-690, 550 was administered based on a mg/kg dose), and was related to dystocia since the litter observations (i.e., dead fetuses) were similar to those of dams that delivered a litter. The adverse clinical signs of this rat were ataxia, decreased motor activity, ptosis, pale extremities, red perivaginal substance and bradypnea. Body weight and feed consumption values for this rat were comparable to values for other rats in this dosage group. All tissues appeared normal at necropsy examination. The litter for this rat consisted of 15 dead fetuses *in utero*.

Clinical Signs

There were no CP-690550-related effects on clinical signs.

The most commonly observed clinical observations were a scab on the tail, mild dehydration (based on skin turgor), localized alopecia on the limbs, pale ears or eyes, hunched posture, sparse hair coat (limbs, underside and/or neck), soft or liquid feces, urine-stained abdominal fur, chromorhinorrhea and chromodacryorrhea, and ungroomed coat.

Body Weight

During Gestation: There were no CP-690550-related effects on body weight or body weight gain.

During Lactation: There were no CP-690550-related effects on body weight or body weight gain for the lactation period (postpartum days 1 to 21).

MATERNAL BODY WEIGHTS - F0 GENERATION FEMALE RATS

Figure 1

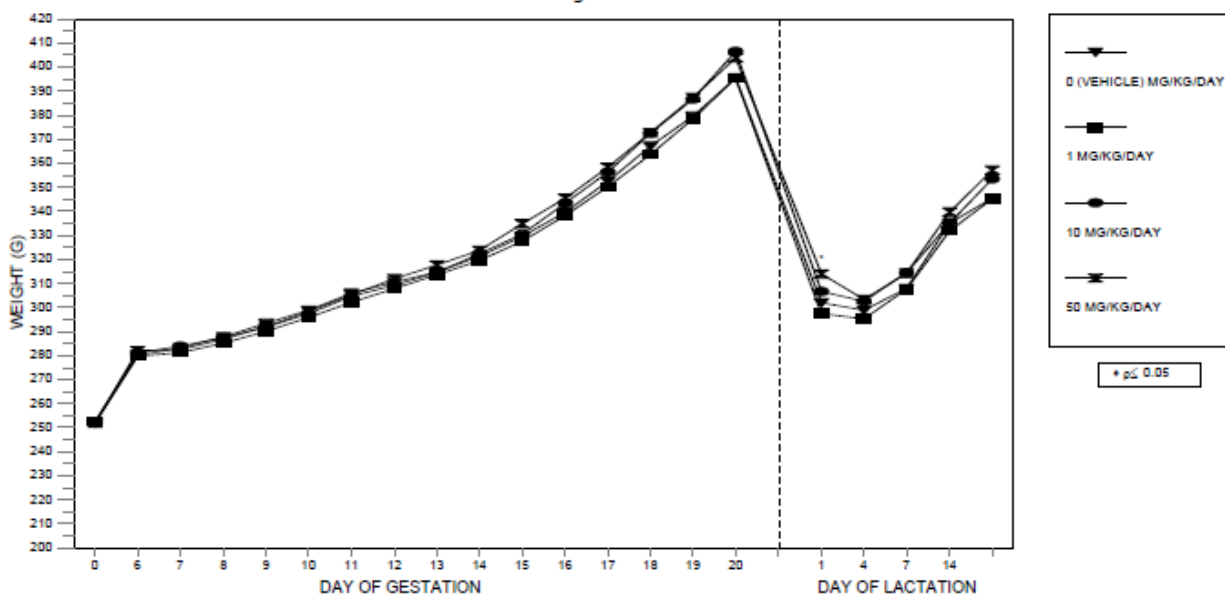


TABLE A4 (PAGE 1): MATERNAL BODY WEIGHT CHANGES - GESTATION - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) a		0 (VEHICLE)	1	10	50
RATS TESTED	N	25	25	25	25
PREGNANT	N	25	24	23	23
MATERNAL BODY WEIGHT CHANGE (G)					
DAYS 0 - 6	MEAN±S.D.	+28.2 ± 7.3	+27.0 ± 5.5	+29.0 ± 6.6	+30.0 ± 7.4
DAYS 6 - 9	MEAN±S.D.	+13.2 ± 5.0	+10.5 ± 5.8	+10.9 ± 4.0	+10.1 ± 6.3
DAYS 9 - 12	MEAN±S.D.	+17.2 ± 3.5	+17.8 ± 5.4	+17.4 ± 4.0	+19.7 ± 3.7
DAYS 12 - 15	MEAN±S.D.	+18.9 ± 5.5	+19.5 ± 5.9	+21.1 ± 4.4	+23.0 ± 6.0
DAYS 15 - 18	MEAN±S.D.	+37.6 ± 6.5	+36.2 ± 6.0	+42.2 ± 7.5*	+37.9 ± 7.4
DAYS 18 - 20	MEAN±S.D.	+29.4 ± 5.3	+31.8 ± 5.7	+33.9 ± 8.0	+31.2 ± 12.8
DAYS 6 - 20	MEAN±S.D.	+116.3 ± 16.5	+115.9 ± 13.6	+125.5 ± 18.4	+121.9 ± 21.0
DAYS 0 - 20	MEAN±S.D.	+144.0 ± 20.3	+142.9 ± 16.0	+154.4 ± 21.8	+151.9 ± 26.0

DAYS = DAYS OF GESTATION

[] = NUMBER OF VALUES AVERAGED

a. Dosage occurred on day 6 of gestation through day 20 of lactation.

b. Excludes values for the dam that was found dead.

* Significantly different from the vehicle control group value (p<0.05).

TABLE A6 (PAGE 1): MATERNAL BODY WEIGHT CHANGES - LACTATION - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) ^a		0 (VEHICLE)	1	10	50
RATS TESTED	N	25	25	25	25
PREGNANT	N	25	24	23	23
INCLUDED IN ANALYSES	N	24 ^b	24	23	22 ^b
DELIVERED A LITTER	N	24	24	23	22
MATERNAL BODY WEIGHT CHANGE (G)					
DAYS 1 - 4	MEAN±S.D.	-3.1 ± 9.6	-2.2 ± 9.5	-4.2 ± 11.4	-11.0 ± 11.0 [8] ^c
DAYS 4 - 7	MEAN±S.D.	+9.0 ± 6.1	+12.2 ± 6.5	+11.8 ± 9.7	+10.8 ± 5.4 [6] ^c
DAYS 7 - 14	MEAN±S.D.	+27.2 ± 8.1	+24.3 ± 10.0	+20.7 ± 17.4	+25.5 ± 5.3 [6] ^c
DAYS 14 - 21	MEAN±S.D.	+10.3 ± 14.3	+13.5 ± 11.7	+18.4 ± 20.0	+17.3 ± 8.9 [6] ^c
DAYS 1 - 21	MEAN±S.D.	+43.4 ± 19.4	+47.8 ± 13.6	+46.8 ± 11.1	+43.0 ± 17.0 [6] ^c

DAYS = DAYS OF LACTATION

[] NUMBER OF VALUES AVERAGED.

a. Dosage occurred on day 6 of gestation through day 20 of lactation.

b. Excludes values for dams that were found dead or euthanized due to adverse clinical observations during the gestation period.

c. Excludes values for dams that were euthanized due to no surviving pups.

Feed Consumption

During Gestation: There were no CP-690550-related effects on absolute and relative feed consumption values during the gestation period. Absolute feed consumption values in the 1, 10 and 50 mg/kg/day dosage groups were 98%, 98% and 100% of the vehicle control group value, respectively, for the entire gestation dosing period (GD 6 to 20).

During Lactation: Absolute and relative feed consumption values were significantly lower in the high dose group, 50 mg/kg/day, over the entire lactation period (DLs 1 to 14) and also at each tabulated interval within this period, in comparison to the vehicle control values. Absolute feed consumption values in the 1, 10 and 50 mg/kg/day dosage groups were 102%, 98% and 79% of the vehicle control group value, respectively, for lactation days 1 to 14. These reductions were probably secondary to the lower energy requirements for the dams in the high dose group due to the smaller litter size of the surviving pups.

TABLE A7 (PAGE 1): MATERNAL ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - GESTATION - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY) ^a		I 0 (VEHICLE)	II 1	III 10	IV 50
RATS TESTED	N	25	25	25	25
PREGNANT	N	25	24	23	23
MATERNAL FEED CONSUMPTION (G/DAY)					
DAYS 0 - 6	MEAN±S.D.	22.1 ± 2.3	22.2 ± 1.5	22.2 ± 1.7	23.0 ± 2.2
DAYS 6 - 9	MEAN±S.D.	23.9 ± 2.9	22.9 ± 1.9	23.6 ± 2.0	24.2 ± 3.2
DAYS 9 - 12	MEAN±S.D.	25.6 ± 3.3	24.8 ± 2.6	25.0 ± 2.6	26.1 ± 3.3
DAYS 12 - 15	MEAN±S.D.	26.0 ± 2.9	26.1 ± 2.7	26.1 ± 2.7	27.1 ± 3.4
DAYS 15 - 18	MEAN±S.D.	25.6 ± 3.1	25.3 ± 3.3	25.4 ± 2.8	24.7 ± 2.8
DAYS 18 - 20	MEAN±S.D.	25.2 ± 3.5	24.4 ± 2.8	24.4 ± 3.2	23.1 ± 5.3
DAYS 6 - 20	MEAN±S.D.	25.3 ± 2.8	24.8 ± 2.0	24.9 ± 2.2	25.2 ± 2.6
DAYS 0 - 20	MEAN±S.D.	24.4 ± 2.5	24.0 ± 1.7	24.2 ± 2.0	24.5 ± 2.3

DAYS = DAYS OF GESTATION

[] = NUMBER OF VALUES AVERAGED

a. Dosage occurred on day 6 of gestation through day 20 of lactation.

b. Excludes values that appeared incorrectly recorded, as well as those associated with spillage.

c. Excludes values for dam 18821, which was found dead on day 18 of gestation.

TABLE A9 (PAGE 1): MATERNAL ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - LACTATION - SUMMARY - F0 GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY) ^a		I 0 (VEHICLE)	II 1	III 10	IV 50
RATS TESTED	N	25	25	25	25
PREGNANT	N	25	24	23	23
INCLUDED IN ANALYSES	N	24 ^b	24	23	22 ^b
DELIVERED A LITTER	N	24	24	23	22
MATERNAL FEED CONSUMPTION (G/DAY)					
DAYS 1 - 4	MEAN±S.D.	27.1 ± 4.2	28.3 ± 4.2	27.2 ± 3.3	21.6 ± 2.3**
DAYS 4 - 7	MEAN±S.D.	38.4 ± 5.1	40.0 ± 4.7	39.0 ± 5.3	32.3 ± 5.1*
DAYS 7 - 10	MEAN±S.D.	51.2 ± 5.8	50.9 ± 5.8	49.6 ± 5.5	40.6 ± 6.1**
DAYS 10 - 14	MEAN±S.D.	60.6 ± 5.6	61.6 ± 5.8	58.3 ± 6.8	44.4 ± 8.3**
DAYS 1 - 14	MEAN±S.D.	45.4 ± 4.5	46.4 ± 4.2	44.7 ± 4.0	35.8 ± 4.4**

DAYS = DAYS OF LACTATION

[] NUMBER OF VALUES AVERAGED

a. Dosage occurred on day 6 of gestation through day 20 of lactation.

b. Excludes values for dams that were found dead or euthanized due to adverse clinical observations during the gestation period.

c. Excludes values for dams that were euthanized due to no surviving pups.

d. Excludes values that were associated with spillage.

* Significantly different from the vehicle control group value (p≤0.05).

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

Pregnancy/Uterine Content

There was no effect of CP-690550 treatment on pregnancy rates, but there was a reduction in litters born in the high dose group, 50 mg/kg/day. Of the 25 dams per dose group, pregnancy occurred in 25, 24, 23 and 23 rats in the vehicle, 1, 10 and 50 mg/kg/day dose groups, respectively, and the number of dams that littered was 24, 24, 23 and 21 dams, respectively. In addition, the averages for the total number of delivered

pups and the number of liveborn pups were reduced in the 50 mg/kg/day dosage group (12.2 delivered pups and 11.8 liveborn pups, respectively) in comparison to the vehicle control group value (14.2 delivered pups and 13.7 liveborn pups).

Necropsy Observations

The only gross lesions related to CP-690550 treatment was the presence of conceptuses (i.e., fetuses and/or late resorptions) in the left and/or right uterine horn of two dams in the high dose group, 50 mg/kg/day. Other necropsy observations that were not related to dose included a diaphragmatic hernia (i.e., portion of the right lateral lobe of the liver was protruding into the thoracic cavity), mottled (red and dark red) lung lobes and tissue present in the stomach (i.e., presumed to be tissue from cannibalized pups).

Toxicokinetics

Not conducted

Stability and homogeneity

Stability: This had been determined in previous studies and the conducting laboratory procedures appeared to be within those stability boundaries.

Homogeneity: The homogeneity results for CP-690550-10 formulations prepared on the first day of study were within the criteria of <10% difference with means of 3.8%, 7.9% and 6.1%, respectively, for 0.1 mg/mL, 1 mg/mL and 5 mg/mL concentrations.

Concentrations: All concentration samples prepared on the first day of study, mid point of study, and end of study were within specification, 90% to 110% of intended concentration, except for the 0.1 mg/mL samples (mean of 116%) from first day of preparation and 5 mg/mL samples (mean of 111%) from mid point of study. An out-of-specification investigation found no analytical error. The Applicant provided the possible explanation that the results at 0.1 mg/mL may be have larger variability as a normal consequence for such a low concentration formulation, but this is unlikely as the processing and detection method should be optimized for a wide range of sample concentrations. A slightly greater concentration than the acceptable range would not be expected to affect the study results as the dose-response effects (either toxicokinetic or biologic) do not have a steep profile.

F1 Generation

Surviving litters assigned to the 50 mg/kg/day dosage group (Group IV) were not weaned, but were terminated on PPD 21 because of excessive mortality that occurred during parturition and up to day 4 postpartum.

Survival

The high dose 50 mg/kg/day group had a higher incidence of pups found dead on day 1 postpartum (14.0%) and days 2 to 4 postpartum (74.2%), and a higher incidence of dams with all pups dying between days 1 and 4 postpartum (66.7% vs. 0% in vehicle controls). This resulted in a significantly lower viability index in this dosage group was significantly lower than the vehicle control group value (22.1% vs. 97.3% in vehicle controls). In addition, 16 of the 25 dams in the 50 mg/kg/day dosage group had no surviving pups.

Also for the 50 mg/kg/day group, the average number of pups surviving per litter on days 4 (pre- and post-culling), 7, 10 14, 17 and 21 postpartum was lower or significantly lower when compared to the vehicle control group values. On days 1 and 4 (pre-culling) postpartum, the average live litter size at weighing was significantly lower in the 50 mg/kg/day dosage group in comparison to the vehicle control group values.

One F1 generation male rat (#4263) in the 10 mg/kg/day maternal dosage group was humanely euthanized on day 24 postpartum because of adverse clinical signs (ataxia, decreased motor activity, pale eyes and extremities, ptosis, a tip-toe walk, labored breathing, dyspnea, gasping, urine-stained abdominal fur and scant feces). This early euthanasia was attributed to a failure to thrive rather than maternal treatment with CP-690550. This weanling rat weighed 24 g on day 22 postpartum, while other weanling rats in this maternal dosage group weighed between 28 g to 60 g on this day and they survived to scheduled sacrifice. All tissues examined appeared normal at necropsy.

One F1 generation female rat in the 10 mg/kg/day maternal dosage group was found dead on day 25 postpartum. There were no adverse clinical observations for this rat. The weanling weighed 37 g on day 22 postpartum while other weanling rats in this maternal dosage group weighed between 40 g to 61 g on this day and they survived to scheduled sacrifice. At necropsy, this rat was observed with intestines distended with gas; all other tissues appeared normal.

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

DOSAGE GROUP DOSAGE (MG/KG/DAY) ^a		I 0 (VEHICLE)	II 1	III 10	IV 50
RATS ASSIGNED TO NATURAL DELIVERY	N	25	25	25	25
PREGNANT	N	25	24	23	23
DELIVERED A LITTER	N(%)	24(100.0) ^b	24(100.0)	23(100.0)	22(100.0) ^b
INCLUDED IN ANALYSES	N	24	24	23	21 ^c
DURATION OF GESTATION ^d	MEAN±S.D.	22.7 ± 0.5	22.5 ± 0.5	22.7 ± 0.4	22.7 ± 0.6
IMPLANTATION SITES PER DELIVERED LITTER	N MEAN±S.D.	354 14.8 ± 1.9	384 16.0 ± 1.5	348 15.1 ± 2.5	148 13.4 ± 2.6 [11] ^e
DAMS WITH STILLBORN PUPS	N(%)	3(12.5)	1(4.2)	4(17.4)	4(19.0)
DAMS WITH NO LIVEBORN PUPS	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.8)
GESTATION INDEX ^f	% N/N	96.0 24/ 25	100.0 24/ 24	100.0 23/ 23	95.2 20/ 21
DAMS WITH ALL PUPS DYING DAYS 1-4 POSTPARTUM	N(%)	0(0.0)	0(0.0)	0(0.0)	14(66.7) ^{**}
DAMS WITH ALL PUPS DYING DAYS 5-21 POSTPARTUM	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

[] = NUMBER OF VALUES AVERAGED

a. Dosage occurred on day 6 of gestation through day 20 of lactation.

b. Excludes values for dams that were found dead or euthanized due to adverse clinical observations during the gestation period.

c. Excludes values for dam 18895; all conceptuses were presumed cannibalized before the litter was processed.

d. Calculated (in days) as the time elapsed between confirmed mating (arbitrarily defined as day 0 of gestation) and the day the first pup was delivered.

e. Excludes values that were not recorded.

f. Number of rats with live offspring/number of pregnant rats.

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

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

DOSAGE GROUP DOSAGE (MG/KG/DAY) ^a		I 0 (VEHICLE)	II 1	III 10	IV 50
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	24	24	23	20
PUPS DELIVERED (TOTAL)	N	341	363	335	245
	MEAN±S.D.	14.2 ± 2.0	15.1 ± 1.6	14.6 ± 2.6	12.2 ± 3.6
LIVEBORN	MEAN±S.D. N(%)	13.7 ± 2.3 329(96.5)	15.1 ± 1.6 362(99.7) ^{**}	14.3 ± 2.5 329(98.2)	11.8 ± 3.9 235(95.9)
STILLBORN	MEAN±S.D. N(%)	0.5 ± 1.6 12(3.5)	0.0 ± 0.2 1(0.3) ^{**}	0.3 ± 0.6 6(1.8)	0.4 ± 1.2 9(3.7)
UNKNOWN VITAL STATUS	N	0	0	0	1
CULLED	N	129	163	136	8
PUPS FOUND DEAD OR PRESUMED CANNIBALIZED					
DAY 1	N/N(%)	4/329(1.2)	0/362(0.0)	2/329(0.6)	33/235(14.0) ^{**}
DAYS 2- 4	N/N(%)	5/325(1.5)	7/362(1.9)	7/327(2.1)	150/202(74.2) ^{**}
DAYS 5- 7	N/N(%)	0/191(0.0)	1/192(0.5)	0/184(0.0)	1/ 44(2.3)
DAYS 8-10	N/N(%)	0/191(0.0)	0/191(0.0)	0/184(0.0)	0/ 43(0.0)
DAYS 11-14	N/N(%)	0/191(0.0)	0/191(0.0)	0/184(0.0)	0/ 43(0.0)
DAYS 15-17	N/N(%)	0/191(0.0)	0/191(0.0)	0/184(0.0)	0/ 43(0.0)
DAYS 18-21	N/N(%)	0/191(0.0)	0/191(0.0)	0/184(0.0)	0/ 43(0.0)
VIABILITY INDEX ^b	% N/N	97.3 320/329	98.1 355/362	97.3 320/329	22.1 ^{**} 52/235
LACTATION INDEX ^c	% N/N	100.0 191/191	99.5 191/192	100.0 184/184	97.7 43/ 44

DAY(S) = DAY(S) POSTPARTUM

a. Dosage occurred on day 6 of gestation through day 20 of lactation.

b. Number of live pups on day 4 (preculling) postpartum/number of liveborn pups on day 1 postpartum.

c. Number of live pups on day 21 (weaning) postpartum/number of live pups on day 4 (postculling) postpartum.

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

Clinical Signs

There were no clinical observations of the F1 generation due to CP-690550 treatment.

Clinical observations included chromodacryorrhea, a scab on the head or neck, urine-stained abdominal fur, scant feces, localized alopecia on the limbs, sparse hair coat (limbs, neck, head and/or underside), pale extremities, labored breathing, dyspnea, gasping, tip-toe walk, pale eyes, ptosis, ataxia, decreased motor activity, swollen forepaw or snout, chromorhinorrhea, missing/broken/misaligned incisors, bent tail, an abrasion on the mouth or palate, red substance in the cage pan, elongated snout, red perioral substance, lacrimation, swollen periorbital membrane in the left eye, discolored (red) periorbital membrane in the left eye, scratched cornea in the left eye, left eye enlarged, left eye third eyelid discolored (red), mild or moderate dehydration (based on skin turgor), an ulceration on the mouth or head and an umbilical hernia.

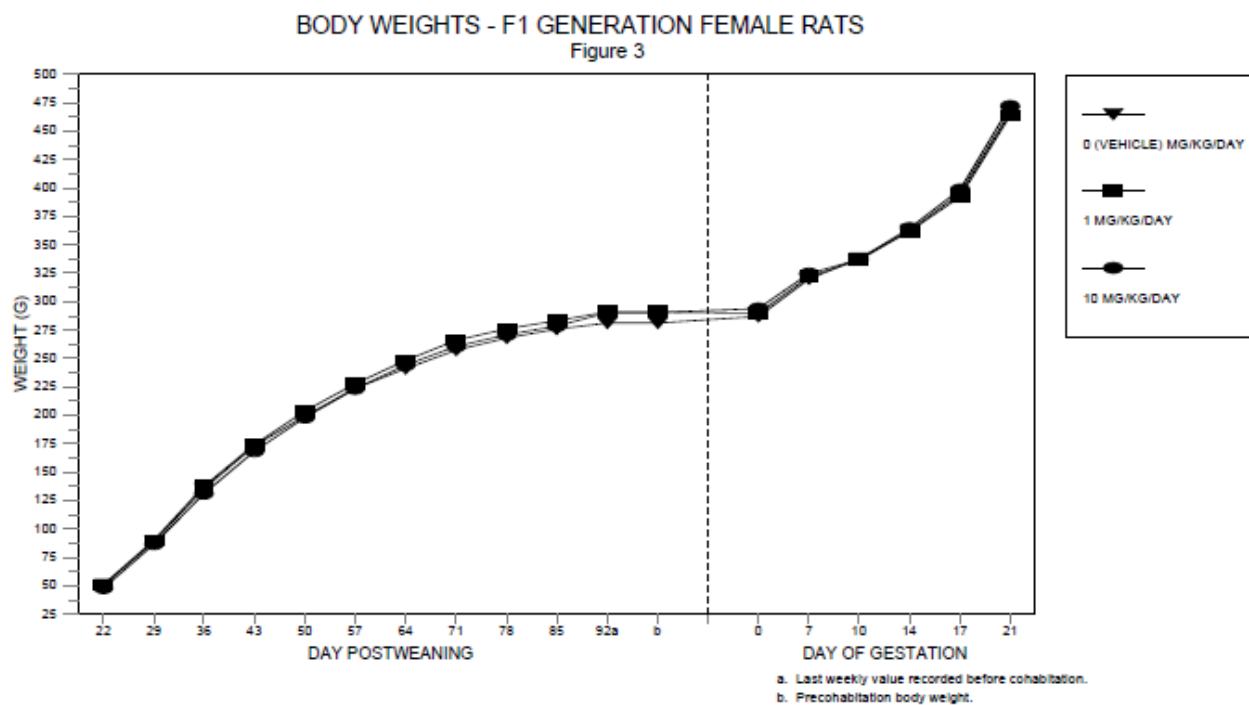
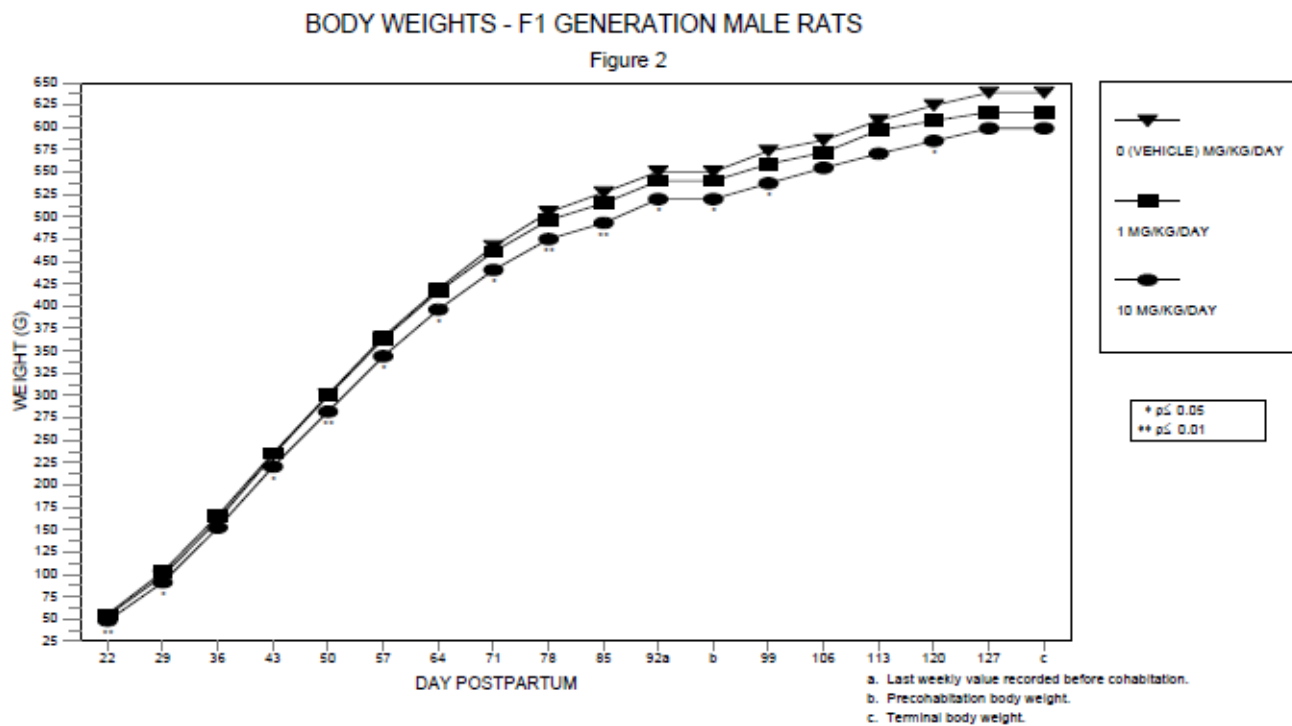
For the low dose (1 mg/kg/day) and mid dose (10 mg/kg/day) treated dams, there was no effects on delivery or litter observations.

Body Weight

The average pup weight per litter between days 1 and 21 postpartum in the 50 mg/kg/day dose group was significantly lower than the corresponding vehicle control group values. The average pup weight on day 4 (pre- and post-culling) was also significantly lower in the 10 mg/kg/day group compared to controls.

Reductions in body weight that occurred in F1 male rats in the 10 mg/kg/day maternal dosage group reflected reductions in body weights that occurred prior to weaning. At 10 mg/kg/day, body weight gains were significantly reduced on PPDs 43 to 50, in comparison to the vehicle control group value.

For F1 female rats, no significant differences occurred among the groups during the postweaning, precohobitation or gestation periods.



Feed consumption

No milk was observed in the stomach of 4, 0, 2 and 53 pups that were found dead in the 0 (Vehicle), 1, 10 and 50 mg/kg/day dosage groups, respectively.

There was no effect of CP-690550 on food consumption in dose group up to 10 mg/kg/day during the postweaning, precohabitation and/or gestation periods

Physical development

Sexual Maturation: There were no effects of up to 10 mg/kg/day CP-690550 maternal treatment on the sexual maturation of F1 rats. The average day on which preputial separation or vaginal patency occurred was similar among the three remaining groups.

Neurological assessment

Motor Activity: There were no effects of CP-690550 maternal treatment on test of F1 on motor activity, as measured by numbers of movements or time spent in motion on PPDs 22 and 54 to 57.

Passive Avoidance: There were no effects of CP-690550 maternal treatment on tests of F1 rats for learning, short-term retention, long-term retention or response inhibition as evaluated by performance in a passive avoidance paradigm (the number of trials to criterion, trial latencies or numbers of rats that failed to learn)

Watermaze: There were no effects of CP-690550 maternal treatment on watermaze performance that involve learning, short-term retention, long-term retention, or response inhibition.

Acoustic Startle Habituation: There were no effects of CP-690550 maternal treatment in the reactivity to auditory stimuli and habituation of responses evaluated by during acoustic startle habituation on PPDs 78 to 81.

Reproduction

Estrous Cyclicity and Fertility: There were no effects of CP-690550 maternal treatment on estrous cycling, mating, and fertility parameters (number of days in cohabitation, the number of rats that mated, confirmed mating, the fertility index, and the number of pregnancies per number of rats in cohabitation).

Cesarean-Sectioning Observations: There were no effects of CP-690550 maternal treatment on Cesarean-section observations conducted on GD 21. The number of pregnant F1 dams with one or more live fetuses in the 0 (Vehicle), 1 and 10 mg/kg/day maternal dosage groups were 23 (92.0%), 21 (84.0%) and 20 (83.3%), respectively. The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, the percentage of resorbed conceptuses, and the percentage of live male fetuses were not different among these three groups. There were no dead fetuses. One dam in the 10 mg/kg/day maternal dosage group had a litter consisting of only resorbed conceptuses (16 total). All placentae appeared normal, with the exception of dam 4354 in the 10 mg/kg/day maternal dosage group that had fused placentae that corresponded to implantation sites 1 and 2.

Necropsy Observations

All necropsy observations in the F1 generation male and female rats were considered unrelated to maternal administration of CP-690550-10 because: 1) the incidences were not dosage-dependent; and 2) the observation occurred in only one or two rats in any dosage group.

In F1 male rats, these gross lesions included the presence of an abdominal mass that was irregularly shaped, firm, lobular and mottled (dark red and red) in appearance. The cut surface of this mass revealed the presence of a firm, tan, red and/or dark red lobular material. This mass also adhered to the adipose tissue. Other gross lesions included large Peyer's patches in the ileum, discolored (red) jejunum, the presence of a firm, tan and red mass on the prostate (the cut surface revealed a firm, tan and red material), a flaccid left testis and a small left epididymis. Gross lesions observed in F1 female rats included slight dilation of the pelvis in the right kidney and gaseous distention of the intestines.

No gross lesions occurred in the F1 pups that survived only to scheduled euthanasia and necropsy on day 21 postpartum, except for a diaphragmatic hernia in 1 rat in the 1 mg/kg/day group.

TABLE A14 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - F1 GENERATION PUPS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY) ^a		0 (VEHICLE)	1	10	50
LITTERS EVALUATED	N	24	24	23	20
TOTAL PUPS STILLBORN					
OR FOUND DEAD ^{b,c}	N	6	6	8	70
STILLBORN	N	1	1	4	7
FOUND DEAD	N	5	5	4	63
NO MILK IN STOMACH ^d	N(%)	4 (80.0)	0 (0.0)**	2 (50.0)**	53 (84.1)
DIAPHRAGMATIC HERNIA	N(%)	0 (0.0)	1 (16.7)**	0 (0.0)	0 (0.0)
PUPS SACRIFICED AND NECROPSIED ON DAY 21 POSTPARTUM ^b					
LITTERS EVALUATED	N	24	24	23	6
PUPS EVALUATED	N	141	141	134	43
APPEARED NORMAL					
LITTER INCIDENCE	N(%)	24 (100.0)	24 (100.0)	23 (100.0)	6 (100.0)
PUP INCIDENCE	N(%)	141 (100.0)	141 (100.0)	134 (100.0)	43 (100.0)

a. Dosage occurred on day 6 of gestation through day 20 of lactation.

b. Restricted to pups in which complete necropsies were performed. Complete necropsies were not performed on pups in which autolysis or cannibalization precluded full evaluation.

c. Refer to the individual pup clinical observations table (Table A25) for external clinical observations confirmed at necropsy.

d. Analysis restricted to pups found dead and necropsied.

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

10 Special Toxicology Studies

Study title: 1-Day In Vitro Blood Compatibility Study of CP-690550 with Human Blood

Study no.:	09GR482
Study report location:	Mod. 4.2.3.7.7
Conducting laboratory and location:	Pfizer Global Research & Development Drug Safety Research & Development Eastern Point Road Groton, CT USA
Date of study initiation:	Dec 21, 2009
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	CP-690550-10, Lot E010009450, Purity 100.2% There was no Certificate of Analysis included with this report. Purity was indicated in Table 2.6.7.4 in Module 2.

Key Study Findings

CP-690550-10 mixed with human whole blood did not result in hemolysis, and mixed with human plasma did not result in precipitation.

Methods

CP-690550-10 and vehicle (10 mM lactic acid in normal saline), were serially diluted from 1 and 5 mg/mL by the addition of heparinized whole blood or plasma (description only states collection was from a human volunteer, hemoglobin 16.9 g/dL, hematocrit 49.6%) as indicated in the table below.

Table 124: Study Design for Blood Compatibility with CP-690550-10

Dilution	Undiluted (mg/mL)	1:2 (mg/mL)	1:4 (mg/mL)	1:10 (mg/mL)	1:20 (mg/mL)	1:40 (mg/mL)	1:100 (mg/mL)
With vehicle (mg/mL)	1.0	0.5	0.25	0.1	0.05	0.025	0.01
With blood (mg/mL)	0.5	0.25	0.125	0.05	0.025	0.013	0.005

The samples were either centrifuged immediately or incubated for 30 minutes then centrifuged. At predetermined time intervals, the tubes were observed and recorded for hemolysis, and precipitation. The extent of hemolysis from each concentration of whole blood and vehicle was determined visually using the positive control (deionized water) and negative control (0.9% sodium chloride) as the baseline. The extent of precipitation from each concentration of plasma and vehicle was determined visually using the negative control (both 10 mM lactic acid in normal saline, and normal saline alone)

Results

There was no hemolysis of whole blood and no precipitation of plasma at various concentrations of vehicle or CP-690550-10.

Study title: Primary Skin Irritation Study in Rabbits (4 Hour Semi-Occclusive Application)

Study no.:	B65935
Study report location:	Mod 4.2.3.6
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Dec 12, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CP-690550-10, Lot E01005898, Purity 99.8% There was no Certificate of Analysis included with this report. Purity was indicated in Table 2.6.7.4 in Module 2.

Key Study Findings

CP-690550-10 is not irritating to rabbit skin

Methods

CP-690550-10 (0.5 g on 2.5 cm x 2.5 cm of surgical gauze) that was moistened with 0.5 mL of purified water was applied by topical semi-occlusive application for a 4 hr duration to the clipped intact left flank of each of three young adult New Zealand White rabbits (1 male, 2 females, 11-12 weeks of age). A separate 1% w/w solution had a pH of 4. The animals were monitored for mortality, body weight, clinical signs and skin reactions. Following removal of the dressing, the skin reaction was evaluated at 1, 24, 48 and 72 hours. The skin reaction was assessed according to the numerical scoring system listed in the Commission Directive 2004/73/EC, April 29, 2004 which was based on the Draize score system. The mean skin reaction (irritation) score was calculated across 3 scoring times (24, 48 and 72 hours after patch removal) for each animal, for parameters of erythema/eschar and edema. A primary irritation index was calculated by totaling the mean cumulative scores at 24, 48 and 72 hr and dividing by the number of observations.

Results

There were no premature deaths, and no changes in body weights related to drug. There were no clinical signs, no staining of the treated skin, and no corrosive effects on the skin. There was no skin reaction at the application site of any animal at any of the observation times (all scores 0). The primary irritation index was 0. Animals were not terminated at the end of the study, but reused in the study examining potential eye irritation.

Based upon the referred classification criteria (Commission Directive 2001/59/BC of August 2001), CP-690550-10 (final) is "not irritating" to rabbit skin, and according to Draize classification criteria CP-690550-10 is "not Irritant" to rabbit skin (P.I.I. = 0). The conclusion is appropriate.

Study title: Primary Eye Irritation Study in Rabbits

Study no.:	B65946
Study report location:	Mod 4.2.3.6
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Dec 12, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CP-690550-10, Lot E01005898, Purity 99.8% There was no Certificate of Analysis included with this report. Purity was indicated in Table 2.6.7.4 in Module 2.

Key Study Findings

- The installation of CP-690550-10 into the rabbit eye resulted in mild to marked, early-onset and transient ocular changes, such as corneal opacity, reddening of the conjunctivae and sclerae, discharge and chemosis. These effects were reversible and were no longer evident 7 to 10 days after treatment.
- According to accepted European standards of evaluation, these effects were insufficient to denote CP-690550-10 as an "eye irritant", however based on the acute reaction with in the first few days, CP-690550-10 is acutely irritating when applied to the rabbit eye.

Methods

CP690550-10 was applied by instillation of 0.1 g into the left eye of each of three young adult New Zealand White rabbits (1 male, 2 females, 13-14 weeks of age at the initiation of this study). The untreated right eye was used as the control. The animals were observed for mortality, body weight, clinical signs and eye reactions. Scoring of irritation effects was performed approximately 1, 24, 48 and 72 hours, and 7 and 10 days after instillation. Eye reactions were assessed according to the numerical scoring system listed in the Commission Directive 2004/173/EC, April 29, 2004. Scleral reddening and ocular discharge were also assessed. A mean score was calculated across 3 scoring times (24, 48, and 72 hours after instillation) for each animal, for parameters of corneal opacity, iris, redness and chemosis of the conjunctivae. No pathology was conducted at the termination of the study.

Results

There were no premature deaths, clinical signs or effects on body weight during the observation period. There was no staining or signs of corrosive effects of the eye. White instillation material was present in the eye or conjunctiva sac at 1 hour after instillation. It is not clear from the results if the statement “A very slight opacity affecting the whole area was observed in all treated animals 1 hour after instillation” refers to the presence of this white substance (probably CP-690550-10) or actual corneal opacities, which was the intent of this category. The text indicated the scores were 0 for each animal for corneal opacity and iris although the table indicated the scores were 1 for the 1 hour observation time point. The Reviewer assumes that the opacity refers to the applied drug, since corneal opacities would persist and they were not present in the later evaluations.

A marked reddening of the conjunctivae was visible in all animals at 1-hour after installation and persisted as slight to moderate up to the 24- or 72-hour reading, respectively. Chemosis of the conjunctivae sufficient to produce half-closed eyes occurred in the three animals 1 hour after treatment and persisted as slight swelling up to the 24-hour reading. Due to the marked swelling of the conjunctivae, the assessment of the sclerae was not possible at the 1-hour reading. At the 24 hour and 48 hour readings, there were moderate to marked reddening of the sclerae, which slowly attenuated as slight to moderate from 72 hours to 7 days when it had resolved. Moderate ocular discharge was present in all animals at the 1-hour observation and persisted as slight up to 24 hours in 2 animals. No abnormal findings were observed in the treated eye of any animal 7 or 10 days after treatment.

The individual mean scores for conjunctivae were 0.33, 1.67, and 1.67 for reddening, and 0.33 for chemosis for each animal.

Table 125: Eye Irritation Scores

Table 1 Eye Irritation Scores – Individual Values

Animal Number	Sex	Evaluation Interval*	Corneal Opacity	Area of Corneal Opacity	Iris	Conjunctivae		Sclera
						Redness	Chemosis	
51	M	1 hour	1	4	0	3	3	n.a.
52	F		1	4	0	3	3	n.a.
53	F		1	4	0	3	3	n.a.
51	M	24 hours	0	0	0	1	1	3
52	F		0	0	0	2	1	2
53	F		0	0	0	2	1	2
51	M	48 hours	0	0	0	0	0	3
52	F		0	0	0	2	0	2
53	F		0	0	0	2	0	2
51	M	72 hours	0	0	0	0	0	2
52	F		0	0	0	1	0	1
53	F		0	0	0	1	0	1
51	M	7 days	0	0	0	0	0	1
52	F		0	0	0	0	0	0
53	F		0	0	0	0	0	0
51	M	10 days	0	0	0	0	0	0
52	F		0	0	0	0	0	0
53	F		0	0	0	0	0	0

* Examinations were performed at the specified times after instillation of the test item.

n.a. = not assessable due to swelling of the conjunctivae

Based on the classification criteria (Commission Directive 2001/59/EC of August 06, 2001), CP-690550-10 is "not irritating" to the rabbit eye. This assessment is based on multiple days of observation that minimize acute eye responses and focus on persistent responses. This does not accurately reflect human response from initial eye contact. Missing in this assessment is an acute evaluation, that suggests based on the finds of ocular swelling, discharge, chemosis, conjunctiva and sclera reddening, semi-solid or suspension of CP-690550 is acutely irritating to the rabbit eye.

Study title: Murine Local Lymph Node Assay with CP-690550

Study no.:	07GR202
Study report location:	Mod 4.2.3.6
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Aug 27, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CP-690550, Lot 121063-100-1 (free base lot), Purity 96.6% There was no Certificate of Analysis included with this report. Purity was indicated in Table 2.6.7.4 in Module 2.

Key Study Findings

- CP-690550 is not a skin sensitizer in the local lymph node assay.
- CP-690550 treatment did not induce proliferation of lymphocytes from the auricular lymph nodes of topically treated CBA/J mice compared to appropriate controls.

Methods	
Doses:	0,10, 20 and 33% (w/v) The maximum feasible concentration of CP-690550 in DMSO was 33%.
Frequency of dosing:	Once daily from day 1 through day 4
Route of administration:	Dermally, by pipette to the dorsal aspect of the ear.
Dose volume:	25 µL
Formulation/Vehicle	dimethyl sulfoxide (DMSO)
Negative Control:	acetone, olive oil (4:1, v/v)
Positive Control:	Hexylcinnamaldehyde
Species/Strain:	Mice, CBA/J females
Number/Sex/Group:	5 females/dose

Age:	8 to 9 week of age
Weight:	18.1 to 23.9 g
Satellite groups:	None
Unique study design:	The in-life portions of the study were divided into a toxicity and irritation screening phase, a local lymph node assay (LLNA) Phase 1, and a repeat portion of the LLNA phase (LLNA Phase 2). Each aspect is described separately below.
Deviation from study protocol:	There were no protocol deviations that affected the study results or conclusions.

Toxicity and Irritation Screening

Methods

Animals were topically dosed on both ears once daily for 4 days and observed for mortality, abnormalities, and signs of pain or distress. The ears of each animal were observed predose, approximately 4 hours postdose, and approximately 22 to 26 hours postdose. Any reactions noted were scored for redness and swelling according to a 4-point scale. The animals were observed twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress. Body weights were recorded on day 1 of the toxicity and irritation screening phase.

Table 126: Lymph Node Assay Study Treatments

Toxicity and Irritation Screening Phase			
Group	No. of Females	Treatment ^a	Dose Level (% w/v)
1 (Control)	2	DMSO	0
2	2	CP-690,550 in DMSO	10
3	2	CP-690,550 in DMSO	20
4	2	CP-690,550 in DMSO	33

^a The dose volume was 25 microliters/ear.

Results

There were no deaths, no changes in body weights, and no clinical observations of toxicity, but redness and swelling occurred in all animals following daily CP-690550 dermal application. Residual CP-690550 was observed on the ears of all animals for all doses. Dose levels of 10, 20, and 33% (w/v) were selected for the LLNA phase based on the absence of dermal irritation and amounts of residual test material observed on the ears of all animals. It is difficult to understand the rationale for future study dose selection when the only doses for comparison are those chosen for future study.

Local Lymph Node Assay 1

Methods

Both ears of each animal were topically dosed once daily for 3 consecutive days. Following the final application, the animals were rested for 2 days without treatment. The animals were observed twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress. The ears of each animal were observed predose and approximately 2 to 4 hours postdose on each dosing day and once on days 4, 5, and 6 for any significant alterations in the appearance of the application sites. Body weights were recorded on days prior to dosing, on day 1, and prior to the ^3H -thymidine injection on day 6. On day 6, animals were given a single tail vein injection of ^3H -thymidine (20 $\mu\text{Ci}/\text{animal}$). Approximately 5 hours following the ^3H -thymidine injection, animals were sacrificed and the auricular lymph nodes were removed intact and pooled for each individual animal. There was no terminal necropsy of the animals.

Cell suspensions were prepared from the auricular lymph node pool for each individual animal, the radiolabeled macromolecules were precipitated with trichloroacetic acid, and the samples were analyzed by liquid scintillation spectrometry.

A stimulation index (SI) was calculated for each group and statistical comparisons were performed on the disintegrations per minute (dpm).

Table 127: Study Design for Local Lymph Node Assay

Local Lymph Node Assay Phase

Group	No. of Females	Treatment ^a	Dose Level (% w/v)
1 (Naïve Control) ^b	5	Naïve	0
2 (Negative Control)	5	DMSO	0
3 (Negative Control)	5	Acetone/olive oil (4:1)	0
4	5	CP-690,550 in DMSO	10
5	5	CP-690,550 in DMSO	20
6	5	CP-690,550 in DMSO	33
7 (Positive Control)	5	Hexylcinnamaldehyde in acetone/olive oil (4:1)	25% (v/v)
Repeat Dose			
1R (Naïve Control) ^b	5	Naïve	0
2R (Negative Control)	5	DMSO	0
3R (Negative Control)	5	Acetone/olive oil (4:1)	0
6R	5	CP-690,550 in DMSO	33
7R (Positive Control)	5	Hexylcinnamaldehyde in acetone/olive oil (4:1)	25% (v/v)

Note: Since the initial dose to Group 7, positive control, did not produce acceptable results (mean Stimulation Index was < 3.0), all controls and the high dose group in the LLNA phase were repeated as Groups 1R, 2R, 3R, 6R, and 7R.

a The dose volume was 25 microliters/ear.

b Animals were not treated.

Results

There were no deaths, no changes in body weights and no clinical observations of toxicity. In all CP-690550 animals there was erythema, but no edema noted, and residual CP-690550 was observed on the ears of all animals. The mean SIs were 0.3,

0.4, and 0.3 for dose groups of 10, 20, and 33% (w/v), respectively, and the mean dpm values were statistically ($p < 0.01$) different from controls. However, the mean SI for the positive control group was 2.0, which did not meet the assay acceptance criteria; therefore the assay was repeated with the high dose group and control groups.

The acceptance criteria as outlined in NIH Publication No. 99-4494, 1999 are:

- SI of 3.0 or greater at any dose level
- Statistically significant differences from control values
- Evidence of a dose response

Local Lymph Node Assay 2

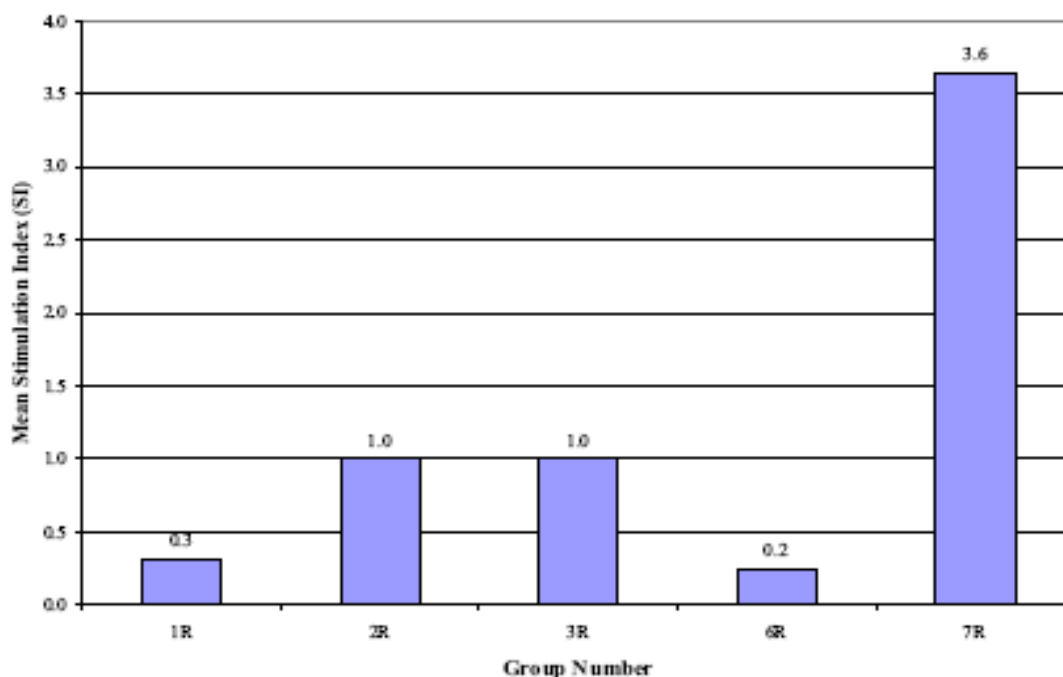
Methods

The procedures were as described for LLNA 1. The dose groups were only the high dose 33% (w/v) CP-690550, the negative control and the positive control groups.

Results

There were no deaths, no changes in body weights and no remarkable clinical observations. There were occurrences of erythema but no edema for the control and CP-690550-treated animals. In the positive control treatment group, there was slight to moderate erythema, but no edema. Residual CP-690550 was observed on the ears of animals in the 33% (w/v) CP-690550 dose group.

The positive control group had an SI of 3.6, thus the assay was acceptable. An SI of 2 was obtained for the 33% (w/v) CP-690550 dose group, and there was a statistically significant ($p < 0.01$) difference in mean dpm values for this dose compared with the vehicle control group which had an SI = 1.0. However, the absence of an SI greater than 3.0 at the 33% dose level indicated that CP-690550 was not a contact sensitizer. Although this second assay was conducted without sufficient dose groups to determine a dose-response relationship, the conclusion that CP-690550 does not induce skin sensitization is justified since the highest dose, although below the SI criteria, was limited due to solubility, and the negative and positive controls indicated assay validity.

Figure 17: Results of Lymph Node Assay 2 (from applicant's figure 2)

AOO
DMSO

Acetone/olive oil (4:1 v/v).
Dimethyl sulfoxide.

Group	Dose Group/ % (w/v)
1R	Naïve control/ 0%
2R	DMSO vehicle control/ 0%
3R	AOO vehicle control/ 0%
6R	CP-690,550/ 33%
7R	Positive control/ 25% (v/v)

SI Stimulation index. Calculation:

(dpm for individual animal)/(Mean dpm of solvent or vehicle control group).

Stability and Homogeneity

Stability: CP-690550 in DMSO at the concentrations used in the assays was stable out to 14 days, with values ranging from 93.8 to 102.4 of expected concentrations.

Homogeneity: Homogeneity was not determined.

Dose Formulation Analysis: Single aliquots were taken from the middle of the formulations prepared for the toxicity and irritation screen which were also used in the first LLNA and single aliquots were taken from the middle of the day 1 dose formulations for Groups 2R, 3R, and 6R. The mean concentrations (samples collected from middle of the dose formulation for the 10-, 20-, and 33%-dose levels) of CP-690550 were within <6% of the theoretical target concentrations which meet the criteria for acceptance.

Study title: Determination of the Phototoxic Potential of CP-690550-10 in the 3T3 Neutral Red Uptake Phototoxicity Assay

Study no.:	07AM087
Study report location:	Mod. 4.2.3.7.7
Conducting laboratory and location:	Pfizer Global Research & Development Amboise, Safety Sciences Europe Route des Industries, Pocé-sur-Cisse 37400 Amboise, France
Date of study initiation:	June 18, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CP-690550-10, Batch number: 043798-002-1H, (Lot E010009921), Purity 100.2%; There was no Certificate of Analysis included with this report. Purity was indicated in Table 2.6.7.4 in Module 2.

Key Study Findings

- CP-690550-10 was tested for photoreactivity using the 3T3 NRU assay and found to have no phototoxic potential in vitro.

Methods	
Doses:	In vitro: 0.061, 0.244, 0.977, 3.906, 15.625, 62.5, 250 and 1000 µg/mL CP-690550-10 was tested at concentration up to 1000 µg/mL and found not to affect cellular viability in the absence of UVA light, therefore 1000 µg/mL was the highest tested concentration.
Frequency of dosing:	Once, incubation 1 hour
Route of administration:	In vitro 96-well microtiter plates
Dose volume:	100 µL
Formulation/Vehicle	1% DMSO in EBSS (Earles Buffered Saline Solution)
Negative Control:	Sodium Lauryl Sulfate (SLS)
Positive Control:	Chlorpromazine hydrochloride (CPZ)
Species/Strain:	Balb/c 3T3 mouse fibroblasts (clone 31)
Unique study design:	The method used in this study followed the draft protocol provided

by the OECD 432 guidelines.

Balb/c 3T3 mouse fibroblasts (clone 31) were purchased from LGC France. Cells were maintained in standard tissue culture flasks and sub cultured into 96-well plates incubated overnight at 37°C in a humidified atmosphere of 95% air and 5% CO₂. The next day, cells were incubated with eight concentrations of CP-690550 and each concentration was tested 6 times. Cells were incubated for about 1 hour at 37°C in a humidified atmosphere of 95% air and 5% CO₂, before irradiation of the cells (5 joules/cm²).

Concurrently, a second plate was prepared under similar conditions and kept in the dark at ambient temperature. The buffer was removed from the 2 plates immediately after irradiation. The cells were further incubated with culture medium free of drug overnight at 37°C in a humidified atmosphere of 95% air and 5% CO₂, and Neutral Red Uptake (NRU) was determined.

<u>Items</u>	<u>Stock solution</u>		<u>Working solutions</u>	
	<u>Concentration</u> <u>(mg/mL)</u>	<u>Solvent</u>	<u>Concentrations tested</u> <u>(µg/mL)</u>	<u>Vehicle</u>
CPZ	10	Water	250 – 62.5 – 15.625 – 3.906 – 0.977 – 0.244 – 0.061 – 0.015	EBSS
SLS	0.7	EBSS	70 – 35 – 17.5 – 8.75 – 4.375 – 2.188 – 1.094 – 0.547	EBSS
CP-690,550-10	100	100 % DMSO	1000 – 250 – 62.5 – 15.625 – 3.906 – 0.977 – 0.244 – 0.061	1% DMSO in EBSS

UV radiation at 365 nm was provided by a solar simulator. Filters restricted irradiation to the UVA region (315-400 nm). The chamber of the simulator was first sterilized for 10 min at 254 nm. A dose of 5 joules/cm² (UVA) is known to be non cytotoxic to Balb/c 3T3 clone 31 mouse fibroblasts and sufficiently potent to excite weakly phototoxic compounds (Spielmann et al, 1998, ATLA **26**: 679-708). To achieve 5 joules/cm², irradiation was adjusted about 4mW/cm², at 365 nm, for approximately 21 minutes (22.0 < temperature < 23.1°C). Measurements were made with the polystyrene lids on the well plates.

The cells were incubated with a solution containing 50 µg/mL of neutral red (3-amino-7-dimethylamino-2-methylphenazine hydrochloride) for about 3 hours at 37°C in a humidified atmosphere of 95% air and 5% CO₂. After incorporation of neutral red into the lysosomes, the solution was discarded and the cells were incubated with ethanol/acetic acid/water. Spectrophotometric reading of the optical density (OD) was determined for the ethanolic extract at 540 nm.

Analysis:

Mean OD values +/- standard deviations (SD) were calculated using 6

<p>measurements per concentration and the percentage of cell viability was expressed relative to untreated controls in the absence (-Irr) or presence (+Irr) of UVA irradiation as: $100 \times \text{mean OD treated} / \text{mean OD control}$. Negative values were reported as 'Zero' in the Group mean data tables.</p> <p>Mean cell viability values greater than 100% are considered to be variations in the test system with no impact on the outcome of the study. Decrease of cell viability is considered significant if it is less than 80%.</p> <p>The IC_{50} (-Irr), IC_{50} (+Irr), PIF and MPE values were calculated. The photo irritation factor (PIF) is based on a comparison of two equally effective cytotoxic concentrations (IC_{50} values, concentrations which induced 50% cell death) obtained in the presence (+Irr) and absence (-Irr) of UVA irradiation. Therefore, the PIF is the ratio: $IC_{50} (-Irr) / IC_{50} (+Irr)$. If an IC_{50} in the presence or absence of light cannot be calculated, no PIF value can be determined. The mean photo effect (MPE) is based on a comparison of the (+Irr) and (-Irr) concentration-response curves on a grid of concentrations chosen from a common concentration range of the dark and light experiments.</p>	
Deviation from study protocol:	There were no deviations that affected the results and conclusions. The report indicated that this is a repeat of the initial study since in that study cell viability was insufficient.

Results

Study validity

The study was valid and the study is acceptable. The applicant provided the following criteria for study validity based on OECD guidelines (2002) and the EU/COLIPA study (Spielmann et al, 1998 Toxicology *In Vitro* **12**: 305-327, 1998), which has also been accepted informally by the FDA.

- A compound is considered to be phototoxic if the PIF value is greater than 5 and/or the MPE value is greater than 0.15.
- A compound is considered to be probable phototoxic if the PIF value is comprised between 2 and 5 and/or the MPE value is comprised between 0.1 and 0.15.
- A compound is considered to be not phototoxic if the PIF value is lower than 2 and/or the MPE value is lower than 0.1.
- Cut-off values of 2 for PIF and 0.1 for MPE.

Phototoxic potential of chemical

<u>Values</u>	<u>No phototoxicity</u>	<u>Probable phototoxicity</u>	<u>Phototoxicity</u>
PIF	< 2	$2 < \text{PIF} < 5$	> 5
MPE	< 0.1	$0.1 < \text{MPE} < 0.15$	> 0.15

- UVA sensitivity of the cells
 - Cellular viability was determined after irradiation from 2.5 to 15 joules/cm². Untreated cells irradiated at 5 joules/cm² showed a viability of more than 80% when compared with non-irradiated cells.
- Vehicle controls
 - If the irradiated vehicle controls show a viability of more than 80% when compared with non-irradiated negative/EBSS control cells, then the test meets the quality criteria.
 - Sodium Lauryl Sulfate (SLS) tested in a full-scale phototoxicity test on 2 plates concurrently with the test chemical serves as negative reference.
 - A test meets acceptance criteria, if the PIF value is less than 2.
- Positive Control
 - Chlorpromazine (CPZ) was tested in a full-scale phototoxicity test on 2 plates concurrently with the test chemical serves as positive reference. A test meets acceptance criteria, if for CPZ:
 - the IC₅₀ (+ Irr) is within the range of 0.1 - 2.0 µg/mL,
 - the IC₅₀ (- Irr) is within the range of 7.0 - 90.0 µg/mL, the PIF is at least 6

Negative and Positive controls

<u>Products</u>	<u>IC₅₀ (-Irr)</u> <u>µg/mL</u>	<u>IC₅₀ (+Irr)</u> <u>µg/mL</u>	<u>PIF</u>	<u>MPE</u>
Chlorpromazine	12.4	0.8	15.51	0.362
Sodium Lauryl Sulfate	13.5	13.1	1.03	0.000

Assay Results

The 5 joules/cm² UVA dose had no effect on cell viability (96% viability relative to non-irradiated cells) for untreated cells in EBSS. The IC₅₀ (-Irr), IC₅₀ (+Irr) and PIF values with CPZ and SLS also met acceptance criteria. These results indicate that the test conditions were acceptable to determine the *in vitro* phototoxic potential of CP-690550.

The vehicle (1% DMSO in EBSS) used to dissolve CP-690550 had no effect on cell viability either in the presence or absence of UV light, with 95 and 100% of cell viability

relative to untreated cells, respectively. CP-690550 reduced cellular viability (below the threshold of 80%), in the presence or absence of UV light, from concentrations of >1000 µg/mL, respectively. The PIF and MPE values were below the cut-off values of 2 and 0.1, respectively (Table below).

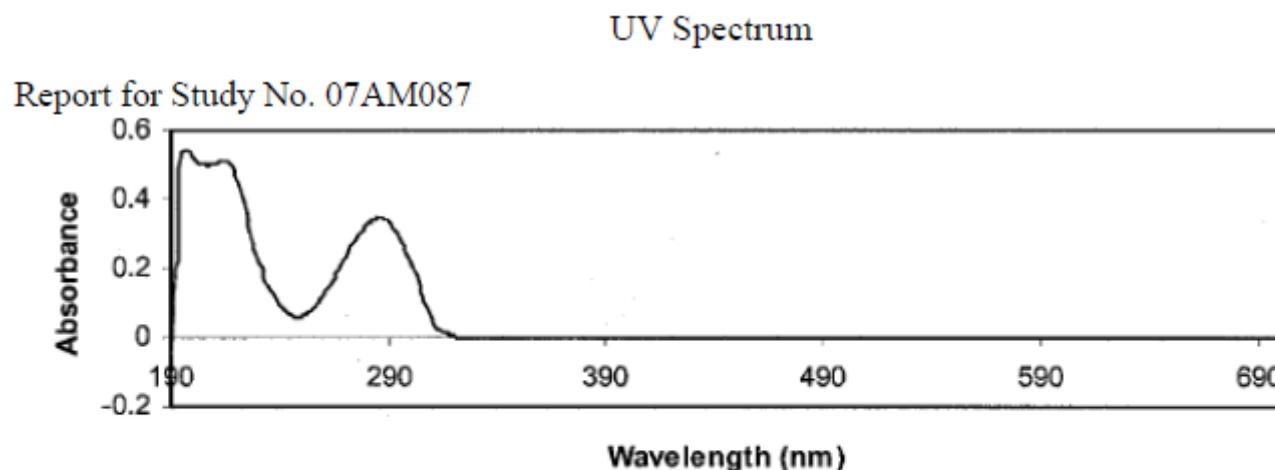
IC50, PIF and MPE values for CP-690,550-10

<u>Test chemical</u>	<u>IC50 (-Irr)</u> <u>µg/mL</u>	<u>IC50 (+Irr)</u> <u>µg/mL</u>	<u>PIF</u>	<u>MPE</u>
CP-690,550-10	>1000 ^a	>1000 ^a	1.00 ^b	0.025

a: Since the highest tested concentration did not reduced cellular viability by 50%, the IC50 was not calculated.

b: The PIF value was calculated based on the extrapolated IC50 values.

Figure 18: Absorption Spectrum for CP-690550



Solution	MEC (M⁻¹cm⁻¹) 290 nm	MEC (M⁻¹cm⁻¹) 286/287
pH 7.5 PBS	16005	16472 (286 nm)
pH 5.5 PBS	15892	16254 (287 nm)

Study title: Phototoxicity Study to Determine the Effects of Seven Days of Oral (Gavage) Administration of CP-690550 on Eyes and Skin in Pigmented Rats

Study no.:	10GR350
Study report location:	Mod. 4.2.3.7.7
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Jan 11, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CP-690550-10, Lot E010009921, Purity 100.2% Composition: 62.0% active moiety There was no Certificate of Analysis included with this report. Purity was indicated in Table 2.6.7.4 in Module 2.

Key Study Findings

- The study was not an adequate assessment of phototoxicity due to the insufficient intensity of radiation at the wavelengths of concern, those around the lower limit of UVB spectrum, 290 nm, based the absorption spectrum of CP-690550.
- CP-690550 did not produce evidence of induced cutaneous or ocular phototoxicity after administration of 10, 30 and 100 mg/kg/day once daily for 7 days to Long-Evans pigmented rats followed by a single exposure to simulate sunlight for 30 min.

Methods	
Doses:	0, 10, 30 and 100 mg/kg/day Doses in mg/kg are based on mg of the active moiety of the drug substance.
Frequency of dosing:	Once daily for 7 days
Route of administration:	Oral, by gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% methylcellulose
Negative Control	0.5% methylcellulose
Positive Control	8-Methoxypsoralen (8-MOP) in corn oil, 50 mg/kg of a 5 mg/mL solution
Species/Strain:	Rat, Crl:LE, females
Number/Sex/Group:	5 females/dose group

Age:	~9 weeks of age at arrival
Weight:	200-259 g at study assignment
Satellite groups:	Toxicokinetic: 6 females/dose group
Unique study design:	Refer to the design table below and the following description:
<p>After administration of the final dose on day 7, rats were exposed to UVR or sham exposed to UVR. Rats assigned to Group 6 were administered a single oral dose of the positive control, 8-MOP.</p> <p>Before UVR exposure, hair of rats assigned to the phototoxicity phase was removed from backs with electrical clippers. In addition, the backs of these rats were clipped as necessary during the course of the study to evaluate the skin sites. After formulation administration but before UVR exposure, all rats were lightly anesthetized using a mixture of ketamine and xylazine administered via the intramuscular route. Each rat was positioned on a plastic tray. An aluminum foil mask with two holes, each with a diameter of 1.3 cm (1.3 cm²) that served as sites of cutaneous UVR exposure was placed over each rat before UVR exposure immediately caudal to the appropriate tattooed spot on the mid-dorsal area.</p> <p>In order to expose the eyes to a UVR intensity comparable to that of the skin sites, the head of each rat was elevated so that the eyes were on a plane equivalent to the skin sites. The eyes were held open by clasping the head and neck skin with tape and gently pulling the clasped skin caudally. The eyes were lubricated with a sterile ophthalmological solution immediately before, during, after and 30 ± 5 minutes following exposure. The rats in groups 1-4 and 6 were placed 1.2 meters from the UVR source at the time of exposure. An instrumental UVR exposure dose equivalent to 0.5 minimal erythema dose (MED, a UVR dose adequate to elicit a barely perceptible response in skin) was delivered to each rat as shown in the Study Design Table over a period of 30 ± 5 minutes. The rats in group 5 had all the above procedures performed with the exception of UVR exposure. These rats were sham-exposed in the same room as the other rats that were exposed to UVR.</p> <p>The interval between test article administration and UVR exposure was based on information indicating that CP-690550 concentrations reached a peak in the skin of rats approximately 0.5 hr after a single oral administration [refer to Report: DM2004-690550-041: Tissue Distribution of CP-690550 (Pyrrolo[2,3-d]pyrimidine) in Long-Evans Male Rats]. The interval between 8-MOP administration and UVR exposure was based on previous experience at the Testing Facility</p>	

Group	No. of Rats	Descriptor	Dose (mg/kg)	Concentration (mg/mL)	Dose Volume (mL/kg)
1	5+6 ^a	Vehicle	0	0	10
2	5+6 ^a	CP-690,550	10	1	10
3	5+6 ^a	CP-690,550	30	3	10
4	5+6 ^a	CP-690,550	100	10	10
5	5 ^b	CP-690,550	100	10	10
6	3	8-MOP	50	5	10

- a. Rats assigned to the toxicokinetic sample collection were not anesthetized, restrained, or exposed to UVR.
b. Group 5 were sham-exposed to UVR.

Group	No. of Rats	Descriptor	Instrumental UVR Dose (MED)	Interval Between Formulation Administration and UVR Exposure (Minutes) ^a	Assigned Rat Numbers	
					Phototoxicity Phase	Toxicokinetic Phase
1	5+6 ^b	Vehicle	0.5	30 ± 5	1272 - 1276	376 - 381
2	5+6 ^b	CP-690,550	0.5	30 ± 5	1277 - 1281	382 - 387
3	5+6 ^b	CP-690,550	0.5	30 ± 5	1282 - 1286	388 - 393
4	5+6 ^b	CP-690,550	0.5	30 ± 5	1287 - 1291	394 - 399
5	5 ^c	CP-690,550	0	-	1292 - 1296	-
6	3	8-MOP	0.5	60 ± 10	1297 - 1299	-

- a. The interval between formulation administration and UVR exposure was based on the median dosing time for each group.
b. Rats assigned to the toxicokinetic sample collection were not anesthetized, restrained, or exposed to UVR.
c. Group 5 were sham-exposed to UVR.

UVR Source

The source of irradiation, a 6.5 kw long-arc xenon water-cooled lamp, was used to simulate mid-latitude summer sunlight. One filter (Schott WG 320, doped glass, 1 mm thick) was used to attenuate mid-range ultraviolet (UVB). This filtered source includes ultraviolet B radiation from 290 to 320 nm, ultraviolet A radiation from 320 to 400 nm, and visible radiation. A Solar Light detector was used to monitor the incident UVR.

The UV spectrum below (bottom) was submitted to the previously reviewed study, Report 07AM087. Note that the reason for phototoxicity study of CP-690550 is the absorption of UV light around the lower limit of UVB spectrum, 290 nm. However, in this study, despite the common use of this type of solar stimulated light source, the intensity of radiation at the wavelengths of concern are minimal and insufficient to properly assess phototoxicity at the absorption spectrum for CP-690550

Details for UVR Exposure

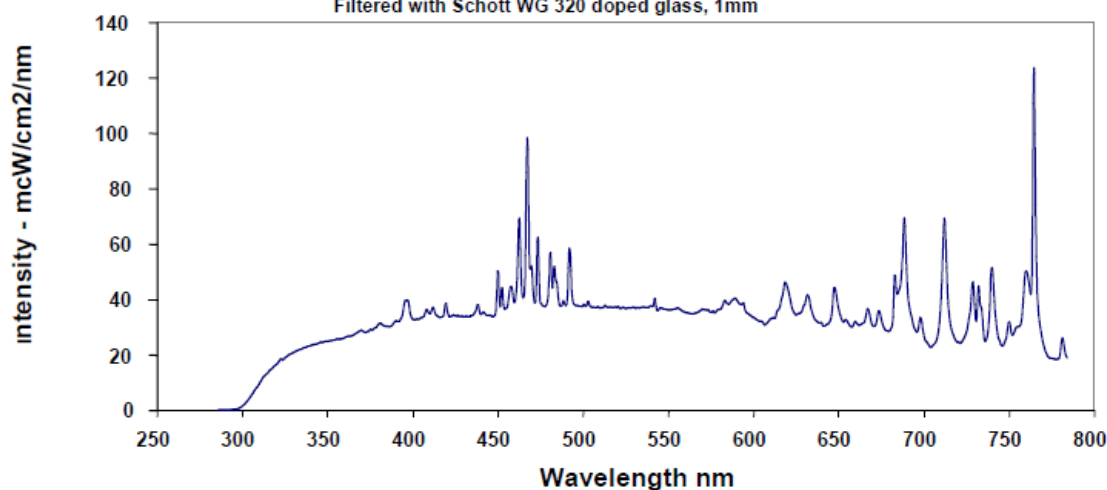
UV Source: Atlas Xenon Arc Lamp

STP Version: 20008434 (25 OCT 2010)

Emission Spectrum of the Irradiation Source

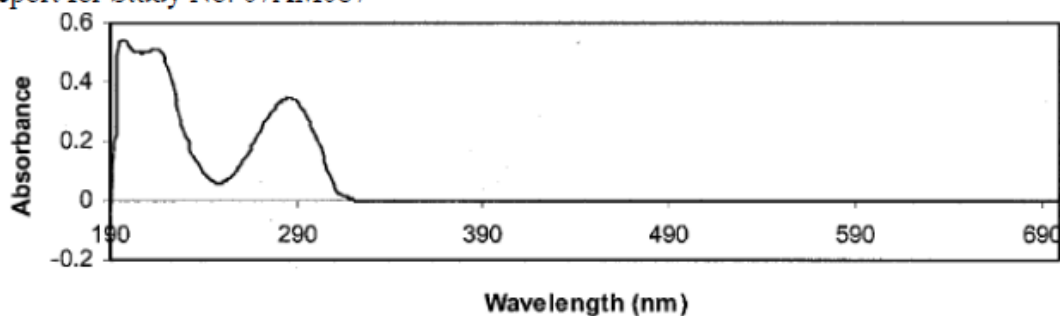
6500 Watt Atlas Xenon Arc Lamp

Filtered with Schott WG 320 doped glass, 1mm



UV Spectrum

Report for Study No. 07AM087



Solution	MEC ($M^{-1}cm^{-1}$) 290 nm	MEC ($M^{-1}cm^{-1}$) 286/287
pH 7.5 PBS	16005	16472 (286 nm)
pH 5.5 PBS	15892	16254 (287 nm)

Deviation from study protocol:

There were no protocol deviations that affected the study results or conclusions.

Observations and Results

Mortality

All rats were observed for viability at least twice daily.

There was 1 death (#1278) in the 10 mg/kg/day dose group, found on day 7 at the completion of the UVR exposure. There were no adverse clinical observations and no gross pathology abnormalities. While the lack of evidence for an explanation of this death raises concern for it to be related to treatment, the fact that 10 other rats receiving this dose did not die and 27 rats administered higher doses (up to 10-fold higher) survived, suggests the death was unrelated to CP-690550.

Clinical Signs

Clinical observations and general appearance checks were performed twice daily. Rats were observed prior to anesthesia administration for clinical observations only and were monitored constantly during and until recovery from anesthesia. Rats were individually examined 30 ± 5 minutes and 4 hours ± 30 minutes after the completion of UVR exposure or sham UVR exposure for general appearance and signs of skin responses at the site of UVR exposure or sham UVR exposure, as well as 1, 2 and 3 days after UVR exposure.

There were no clinical observations related to CP-690550 administration.

Skin Reactions

There were no skin reactions related to CP-690550 administration in either light or darkly pigmented regions of skin exposed or not exposed to UVR. Skin reactions in both light and dark skin regions were observed in the positive control, 8-MOP, treated animals exposed to UVR and these included erythema grade 1 (barely perceptible light redness), erythema grade 2 (distinct redness), edema grade 1 (mild, raised < 1 mm) and edema grade 2 (moderate raised 1 mm to 2 mm).

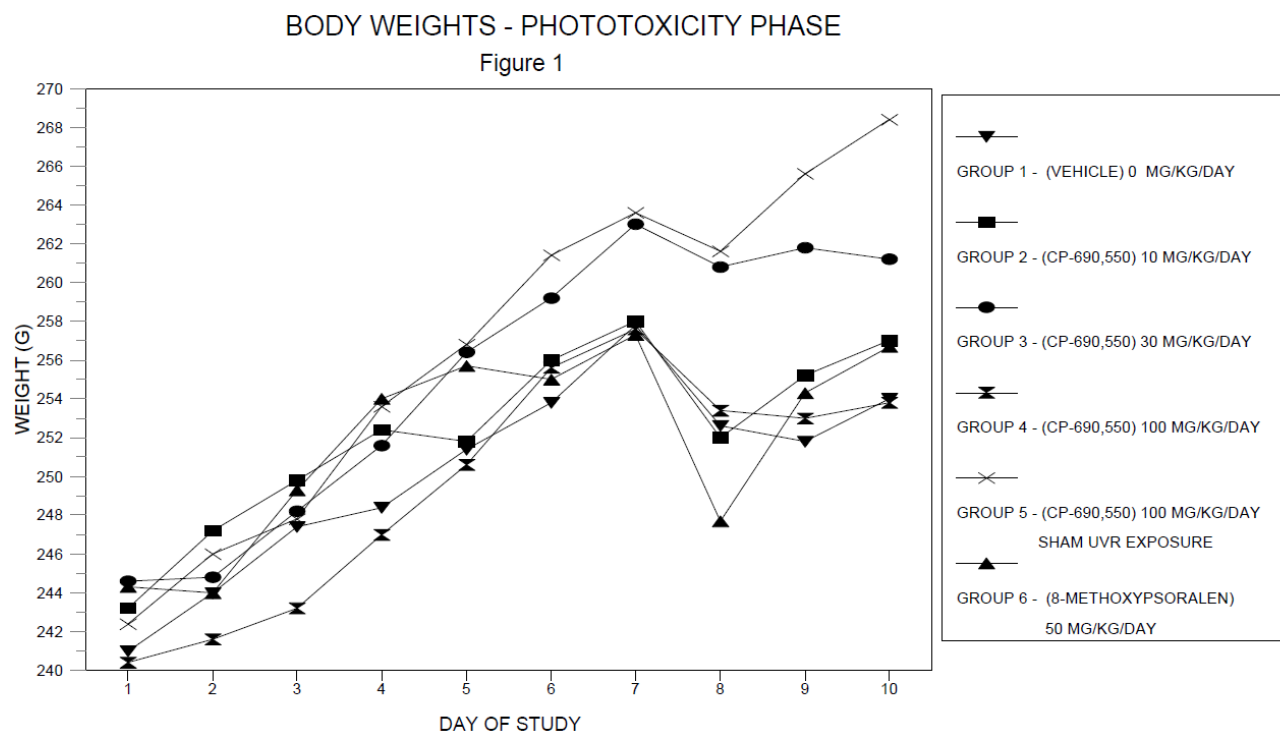
Based on the response of the positive control the study of phototoxicity would be acceptable, however the wavelength of concern at the lower limit of the spectrum, around 290 nm, was not evaluated appropriately. The absorption spectrum of 8-MOP is different than CP-690550. The Reviewer is not aware if an appropriate positive control for the compounds that absorb the lower end of the UVB spectrum has been identified and validated.

Body Weights

Body weights were recorded twice during acclimation, daily before formulation administration and prior to sacrifice.

There was no effect of CP-690550 on body weights and body weight gain. All groups lost a similar amount of weight over the day 7 to 8 interval. This body weight effect is typical in this study type and is related to the anesthesia and handling required for UVR

exposure. Body weights and body weight gains were comparable among the vehicle and CP-690550 UVR dose groups (dose groups 1 through 4) throughout the study (days 1-10 interval) and following UVR exposure (days 7-10 interval).



Feed Consumption

Feed consumption was not monitored.

Ophthalmic Examinations

Ophthalmological examinations were performed for all rats before assignment to study and three days after UVR exposure as follows: indirect ophthalmoscope in conjunction with a hand-held lens (60 diopter) was used to examine visible ocular structures (lens and fundus oculi), slit lamp biomicroscopy was used to evaluate the lids, adnexal structures, cornea, anterior chamber, lens and anterior vitreous for any abnormalities. The mydriatic solutions used were 1% Tropicamide Ophthalmic Solution and 1% Atropine Sulfate Ophthalmic Solution.

There were no dose-related CP-690550 effects in the ophthalmological evaluation conducted three days after simulated sunlight exposure.

All the positive control rats exposed to UVR had bilateral diffuse corneal edema sufficient to obscure intraocular structures. Two of the 3 rats developed cataracts, which were not present in the initial ocular examination. Both findings were indicative of phototoxicity.

Gross Pathology

Rats were sacrificed by carbon dioxide asphyxiation following completion of the final ophthalmological examination and both eyes were processed for histopathology.

The skin encompassing the UVR exposure sites or sham UVR exposure sites and surrounding unexposed skin were retained in neutral buffered formalin for possible histopathological evaluation.

There were no gross pathological findings presented. From the results of the skin reactions noted above and the eye histopathology, it appears there were no gross pathological findings related to CP-690550.

Histopathology

The following eye tissues were examined: cornea, lens, bulbar conjunctiva (when present), vitreous and aqueous chambers, optic nerve (when present), retina, sclera, iris, ciliary body and choroids.

Adequate Battery: Yes, for this specialized study

Peer Review: Yes, by the applicant's pathologist

There were no CP-690550 -related histopathologic findings observed in the eyes (including the bulbar conjunctiva, cornea, aqueous chamber, iris, ciliary body, lens, vitreous chamber, choroid, retina, sclera and optic nerve) of the rats administered CP-690550 at doses of 10, 30 or 100 mg/kg/day. Also there were no effects in control animal eyes.

Positive Control: Findings in the positive control rats indicative of phototoxicity and that were not present in the other treatment groups included bilateral, marked neutrophilic infiltration of the cornea, together with corneal edema and patchy loss of the corneal endothelium. In addition, there were instances of corneal ulceration, intercellular edema or vacuolar degeneration of the corneal epithelium and single cell necrosis and a focus of minimal hyperplasia of the central lenticular epithelium.

Toxicokinetics

Blood samples were collected from the jugular vein at approximately 0.5, 1, 3, 8 and 24 hours after dosing on day 7. Rats administered CP-690550 were sampled at the times indicated in the following table. Control group 1 was sampled at 0.5, 1 and 3 hours only. The lower limit of quantification was 5.00 ng/mL.

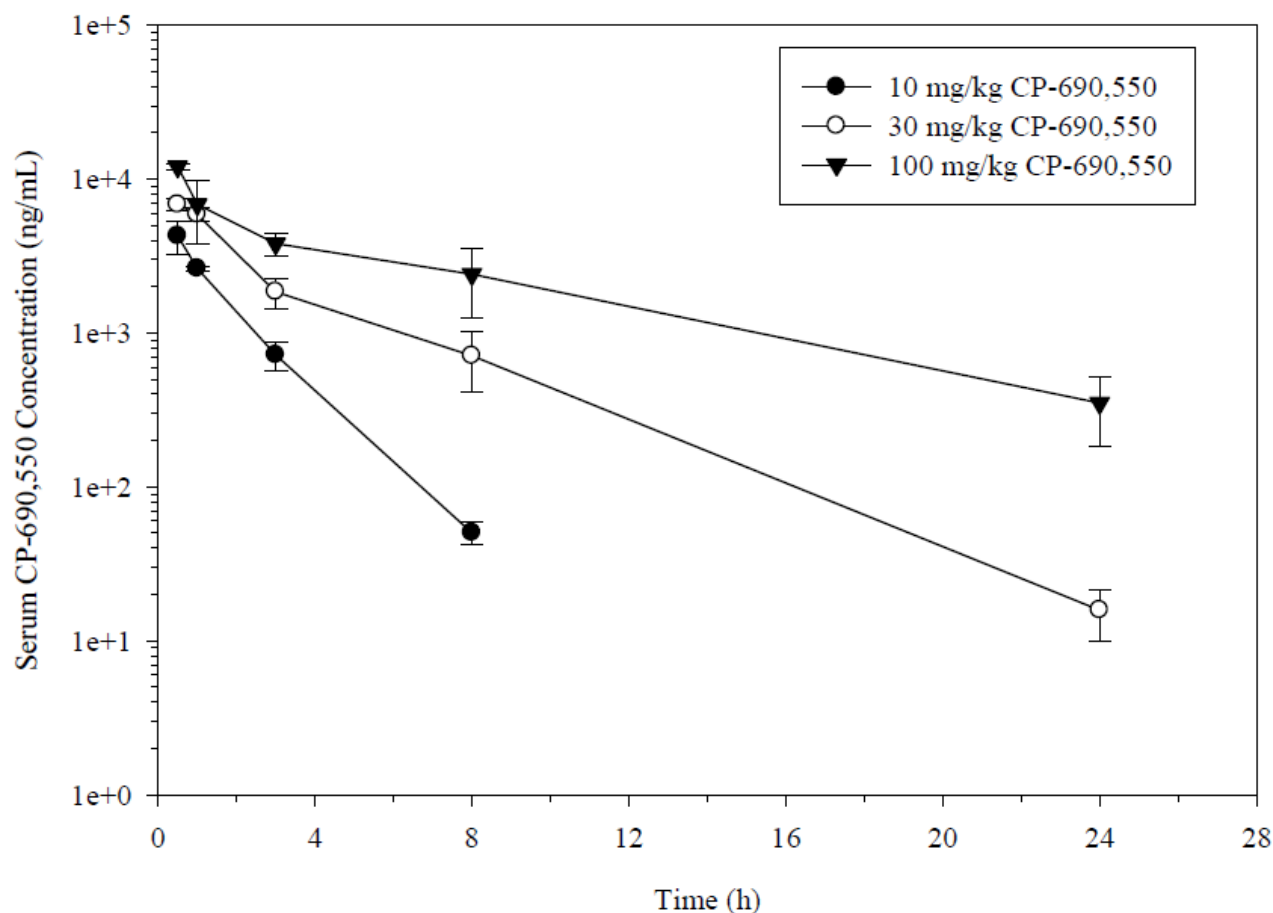
In general, systemic exposure to CP-690550, C_{max} and AUC_{0-24} , increased with the increase in dose, and were dose proportional except for 1 or 2 instances. CP-690550 in samples from the vehicle control group were below the quantifiable limits of the assay. Mean T_{max} at 10, 30 and 100 mg/kg on day 7 occurred at 0.5 hours.

Mean Toxicokinetic Parameters for CP-690550 in Rats after Oral

Administration of CP-690550 on Study Day 7

Dose Group	Dose (mg/kg)	T_{max} (h)		C_{max} (ng/mL)		$C_{max}/Dose$		$AUC_{(0-24)}$ (ng*h/mL)		$AUC_{(0-24)}/Dose$	
		Mean	n	Mean	n	Mean	n	Mean	n	Mean	n
1	0	0.00	3	0	3	0	3	0	3	0	3
2	10	0.50	3	4270	3	427	3	8070	3	807	3
3	30	0.50	3	6830	3	228	3	24900	3	829	3
4	100	0.50	3	12000	3	120	3	56000	3	560	3

Note: The blood from 3 animals per dose group was sampled at each time point (n = 6/dose group).

Mean CP-690550 Concentration in Female Rats versus Time after Oral Administration of CP-690550 on Study Day 7**Stability and Homogeneity***Stability:*

The stability of CP-690550 was not evaluated in this study, as previous studies had established the stability of CP-690550 stored under various conditions. The formulation was stored and used appropriately based on those results. Specific reference to those

studies was not provided in this report, but were noted in other studies of this NDA review.

Homogeneity: Samples obtained from the top, middle, and bottom locations of the dosing formulations ranged from 0.8 to 3.7% of the targeted concentration and met the criteria (relative standard deviation (for the grand mean of the average values for top, middle and bottom locations, is less or equal to 5) for being appropriately homogeneous during dosing.

Formulation Concentration

The overall mean concentration results ranged from 97.9% to 100.2% of the target concentrations and meet the criteria ($\leq 10\%$) for concentration. There was no CP-690550 detected in any control samples analyzed.

11 Integrated Summary and Safety Evaluation

Pharmacology

Tofacitinib (CP-690550 or (b) (4)) were the code names used in all the nonclinical studies and CP-690550 is the term used throughout this review to refer to tofacitinib) is an inhibitor of the Janus associated kinases (JAK) family. CP-690550 is a more potent inhibitor of JAK1, JAK2 and JAK3 kinase activity than TyK2 members in this kinase family. JAKs are involved in myeloid and erythroid cellular development and function by interrupting the signaling pathway from cytokine receptor to STAT (signal transducers and activators of transcription) through its inhibition of JAK activity intracellularly associated with cytokine receptors. Thus CP-690550 inhibition of JAK activity decreased the cellular response to cytokine signaling (IL-2, -4, -6, -10, -15, and -21, (IC_{50} potencies ≤ 500 nM for the cellular forms, i.e., JAK homo or hetero-dimers) resulting in the reduction of the synthesis and secretion of additional cytokines and other inflammatory mediators.

Secondary pharmacodynamic studies evaluated the binding and potency of CP-690,550 on a broad panel of receptors, ion channels, and enzymes that demonstrated a lack of significant binding or activity to these compounds. It was highly specific to the JAK family and did not affect the activity of other classes of kinases. There were a few compounds with which CP-690550 had significant activity (inhibition of $>50\%$) in these screens. These were the MT_3 (Melatonin 3) receptor (K_i 5.2 μM) and VEGFR1 (vascular endothelial growth factor receptor 1 (K_i 3.7 μM), CaMK2 α (calmodulin dependent protein kinase 2 α (K_i 12 μM)), and Lyn A Kinase (K_i 2.3 μM).

Of note is that VEGFR1 activity is mediated through tyrosine kinase signaling, and is essential to maintain vascular integrity (Koch et al 2011). In the initial early single dose toxicology studies at dose up to 2000 mg/kg, internal hemorrhage was present in the gastrointestinal tract and pulmonary systems and probably cause of death. In the the 6-

month study in the rat at substantially lower doses ≤ 100 mg/kg, there were infrequent and not dose-related incidences of hemorrhage associated with lymphoid organ atrophy (lymph nodes, thymus), as well as liver and lung. These were unexplained finding in the study reports, but could be attributed to weakening the vascular structural integrity, due to cellular depletion, anoxia and organ atrophy. In this regard, tissue distribution studies (Report DM2004-690550-041) found that [^{14}C]CP-690550 radioactivity was detected in vessel walls up to 504 hour after oral administration of 10 mg/kg (454 $\mu\text{Ci/kg}$), the last timepoint examined.

In two animal models of arthritis, collagen-induced arthritis model in male DBA/1J mice and adjuvant (*Mycobacterium byturium*)-induced arthritis model in female Lewis rats, tofacitinib demonstrated efficacy by attenuating paw arthritis scores (swelling, edema and paw volumes). Based on studies of the collagen-induced arthritis in mice the applicant demonstrated that suppression of the inflammatory response did not require continuous exposure to CP-690550 for effectiveness.

The specific mechanism for preventing rheumatic changes in bone and cartilage were not addressed in detail, other than to associate tofacitinib inhibition of numerous interleukin compounds' signaling with the reduction in synthesis and secretion of various inflammatory compounds. The two pharmacology studies, the rat adjuvant-induced arthritis model (Report 160531) and mouse collagen-induced arthritis (Report 160243) investigated CP-690550 for histological effects of cartilage damage, bone resorption, inflammation, pannus and CD68 and CD3 cellular infiltrates (rat) or F4/80 and CD3 cellular infiltrates (mice). However both of these studies were flawed with regards to interpretation of the effects on cartilage. At the time of the initial tofacitinib treatment (actually 4 hours after the start of treatment tofacitinib or vehicle), approximately half the animals in the first timepoint group had no evidence of cartilage damage (ankle and wrists). This limited not only this groups but sequent timepoint evaluations since any effect in reduction of arthritic disease is confounded with possibility the animal never had signs of disease. At this time, it is not known if tofacitinib has or does not have activity on cartilage, the studies did not allow either conclusion.

At this time, the clinical radiographic evidence for prevention of disease progression is inadequate to address this claimed benefit (Refer to the Clinical Review by Dr. Nikolov). The nonclinical toxicology studies did not contribute substantially to understanding the proposed mechanistic effects of CP-690550 on cartilage or bone, however the studies do support the safety of CP-690550 on normal bone structure.

- Adult monkey study 9 month toxicology study: no effect on sternum histopathology
- Rat 6 month toxicology study: no effect on sternum and distal femur and stifle (knee) joint histopathology.
- Rat (2 year) carcinogenicity study no effect on sternum or femur examined microscopically, or joints (stifle) examined macroscopically in aged rats.
- Juvenile monkey study, 9 month treatment starting at month 13-14 of age, no effect on sternum histopathology, and as an exploratory non-GLP aspect of this

study, radiographic analysis of radius and tibia length found no effect on growth of bones.

- Embryofetal developmental studies indicating numerous bone abnormalities and delay ossification in rats and rabbits. These only assess macroscopic changes, there was no histopathology which is standard and acceptable practice.
- No effect on rat growth and behavior in postnatal studies.

Thus CP-690550 produced no pathologies on bone and cartilage in juvenile or adult, including aged healthy animals, supporting CP-690550 safety. In contrast, detrimental bone and possibly cartilage effects were evident in studies of fetal development.

Safety pharmacology studies of tofacitinib found no effect on hERG channel current or electrophysiological characteristics of cardiac Purkinje fibers. In vivo assessment of cardiovascular effects in rats found dose dependent reductions in blood pressure associated with increased heart rate. At 10 and 100 mg/kg doses, mean blood pressure was reduced 5 and ~37 mmHg, respectively, and heart rate increased 30 and 100 bpm. respectively. Monkey EKG studies were incorporated into the long term toxicology studies, but there was insufficient information provided to adequately analyze the cardiovascular and EKG parameters, and this was superseded by the clinical TQT study. An additional major problem with monkey EKG was that heart rates of all animals were markedly elevated (>200 bpm) and literature indicates that these values are near or outside the validation range for standard QT correction methods. Neurobehavioral assessments in mice found a reduction in spontaneous activity and signs of general toxicity. There were no significant effects on respiratory characteristics, kidney function, or gastrointestinal transit time.

Pharmacokinetics

Pharmacokinetic parameters were determined for all of the species used in the toxicology studies mouse, rat, rabbit, and cynomolgus monkey. The dog was not used in toxicology studies but parameters were determined to aid with pharmacokinetic predictions in humans. Tofacitinib was rapidly absorbed T_{max} values of 0.3 to 1.5 hours. The bioavailability was highly variable in the rat (11 to 129%) and this may be due to issues with early assay development. In the dog and monkey bioavailability was 43% and 48%, respectively, and in one of the higher dose rat studies values of 42.9 to 43.3 were attained in one rat study. For comparison, the human bioavailability is about 75% (refer to the Clinical Pharmacology Review of Dr. Jain). The clearance in the rat (29-42 mL/min/kg), and monkey (18 mL/min/kg) were moderate to high with a low to moderate volume of distribution (rat: 1.4-1.6 L/kg, monkey 1.7 L/kg). The percentage of tofacitinib bound to plasma proteins varied with species, mouse 33%, dog 20%, monkey 35%, and human 39%, with the rat dependent on tofacitinib (ranging from 31% bound at low tofacitinib concentrations to 91% bound at high concentration). It was determined that tofacitinib bound mostly to albumin, and that α 1-acid glycoprotein did not have appreciable binding. Tofacitinib distributed approximately equally between red blood cell and plasma compartments indicating red blood cells were not accumulating it.

In the toxicology studies, plasma concentrations, both C_{max} and AUC, increased with increasing dose. It is noteworthy that only the highest administered dose in the rat and monkey toxicology studies provided for a detectable plasma level over a 24 hour period, with the low dose detectable only up to approximately 6 hours per day and 8 to 12 hours for mid doses. Thus the effects observed at lower doses during and at the end of the study occurred in the absence of continuous tofacitinib exposure. In the rat studies the entire daily dose was administered at one time, while in the monkey studies the daily dose was administered as a split dose either 2 or 3 times per day, evenly distributed temporally. In monkeys there was no effect of sex on tofacitinib levels, but in rats females tended to have 2 to 3-fold higher AUC levels than males in the low and mid dose groups. There was no systemic accumulation of tofacitinib during the duration of the studies. Comparison of tofacitinib exposures between juvenile and adult monkeys in separate toxicological studies (Reports 09GR248 and 2003-0301, respectfully) receiving the same dosages found reasonably similar C_{max} and AUC exposures.

Tissue distribution studies were conducted in pigmented rats with [^{14}C]CP-690550 (Report DM2004-690550-041). There was rapid distribution to all tissues except the brain, testis and adipose tissue at the first timepoint 30 min after oral dosing. For most tissues there was a gradual decline in radioactive levels over the following days, however at the last timepoint 504 hours, blood vessels and ocular tissue containing melanin were the only tissues with detectable activity. Ophthalmologic examinations during toxicology studies in rats and monkeys (Reports 77435 and 2003-0301, respectively) found no effects of tofacitinib treatments. A study in lactating rats found milk to plasma ratios of tofacitinib of approximately 2:1 indicating the concentrating potential in active mammary glands (Report 103847). In vitro studies found that P-glycoprotein but not breast cancer resistance protein is an efflux transporter for tofacitinib (Reports 060532 and 175813, respectively). CP-690550 inhibited the human hepatic uptake transporter OATP 1B1 in an in vitro study of human HEK293 cells (Report 192119) and inhibited human OCT2 mediated uptake of creatinine (Report 13323).

Metabolism and excretion studies were conducted with radioactive [^{14}C]CP-690550 in mice (Report 140653), rats (Report DM2005-CP690550-055), rabbits (Report DM2005-CP690550-064), and monkeys (Report DM2005-CP690550-052) and compared with studies in humans (Report DM2005-CP690-049, and refer to the Clinical Pharmacology Review of Dr Jain for Report A3921010 for further details about the human results). In the rat and monkey elimination was primarily through urine with the unmetabolized drug comprising approximately 10% of total radioactivity recovered in urine in the monkey and male rat, and 30% in the female rat. The circulating levels of unmetabolized drug ranged from 58-60% in the rat and 31-49% in the monkey as a percentage of total blood radioactivity. The primary metabolic pathways were due to oxidation of the pyrrolopyrimidine ring (forming metabolite M9), oxidation of the piperidine ring (forming M6 and M18), *N*-demethylation (forming M1), oxidation of the piperidine ring side chain (forming M2), and glucuronidation (forming M20). In comparison, more than 65% of the circulating radioactivity in humans was unmetabolized CP-690550 and all human metabolites were present at <10% of total circulating activity. While all the human

metabolites were not present in a single animal species, they were identified in at least one animal species studied. The Applicant indicated that all metabolites have or are predicted to have ≤ 10 -fold the potency of CP-690550 for Janus kinase (JAK) 1/3 inhibition, but there was no empirical data or references to supporting this claim in the nonclinical submissions. In vitro CYP 450 phenotyping studies indicated CP-690550 was primarily metabolized by CYP3A4 and 2C19 (Report DM2007-(b) (4) 001).

General Toxicology

General toxicology studies were conducted in Spague-Dawley rats and cynomolgus monkeys.

Rat

Two GLP single dose studies were conducted in rats. The initial study (Report 01-2063-07), single oral CP-690550 doses of 500, 1000 and 2000 mg/kg resulted in mortalities at the two higher doses within hours with pathology consistent with gastrointestinal and pulmonary hemorrhage as cause of death. A single dose intravenous administration study at 3 mg/kg (Report 09GR453) examined only the local toxicity and found minimal perivascular hemorrhage, not an unexpected finding, usually unavoidable when penetrating the vascular wall with a needle. A repeated oral dosing non-GLP exploratory study resulted in the death of 1 of 5 females on day 2 of the 1000 mg/kg/day dose. The animal had stomach ulcerations and all animals at this dose administered for 4 days had necrosis of the stomach mucosa (Report 00-2063-03). These early studies characterized lethal and high dose toxicity and helped identify a maximal tolerated dose in rats. In addition they helped identify the critical hematology and clinical chemistry parameters, for which tofacitinib's effects were generally consistent throughout the developmental program across both rats and monkeys.

The pivotal toxicological studies in rats were 6-weeks and (Report 01-2063-06) and 6-month (Report 02-2063-20) repeated dosing studies. Only the 6-week study included recovery groups, which was 1 month duration. In the two pivotal toxicology studies, a 6-month study in rats (Report 77435) and a 9-month study in monkeys (Report 2003-0301, discussed below), NOAELs could not be determined as adverse effects were noted in each of the lowest doses tested. These were suppression of the immune and hematopoietic systems evidenced by suppression of myeloid and erythroid bone marrow production, reduced or atrophied lymphoid organs, and reductions in circulating red and white blood cells. The effects were minimal at the low dose (only hematological changes) and reversible in rats such that with careful hematology monitoring, together with previous human experience in the development program for renal transplantation, the initial clinical study was allowed to proceed.

6-week study: In the 6-week study (Report 01-2063-06) with 1-month recovery, Sprague-Dawley rats were administered oral doses of CP-690550 at 0, 1, 10, or 100 mg/kg/day. There were no CP-690550-related effects on mortalities, clinical signs, and body weight, food consumption, or urinalysis. The major treatment related hematologic

findings included marked decreases in white blood cell parameters and slight time dependent decreases in red blood cell count, hemoglobin, and hematocrit. Compared to control values, for the high dose on day 43 the changes included red blood cell counts reduced red blood cell counts (M/F 93%/92%), hematocrit (M/F 93%/93%), hemoglobin (M/F 94%/94%), and reticulocytes (M/F 80%/85%), all of which returned to control levels by the end of the recovery period. For white blood cells, a similar comparison, revealed reductions in white blood cell counts (M/F 45%/50%), lymphocyte counts (M/F 36%/36%), eosinophil counts (M/F 69%/44%), basophil counts (M/F 18%/19%), large unstained cells (M/F 15%/20%), and an increase in neutrophils (M/F 122%/169%). Except for neutrophils which recovered completely the other white blood cell parameters only partially recovered by the end of 1 month. Macroscopic changes were observed in the thymus and spleen of animals in the 100 mg/kg dose group, and histopathological changes included lymphoid depletion in lymphoid tissues, thymus, spleen, mesenteric lymph node, and bone marrow. A NOAEL was not identified in the study since adverse hematological changes were noted at the lowest dose level examined, 1 mg/kg, but histopathology was not conducted in this group.

6-month study: In the pivotal 6-month repeated dose study, CP-690550-10 was orally administered to Sprague Dawley rats, daily, at doses of 0, 1, 10, and 100 mg/kg/day. There were no recovery groups. There were 5 deaths in the CP-690550 treatment groups, at least one at each dose, but none of these deaths could be clearly attributed to CP-690550 treatment, rather the histopathology findings suggest they were related to the numerous jugular vein blood collections during the study. The major clinical sign was salivation at the high dose, which occurred in all animals for most of the study and might have contributed to dehydration in this dose group, evidenced by increased sodium and total protein levels during the second half of the study. Except for the high dose male group, there was no effect on body weight and weight gain. The mean weight of the high dose male group was 86% of the control males, and mean body weight gain was 82% of the control males.

The major treatment-related hematologic findings were similar to the 6-week rat study and included reduction in white blood cell counts (total WBC 32% of control, lymphocytes 55% of control), increases in neutrophils (398% of control) and monocytes (259% of control) and decreases in red blood cell parameters (RBC 85% of control, Hb 90% of control, Hct 88% of control) at doses ≥ 10 mg/kg/day in males and females. For doses ≥ 10 mg/kg/day, the lower dose generally required a longer treatment period to reach similar effects in the high dose group. Analysis of lymphocyte subset populations by cell surface markers found dose-dependent decreases in all the evaluated lymphocytes subpopulations, helper T cells, cytotoxic T cells and a small subset of NK cells, total T cells, total B cells, and NK cells. These populations were ranged from 5% to 77% of controls.

Clinical chemistry findings included Increased in total protein, mainly due to an increase in albumin and this was reflected in an increase in A/G ratio. Calcium levels were also increased in association with albumin concentrations. The reported changes in glucose, triglycerides, and alkaline phosphatase were within normal variation and not

toxicologically significant. Thus, the observed changes in clinical chemistry parameters were likely related to dehydration, possibly do to excessive salivation from drug administration.

Atrophy in lymphoid tissues was prominent in the lymph nodes (inguinal, ileo-femoral and mesenteric), spleen, and thymus. This was associated with reduced organ weights and macroscopic observations of small size. In the lungs, pulmonary histiocytosis with interstitial inflammation occurred at the high dose. There was a dose-related increase in liver weight (when expressed as % body weight) corresponding with hepatocellular hypertrophy in males and females.

Systemic exposure was approximately 2-3.5-times greater in females than males at doses of 1 and 10 mg/kg/day, but was similar in females and males at 100 mg/kg/day. Most samples obtained after the 4 hour timepoint of the low and mid dose groups were below the level of CP-690550 detection, indicating that the lack of toxicity in the low and mid dose groups probably reflects their lack of continuous drug exposure (<8 hours/day). Whether this is also the case for tofacitinib's efficacy is not clear from nonclinical studies, although pharmacology studies of different dosing regimes do support prolonged effects on the activity of some cytokines. Due to tofacitinib's intracellular site of action, its duration of pharmacodynamic activity may not be directly reflected by systemic concentrations, although some degree of association would be expected.

The NOAEL was 1 mg/kg/day due to the lack of histopathological changes and only minor changes in hematology parameters. The lack of evaluation for recovery from observed effects, particularly anatomical pathology, hindered the use of a higher dose as the NOAEL. The associated toxicokinetic parameters at week 26 were C_{max} : male 120 mg/mL female 382 ng/mL, AUC_{0-8} male 255 ng-h/mL, female AUC_{0-24} 710 ng-h/mL.

CYNOMOLGUS MONKEY

In the monkey, studies consisted of a single oral dose GLP study, an exploratory non-GLP 2-week repeated oral administration study, a 1-month repeated oral administration study with a 1-month recovery, and a 9-month repeated oral administration study. All of the studies incorporated a split dosing schedule of either bid or tid dosing, at approximately equally spaced intervals over 24 hours.

In a single oral dose study (Report 00-2063-04) in monkey doses up to 1000 mg/kg/day, but split as three administrations per day of 13, 67, and 333 mg/kg. There were no deaths. Doses of 200 and 1000 mg/kg/day resulted in emesis and reduced activity. Although hematology and clinical chemistry samples were obtained, interpretation of the data was limited due to variation arising from few animals per dose group. These animals were not terminated, so there was no anatomic pathology in this study. This study was followed by a non-GLP, 2-week exploratory repeated oral dosing study (Report 00-2063-05) in which CP-690550-10 was administered at 20, 50, 200 and 500 mg/kg/day as split doses, TID (n=1/sex/dose). On day 8, the 20 mg/kg/day dose group was

escalated to 500 mg/kg/day to determine a MTD. Both animals in the 500 mg/kg/day dose group and the 200 mg/kg/day female were euthanized on days 11 and 12 due to morbidity. The highest tolerated dose was 50 mg/kg/day, and no NOAEL was identified. Clinical signs related to drug treatment included emesis at ≥ 50 mg/kg/day; post-dose salivation, loose stool, and hunched posture at ≥ 200 mg/kg/day; and decreased activity, ataxia, pale skin, and dehydration at ≥ 500 mg/kg/day, similar to the signs of toxicity observed in the early rat studies. Although there was only $n=1/\text{sex}/\text{dose}$, there were reductions in WBC, lymphocytes, red blood cell counts, hematocrit, hemoglobin, and reticulocytes, based on concurrent and historical controls, but not obvious effects on serum chemistry parameters. Lymphoid depletion occurred in the thymus, spleen, mesenteric lymph node, and bone marrow. Viral nuclear inclusions were observed in tissues in some euthanized animals with infections and inflammation (stomach, spleen) that resulted in tissue necrosis (stomach), similar to findings observed in the early rat studies with high doses.

1-month study: In the 4-week toxicity study (Report 01-2063-09) CP-690550-10 was administered orally at doses of 0 (0.5% carboxymethylcellulose vehicle), 10, 50, and 100 mg/kg/day as split doses TID (0, 3.33, 16.67, and 33.33 mg/kg) for 4 weeks followed by a 4 week recovery period. The doses of 50 and 100 mg/kg/day resulted in secondary infections of open wounds, or gastrointestinal erosions or ulcers resulting in all animals in the 100 mg/kg/day group being euthanized by day 12, and 3 animals in the 50 mg/kg/day group being euthanized throughout the dosing phase. Lymphoid depletion occurred in the spleen at 50 and 100 mg/kg/day and in the mesenteric lymph node at 100 mg/kg/day. At 10 and 50 mg/kg/day there was a reduction in the myeloid:erythroid ratio in the bone marrow of 54 to 69%, which recovered in the 50 mg/kg/day group (there was no recovery group for the 10 mg/kg/day dose). This was due to a reduction in % myeloid cells (78-83% of control levels) and an increase in % erythroid cells (108-151% of control levels), both of which recovered to control levels at the end of the recovery period. There was an increase in mononuclear cell infiltration in a number of organs that exceeded control levels. The 10 mg/kg/day dose was a tolerated dose; effects included slight to mild reductions in red blood cell counts, hematocrit, and hemoglobin, lymphocytes, and lymphocyte subsets (T-helper, cytotoxic/suppressor T-lymphocytes and natural killer cells). Parameters that did not recover (50 mg/kg/day dose group) within 1 month included the following (values are end of recovery as a % of predose): lymphocytes (153-159% of control) reticulocytes (58% of control in females), ALT (150-263% of control levels), and decreases in RBC (88% of control in males), natural killer cells (1-28% of predose levels in 2 of 4 animals), T helper-lymphocytes, cytotoxic/suppressor T-lymphocytes (210-330% of predose levels). A NOAEL was not identified. The low dose, 10 mg/kg/day, was the MTD in this study. The systemic exposure, AUC_{0-24} , at 10 mg/kg/day was 3440 and 2090 ng-h/mL for males and females, respectively.

9-month study: In the pivotal 39-week study (Study 2003-0301), cynomolgus monkeys were administered CP-690550 orally for 39 weeks as BID at 0, 0.25, 1, and 5 mg/kg/day 12 hours apart for a total daily dose of 0, 0.5, 2, and 10 mg/kg/day. There were no recovery groups. One female in the high dose group was euthanized on day

214. A lymphoma had infiltrated the gastric wall resulting in ulceration and erosion. At the 9 month scheduled necropsy, 2 additional animals with lymphomas were identified, one male and another female both in the 10 mg/kg/day dose group. Of the 3 lymphomas, 2 were identified as B cell origin, 1 was T cell origin, and all were in animals positive for lymphocryptovirus.

CP-690550 treatment resulted in dose-dependent reductions in red blood cell parameters (red blood cell counts, hemoglobin concentration, and hematocrit). At 9 months, a small dose-response relationship was apparent for all three parameters, high dose means were approximately 80-90%, mid dose 91-93% and the low dose 95-96% of control means. The reticulocyte levels were more variable, but after 9 months the mean reticulocyte levels in the high dose were 151-167% of the control means. White blood cell counts and lymphocytes were also reduced (up to 62% of control values) during the study by all doses of CP-690550. Although the reduction in total white cells did not always follow a dose-response relationship, the reduction in lymphocytes did exhibit dose-response dependency. By week 38, the low dose lymphocyte population was reduced to 73-74% of controls, the high dose 57-60% of control, with the mid dose intermediate and more variable (63-79% of control).

Other than the invasive findings due to lymphomas, the histological effects of CP-690550 were limited to lymphoid tissues. Lymphoid hyperplasia involving either lymph nodes, spleen, or gut associated lymphoid tissue was present in almost all treated animals, particularly all high dose animals. Organ weights of the spleen and thymus were reduced, although thymus effects were problematic to interpret since thymus involution apparent by histopathology was present in many animals. Weights of reproductive organs were also reduced, but the presence of numerous immature animals in the high dose group and their lack in the control group made prevented any conclusion about CP-690550 effects.

The heart ECG assessment of this report were deficient in methodology or data. The limb radiograph sections were exploratory in nature and not conducted as a GLP regulated study. Therefore, neither of these aspects of the study will be used to support CP-690550 safety or regulatory decisions. Exposure was dose-related and similar between sexes. However, daily exposure for the low and mid dose was limited to two daily periods of 6 to 8 hours duration, since most samples beyond 6 to 8 hours were below the level of quantification of CP-690550 (<5 ng/mL). There was no serum CP-690550 accumulation over the duration of the study. Due to the presence of lymph node lymphocyte hyperplasia at the low dose, 0.5 mg/kg/day (0.25 mg/kg BID), the lack of any recovery assessment, and the fact that lymphomas developed at high doses with death in one female, there was no NOAEL dose.

Juvenile Animal Studies

Studies were conducted in the rat and monkey. The rat studies consisted of a dose-range finding study and a 1-month repeated oral dosing study in which the emphasis was on hematological and immunological effects, rather than a complete toxicological

evaluation. The monkey study was a GLP 39-week repeated oral dosing study with 6-month recovery, which was a complete toxicological assessment and included additional immunotoxicological assessments. Another juvenile study was conducted in rats to assess effects on reproduction and fertility which is summarized in the reproductive and developmental toxicology section below.

Rat

The dose-range study (Report 09GR249) administered CP-690550 orally once daily from PND 12 through PND 35 at doses of 1, 10 and 100 mg/kg/day. There were no mortalities and no effects on clinical signs, body weight, or body weight gain. Clinical and anatomic pathology were not conducted. CP-690550 plasma concentration, determined from a single blood sample obtained 0.5 hours after administration, increased with increasing dose and at the low and mid doses exposures were greater in females than males, similar to the findings in adult rats in the pivotal studies. There was no dose accumulation.

In the 1-month repeated dose study (Report 01-2063-09) CP-690550 was administered orally once daily to juvenile rats during PND 21-49 at dose levels of 0, 1, 10 and 100 mg/kg/day. A 2-month recovery period followed with animals sacrificed on PND 111. CP-690550 administration produced dose dependent reductions and time dependent reductions in hematological parameters although these were not large changes, a slight reduction in red blood cell counts by day 30 (PND 50, M/F 93%/95%) and in reticulocytes on day 15 (M/F, 85%/83%). The mean white blood cell count was reduced in a dose-dependent manner by CP-690550 administration and this was attributed to dose-dependent reductions in lymphocytes (maximal reduction at the high dose: M/F 34%/24% of control on day 30), eosinophils (high dose: M/F 29%/19% of control on day 30), and basophils (high dose: M/F 25%/17% of control on day 15). These effects were generally reversible by the end of the recovery period. CP-690550 decreased the mean percentage of lymphocyte subpopulations. These effects were sometimes dose- or time-dependent with further reduction on day 30 (PND 50) compared to day 15 (PND 35). The maximal reductions at the high dose for males and females compared to control values were total T cells (M/F 90%/88% on day 15), cytotoxic T cells (M/F 48%/44% on day 30), natural killer cells (M/F 28%/41% on day 15) and NKT cells (M/F 31%/23% on day 30). There were no effects on B lymphocytes.

Thymus and spleen weights were reduced in a dose dependent manner compared to control values in males and females, with maximal reductions at the high dose: spleen (M/F 50%/45% of control) and thymus (M/F 63%/49% of control). The reduction in organ weights were reflected in histopathological findings that all lymphoid organs examined (thymus, spleen, mesenteric lymph node, inguino-femoral lymph node and mandibular lymph node) had decreased cellularity. This was reversible by the end of the 2-month recovery period.

The effects on hematology and lymphoid organ weights were similar to those observed in adult rats (see Reports 01-2063-06 and 02-2063-20). Since similar doses were administered in the adult and juvenile studies and generally similar magnitude of effects

were observed, the juvenile and adult rat appear to have similar sensitivity to CP-690550. The LOAEL for this juvenile rat study is 100 mg/kg/day, based on the findings of reversibility of CP-90550-related effects and lack of clinical signs of adverse effects or mortality. Since the study focused on hematological aspects of CP-690550 and lacked analysis of serum chemistry, urinalysis, complete anatomical pathology analysis, toxicokinetic, this study would not support clinical studies in pediatric populations.

Juvenile Cynomolgus Monkey

Juvenile cynomolgus monkeys, 13-14 months of age, were administered CP-690,570 twice daily at a total daily dose of 0, 0.5, 2 and 10 mg/kg/day for 39 weeks (Report 09GR248). A 6-month recovery period followed the dosing phase for all doses except for the lowest dose 0.5 mg/kg/day group. There were no effects of CP-690550 on mortality, body weights or weight gain, clinical chemistry, coagulation parameters, bone growth, and cardiovascular and ECG parameters. The mid and high doses resulted in reduced thymus (50% of control) and spleen (66% of control) weights, with full recovery in females and partial recovery in males. There were no histopathological correlates to these changes. Compared to effects in the control group, CP-690550 treatments resulted in an increased incidence in inflammatory cell foci of the heart at the high dose, an increase in ulcers of the tail at the mid and high doses, and in increase in lymphoid hyperplasia at the mid and high doses. A full histopathology assessment was not conducted due to the lack of findings in previous toxicology studies of adult monkeys at these doses. In light of the fact that some of the males in the adult study were actually sexually immature, and that in both and rat monkey similar target organs and effects were found, the lack of a full histopathological assessment was appropriate for the submitted study. It is also important to note the proposed label was for treatment of adults, not a pediatric use, and they did not mention pediatric development of tofacitinib with regards to the juvenile animal studies.

There were CP-690550 dose-related changes in both red blood cell and white blood cell parameters. Red blood cell, hemoglobin and hematocrit were 84-86% of control values at the high dose. Total white blood cells were not altered, but total lymphocytes were reduced 31% (69% of control for males, 81.5% for females). Immunophenotyping indicated that most lymphocyte subsets were reduced (NK cells 9% of predose levels; CD4+ T cells 52%, CD8+ T cells 49%, CD4+ naïve T cells 42%, CD8+ naïve T cells 32%, memory central CD8+ T cells 48% and memory effector CD8+ T cells 69%). There was full or partial recovery of lymphocyte subsets over the 6 month recovery phase with females demonstrating full recovery or more complete recover than males. Bone marrow analysis found no changes in cellular morphology or evidence of substantial erythroid or myeloid cellular lineage, although at the high dose there was a reduction in M:E ratio (77% of control value) due to an increase in erythroid precursors. Additional studies to address immunotoxicity included a T-cell dependent antibody response study using keyhole limpet hemocyanin (KLH). CP-690550 reduced the anti-KLH IgM response and completely inhibited of the anti-KLH IgG response at the high dose. The response to KLH was restored when tested during the recovery phase in previously untested monkeys. The response to a second KLH challenge was evaluated in vitro from peripheral blood mononuclear cells (PBMC) collected at necropsy. There

was no effect of CP-690550 to suppress the PBMC proliferative response or the response to a mitogen, concanavalin A. The NOAEL was 0.5 mg/kg/day, corresponding to AUC₀₋₂₄ of 62 ng-h/mL at week 36 (2 x AUC₀₋₁₂ of 31.1 ng-h/mL), which is approximately 0.11-fold of the systemic exposure as the maximally recommended human dose of 10 mg bid.

Genetic Toxicology

The genetic toxicology of CP-690550 was assessed in a bacterial reverse mutation (Ames) assay, an *in vitro* chromosome aberration assay with human lymphocytes, and an *in vivo* rat micronucleus assay. CP-690550 caused a statistically significant increase in chromosome aberrations in cultured human lymphocytes (Report 01-2063-10) in the 3-hour test with metabolic activation, but not in the absence of the addition of induced liver enzymes. The dose at which the positive response occurred, $\geq 1700 \mu\text{g/mL}$, corresponded to 48% mitotic suppression. The results suggest a metabolite may have clastogenic activity. Additional follow-up assays were also conducted, *in vitro* CHO/HGPRT assay (Report 01-2063-16) to assess for mammalian gene mutations and an *in vivo* rat hepatocyte unscheduled DNA synthesis assay (Report 01-2063-17), which were both negative. Since the reason for these two follow-up assays was to confirm the clastogenic finding in the chromosomal aberration assay with added metabolic enzymes, neither assay was an optimal follow-up, being assays for mutagenic rather than clastogenic signals. These assays did confirm the lack of CP-690550-induced point mutations in mammalian cells, as was found in the bacteria reverse mutation assay.

Overall, the evidence indicated no mutagenic or clastogenic activity of tofacitinib and absence of an increase in liver tumors in the rat and mouse carcinogenicity studies support the conclusion that the positive chromosomal aberration finding in the presence of metabolic enzymes was likely due to a more generalized drug toxicity resulting in false positive rather than a specific carcinogenic mechanism.

Carcinogenicity

Lymphomas were observed in the 9-month general toxicology study in cynomolgus monkeys (Report 2003-0301). They occurred in 3 of 8 adult monkeys dosed orally with tofacitinib at 5 mg/kg BID (10 mg/kg/day) for 39 weeks. They were not present at the lower dose corresponding to an exposure margin of 1X for the maximal recommended human dose of 10 mg BID. In a 9-month study in juvenile monkeys (Report 09GR248), 13-14 month of age at the start of dosing, dosed with the regimen and dose levels as in the adult study, no lymphomas or lymphoid hyperplasia was evident. The lymphomas in the adult monkey study were associated with lymphocryptovirus and thought to occur due to tofacitinib mediated immune suppression allowing for viral reactivation. The juvenile monkeys were selected for positive exposure to lymphocryptovirus, but did not develop lymphomas. It's possible the juvenile animals due to the relatively shorter interval from colony vaccinations had a "more robust" immunologic defence despite the presence of immunosuppressive doses of tofacitinib. These lymphoma findings in the non-human primate support the human clinical trial occurrences of lymphoproliferative

disease (refer to the Clinical Review of Dr. Nikolov) that they were due to tofacitinib, likely through immunosuppression providing a window for reemergence of opportunistic infectious agents.

The carcinogenic potential of tofacitinib was assessed in a 2-year duration carcinogenicity study in rats (Report 07GR439) and in a 6-month duration study in rasH2 transgenic mouse (Report 08GR481). In the rat study, the findings were sex specific and included benign Leydig cell tumors, (at an exposure margin 23-times MRHD), benign thymomas in females (at an exposure margin 124-times MRHD), and hibernomas in females (malignancy of brown adipose tissue, at an exposure margin 23-times the MRHD). The lowest AUC exposure at which malignancy occurred was in males due to sex differences in systemic exposure. The absence of malignancies occurred at the 10 mg/kg/kg dose, and based on male rat AUC values corresponded to an exposure margin of 7-fold the maximal recommended human dose of 10 mg BID. The systemic tofacitinib exposure in comparison to human exposure at the MRHD are based on values obtained at 26 weeks in the 2 year rat study and were reasonable similar to values in the 6-month rat general toxicology study (Report 77435).

Thymomas have been previously reported in association with immunosuppressive drugs. For Leydig cell adenomas, the applicant did additional studies to address potential mechanism for the occurrence of these malignancies. In in vitro studies of primary rat Leydig cells (Report 11GR016), tofacitinib blocked prolactin mediated signaling, the prolactin-induced increase in luteinizing hormone receptor mRNA. The relevance of this mechanism of Leydig cell adenoma for man has been argued in the scientific literature, but is considered a realistic possibility by the Reviewer. A mechanism for the occurrence of tofacitinib-related hibernomas in female rats was studied in cultured rat brown adipocytes (Report 11GR015) and an in vivo study in female rats (Report 10GR431). In the in vitro study, CP-690550 inhibited prolactin-induced increase in phosphorylated STAT5 and basal phosphorylated STAT-3 in a concentration-dependant manner at CP-690550, and a similar STAT-3 and STAT-5 inhibition occurred in vivo associated with increased brown adipose tissue weight and cell proliferations. These results suggest that suppression of prolactin-induced increase in signalling molecules allows for proliferative growth. An additional study (Report 11GR383) to examine the role of sympathetic stimulation measured plasma norepinephrine levels and although these were increased correlations with CP-690550 exposure were not obvious. Brown adipose tissue is present during fetal development but is generally lost soon after birth, so this is not a major concern for therapeutic use, although there may be some as yet unidentified mechanism that is applicable to other human adult tissue.

Tofacitinib was not carcinogenic in rasH2 transgenic mice with exposures 32-times the maximal recommended human dose of 10 mg BID, the highest administered dose. The non-neoplastic findings in this study did fit with the known pharmacology of tofacitinib in that femoral bone marrow was affected in which focal subphyseal hypocellularity was characterized by an increased prominence of adipocytes in the mid and high dose males and high dose females, and that there was cellular depletion in the red pulp

regions of the spleen. The transgenic rasH2 mouse model was developed to reveal the direct carcinogenic potential of compounds during a shorter (6-month) period of study than the classical 2 year bioassay. This may be insufficient to determine carcinogenicity due to immunosuppression, the demonstrated mechanism of action of CP-690550, particularly in animals with standard and necessary vaccinations that stimulate the immune system prior to being distributed for studies. It's also notable that in the 2-year (lifetime) rat carcinogenicity study, lymphomas did not develop.

Overall, results from both the genetic toxicology and carcinogenic studies indicate a low risk of direct drug-induced carcinogenicity for patients, due to the large exposure margins relative to the therapeutic dose. However, this cannot be applied for immunosuppression-associated malignancies, since they occurred in the monkey toxicology study and in clinical trials.

Reproductive and Developmental Toxicology

Fertility in female rats was reduced with tofacitinib administration (Report 05GR051). An increase in postimplantation loss occurred with at exposure 14-times the MRHD of 10 mg BID, and a reduction in pregnancy rate due to reduced numbers of corpora lutea, implantation sites, early resorptions and pre- and post-implantation loss occurred at exposure 125-times MRHD. The NOAEL for postimplantation loss was 1 mg/kg/day producing an exposure margin of 1.3-times the maximal recommended human dose of 10 mg BID. Since in the rat prolactin is necessary for maintaining the corpora luteum which in turn is necessary to maintain pregnancy, the reduction in pregnancy is a predictable outcome of tofacitinib's inhibition of prolactin stimulated JAK signaling pathway. Prolactin does not maintain the corpus luteum in humans, but is relegated to a supportive role for many reproductive-related events.

The fertility assessment for males was atypical (Report 05GR051). In this study, males were administered tofacitinib for at least 63 days, but matings occurred after 1 month of treatment, with no effect on pregnancy assessment in the untreated female partners. The duration of treatment at the time of mating was insufficient for drug exposure for a complete spermatogenic cycle. However after 63 days of treatment, there was no effect on sperm motility or concentrations, but sperm morphology was not assessed. Another study in juvenile male rats (Report 05GR051) that were dosed from day 21 of age through expected puberty, had no effect on subsequent pregnancy in untreated female partners when mating occurred a few weeks after tofacitinib treatments ended. However, the fertility assessment for males occurred after 1 month of treatment, an insufficient duration of drug exposure for a complete spermatogenic cycle and therefore does not meet regulatory requirements for safety of male fertility. Therefore, at least the study in adult males should be repeated and conducted in accordance with ICH5(R2), refer to Note 12 in Section 4.1.1. The maximal dose resulted in an exposure margin 78-times the MRHD. In studies with cynomolgus monkeys (Report 2003-0301) there were too few animals that were sexually mature to assess effects on mature testis and epididymis, and although spermatogenic staging was not evaluated, there were no

obvious histopathological changes in sexual organs in males attributed to tofacitinib treatment.

In a fertility study in juvenile rats (Report 05GR051) tofacitinib was administered at the same doses as in the study with sexually mature animals, to females from days 21 to 55 of age and to males from days 21 to 70 days of age, i.e. up to expected puberty and through expected puberty, respectively. There was no effect in females on indicators of sexual development and subsequent estrous cyclicity. However, there was a similar reduction in fertility at doses similar to those in the study of sexually mature females. For males, there was no effect on mating, sperm motility or sperm concentrations. However, the mating assessment for males and females occurred weeks after CP-690550 treatment had ended, allowing for recovery of any potentially adverse effects on fertility. Therefore, this study does not meet regulatory requirements for safety of either male or female fertility evidenced by mating studies. The aspects of the study characterizing estrous cyclicity and spermatozoa are acceptable. Since this study was optional, although useful to provide safety for the treatment of a pediatric population, the mating aspects of this study should be repeated and conducted in accordance with ICH5(R2), refer to Note 12 in Section 4.1.1.

Toxicokinetic parameters were not determined in the study of juvenile rats since a single values of serum concentrations was obtained at 0.5 h after dosing on day 12 in females and day 16 in males. The exposure at the end of the study when animals are now sexually mature, would be expected to be similar to the study with sexually mature rats (Report 02-2063-20) so exposure margins were based on AUC of the 6 month duration general toxicology study in Sprague Dawley rat (Report 02-2063-20).

Embryo-fetal development studies were conducted in rats and rabbits with tofacitinib doses administered during the period of organogenesis. In rats orally administered 30, 100, or 300 mg/kg/day, maternal toxicity was observed at doses ≥ 100 mg/kg/day and was associated with postimplantation loss, consisting of early and late resorptions and consequently a reduced number of viable fetuses, and decreased uterine weight. Teratogenic effects were observed at 100 mg/kg/day consisting of anasarca and membranous ventricular septal defect and numerous skeletal malformations. The NOAEL for maternal and developmental toxicity in this study was 30 mg/kg/day, corresponding to an exposure margin of 53-times MRHD based on a systemic exposure AUC comparison. In rabbits, doses of ≥ 30 mg/kg/day produced no evidence of maternal toxicity, but resulted in numerous teratologic findings including thoracogastroschisis, omphalocele, microstomia, microphthalmia, membranous ventricular septal defects, absent gallbladder, and multiple skeletal malformations associated with a minimal exposure margin of 14-times MRHD. The NOAEL margin for the rabbit was 2.7-times MRHD

Embryo-fetal development studies rats (Report 09GR353) and rabbits (Report 05-2063-25) indicate that there is a risk for early embryo loss, and, if pregnancy is maintained, a risk for life-threatening embryo-fetal malformations. The rabbit was the more sensitive species to tofacitinib since the NOAEL dose corresponded to an exposure margin of

only 2.7-fold greater than the MRHD. Since this exposure difference is relatively small and the sensitivity of human fetal development for tofacitinib is unknown, it was recommended to avoid use of tofacitinib during pregnancy.

A postnatal study in rats (Report 08GR095) found no effect on pup delivery, standard landmarks of development, growth, sexual, or behavioral development at doses up to 50 mg/kg/day administered to dams from late gestation through lactation (up to 21 days after delivery resulting in a NOAEL of 76-times the maximal recommended human dose of 10 mg BID. In a study with lactating rats, a single oral dose of tofacitinib increased tofacitinib in secreted milk approximately 2-fold greater than serum concentrations, indicating that tofacitinib can be concentrated in actively secreting mammary glands.

Special Toxicology Studies

Studies demonstrated that tofacitinib was compatible with whole human blood and plasma, in separate assays (Report B65935). Tofacitinib was acutely irritating when applied to the rabbit eye (Report B65946). Tofacitinib was not a skin sensitizer in the local lymph node assay (Report 07GR202) a measure of its ability to induce hypersensitivity reactions.

Tofacitinib lacked evidence of phototoxic potential when tested in the *in vitro* neutral red uptake assay (Report 07AM087) and in a 7-day *in vivo* assessment in rats (Report 10GR350). However, these were not an adequate assessment of phototoxicity due to the insufficient intensity of radiation in the simulated solar sunlight at the wavelengths that are absorbed by tofacitinib, those around 290 nm. Therefore the concern of phototoxicity has not been adequately addressed if exposed to sunlight. This wavelength of tofacitinib absorption is at the lower limit of the UVB spectrum and at this and lower wavelengths the penetration through skin layers is weak and the wavelength is unlikely to penetrate into the dividing basal cell layer. Therefore, after consultation with the associate pharmacology/toxicology director Dr. Brown and the clinical reviewer, Dr. Nikolov, the absence of any signal for serious adverse skin effects such as melanoma in the safety database, together with physical properties of this end of the UVB spectrum provided sufficient evidence to conclude that tofacitinib is not phototoxic.

Table 128: Table of Exposure Margins

Adverse Finding	Dose and (AUC) at NOAEL	Human Exposure Margin ¹	
		5 mg BID	10 mg BID
Fertility, adult, rat² female mating, pregnancy rate, postimplantation loss male mating female estrous cycle male sperm motility and counts	1 mg/kg/day (742 ng-h/mL) nd 100 mg/kg/day (~68000) 100 mg/kg/day (~43000)	2.6X nd 250X 156X	1.3X nd 125X 78X
Embryo-Fetal Development Rat teratogenic findings	30 mg/kg/day (29400 ng-h/mL)	106X	53X
Rabbit teratogenic findings	10 mg/kg/day (1470 ng-h/mL)	5.4X	2.7X
F₁ Postnatal Development	50 mg/kg/day (~42000 ng-h/mL ³)	152X	76X
Carcinogenicity			
monkey: lymphomas in a 9-month repeated dose study	2 mg/kg/day (524 ng-h/mL)	~2X	~1X
rat: Leydig cell tumors Benign thymomas in females Hibernomas in females	10 mg/kg/day 3880 ng-h/mL 10 mg/kg/day 30 mg/kg/day	14X ⁴	7X ⁴
rasH2 transgenic mouse: no malignancies	200 mg/kg/day (17250 ng-h/mL)	64X	32X
¹ The human AUC value for calculation of exposure ratio (animal AUC/human AUC) was obtained from Clinical Report A3921005. A single 10 mg tablet produced a AUC ₀₋₂₄ of 276 ng-h/mL, this was doubled, ~550 ng-h/mL, for a proposed maximal recommended dose of 10 mg CP-690550 BID. For a human recommended dose of 5 mg CP-690550 BID, the ~550 ng-h/mL value for 10 mg BID, was halved to 275 ng-h/mL. ² AUC for rat was obtained from the end of the 6-month repeated dosing toxicology study ³ Value from interpolated from the rat embryo-fetal developmental study (Report 09GR353) ⁴ For carcinogenicity, the lowest exposure is provided at which no findings were observed in the rat study of either sex nd, not determined, the study was not valid			

12 Appendix/Attachments

The following is the review of the Pharmacology Section of the NDA submission by Dr. Pei.

**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:	203,214
Supporting document/s:	Sequences 0000
Applicant's letter date:	October 21, 2011
CDER stamp date:	October 21, 2011
Product:	Tofacitinib (CP-690,550)
Indication:	Rheumatoid arthritis
Applicant:	Pfizer
Review Division:	Pulmonary, Allergy and Rheumatology Products
Reviewer:	Luqi Pei, Ph.D.
Team Leader:	Timothy Robison, Ph.D.
Division Director:	Badrul Chowdhury, M.D., Ph.D.,
Project Manager:	Philantha Bowen

Template Version: September 1, 2010

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25 Pages Have Been Withheld As A Duplicate Copy Of The Upcoming Review Dated May 14, 2012

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

/s/

LAWRENCE S LESHIN
07/03/2012

MOLLY E SHEA
07/03/2012
I concur.

**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: 203,214
Supporting document/s: Sequences 0000
Applicant's letter date: October 21, 2011
CDER stamp date: October 21, 2011
Product: Tofacitinib (CP-690,550)
Indication: Rheumatoid arthritis
Applicant: Pfizer
Review Division: Pulmonary, Allergy and Rheumatology Products
Reviewer: Luqi Pei, Ph.D.
Team Leader: Timothy Robison, Ph.D.
Division Director: Badrul Chowdhury, M.D., Ph.D.,
Project Manager: Philantha Bowen

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1 Executive Summary

1.1 Introduction

Pfizer is applying for the registration of Tofacitinib (CP-690,550, NDA 203214) indicated for the rheumatoid arthritis. Rheumatoid arthritis is a chronic, systemic autoimmune disease which typically shows inflammation of joints. Tofacitinib possesses anti-inflammatory properties because it disrupts intracellular signaling transduction of cytokines by inhibiting Janus associated kinases (JAK).

JAK kinases are a family of intracellular non-receptor protein tyrosine kinases. The JAK family consists of four members: JAK1, JAK2, JAK3, and TyK2. Binding of cytokines to their receptors results in dimerizations and activations of JAKs. Activated JAK dimers phosphorylate signal transducer and activation of transcription (STAT) which leads to increases in the production and release of pro-inflammatory cytokines.

This document reviews the pharmacological studies of the NDA submission. See nonclinical original review of NDA 203,214 by Dr. Steve Leshin for other information.

1.2 Brief Discussion of Nonclinical Findings

Tofacitinib inhibited cytokine-induced production and release of cytokines and other inflammatory mediators in vitro. Oral and parental administrations of the drug attenuated arthritic disease in rodent models in vivo. The drug also decreased some cytokine concentrations in the plasma and paw tissues in animal models of disease. Tofacitinib also inhibited cytokines-induced STAT phosphorylation in vitro.

1.3 Recommendations

1.3.1 Approvability

See the nonclinical original review of NDA 203,214 by Dr. Steve Leshin for recommendation regarding approvability.

1.3.2 Additional Non-Clinical Recommendations

None

1.3.3 Labeling

[REDACTED] (b) (4)

[REDACTED]

[REDACTED]

(b) (4)

2 Drug Information

2.1 Generic Name

Tofacitinib

2.2 Code Name

CP-690,550 (free base), CP-690,550-10 (citrate salt)

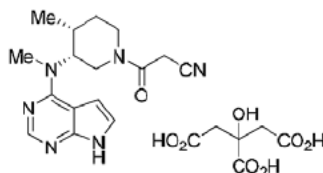
2.3 Chemical Name

3-(((3*R*,4*R*)-4-methyl-3-(methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino) piperidin-1-yl)-3-oxopropanenitrile, 2-hydroxypropane-1,2,3-tricarboxylic acid

2.4 Molecular Formula/Molecular Weight

C₁₆H₂₀N₆O • C₆H₈O₇ (citrate salt) / 504.5 Daltons

2.5 Structure



3 Studies Submitted

3.1 Studies Reviewed

Study No.	Description ^a
	Primary Pharmacology
	<u>In vitro studies</u>
D08A10333	Determination CP-690,550 IC50s for JAK1, 2, 3 and TYK2
D08A10334	Determination CP-690,550 Kis for JAK1, 2, 3 and tyk2
D08A10337	Selectivity of CP-690,550 on JAK1/3 and JAK2/TYK2 in HPBC
D08A10338	Effect of CP-690,550 on STAT phosphorylation in vitro
083053	IC50s of CP-690,550 in kinases
153609	EC50s of CP-690,550 in JAK activity in vivo
165255	Effects on paw arthritis scores and drug concentration after repeat dose (7 days) of CP-690,550 treatment in mice
141423	Effects on paw arthritis scores and drug concentration after single dose of CP-690,550 treatment in mice
150736	The role of JAK1/3 in the efficacy of CP-690,550 in arthritis in mice and humans – PD-PK modeling

	<u>In Vivo Studies in Mice</u>
151443	Prevention of CIA-induced arthritis after oral and SC
141423	Effects on plasma concentrations of cytokine panels at single oral dose treatment
160243	Reduction of tissue inflammation after 7 day treatment
165255	Reduction in paw arthritis severity scores after 7 –day treatment
135046	Reduction in STAT1 responsive gene after 4 and 7 day treatment
150736	PK/PD modeling
	<u>In vivo studies in rats:</u>
100214	Inhibited paw swelling after 10-day treatment (DE50 = 0.06 mg/kg)
102743	Inhibited paw swelling after 7-day treatment (DE50 = 0.02 mg/kg, bid)
141740	Inhibited paw edema at 6.2 mg/kg/day for 4 & 7-day treatment
160531	Inhibited paw inflammation at 6.2 mg/kg/day for 7-day treatment
112613	Inhibited IL-6 mRNA and STAT 1responsive genes at 6.2 mg/kg, qd
	Secondary Pharmacology
NP-02-005	Effects on K ⁺ current in hERG channels in HEK-923 cells
757032	Effects on K ⁺ current in hERG channels in HEK-923 cells
7571347	Effect on action potential in dog purkinje fibers
	Safety Pharmacology
745-03432	Effects on cardiovascular system in monkeys
11GP001	Effects on cardiovascular system in rats
	Effects on CNS and renal systems in mice and rats

a. Each of the study reports was located in Section 4.2.1.1 of the electronic submission.

3.2 Studies Not Reviewed

The following two studies were not reviewed because they do not provide any additional information in the nonclinical safety evaluations of CP-690,550.

Study No.	Description
120754	Effects of CP-690,550 on reverse cholesterol transport in rats
150736	Dose-response modeling in CIA in mice
155854	Reduction of peritoneal macrophage concentration after 4 ad 7 mg/kg/day treatment at 3 and 10 mg/day
NO-02-005	Effect on PO-induced reticulate numbers in monkeys
112613	Gene expression profile in AIA rats

4 Pharmacology

Nonclinical studies were completed to evaluate the primary, secondary and safety pharmacology of CP-690,550. Primary pharmacodynamics of CP-690,550 was studied in vitro and vivo. The in vitro assays assessed potency and selectivity CP-690,550 in recombinant and cellular JAKs by determining IC₅₀s under the testing conditions. The in vivo studies determine efficacy of CP-690,550 in arthritic models in mice and rats. Secondary pharmacodynamic studies evaluated the binding and potency of CP-690,550 on a broad panel of receptors, ion channels, and enzymes. Safety pharmacology studies assessed the potential effects on cardiovascular, respiratory, and CNS systems rats, mice, and monkeys.

4.1 Primary Pharmacodynamics

A number of in vitro and in vivo studies were completed to evaluate the primary pharmacodynamics of CP-690,550. The in vitro studies evaluated the potency and selectivity of the drug in inhibiting the activities of individual recombinant JAK members and JAK dimers. The in vivo studies evaluated the efficacy of the drug in arthritis models in rats and mice.

Pharmacological Activity In vitro:

In vitro pharmacology studies were completed to study the selectivity of CP-690,550 on members of recombinant human JAK family, the JAK functional units, and other kinases. The inhibitory effects (IC₅₀s and K_s) of CP-690,550 on individual JAK family members were determined using recombinant JAK1, JAK2, and JAK 3 and TyK2 enzymes; human peripheral blood cells or whole blood. Table 1 provides an overview of key in vitro pharmacology studies.

Table 1: Overview of CP-690,550 Pharmacology in Vitro

Study No.	Description	Results
D08A10333	Determination of IC ₅₀ s for recombinant JAKs	IC ₅₀ s = 3.2, 4.1, 1.6 and 34 nM for JAKs 1, 2, 3 and TyK2, respectively
D08A10334	Determination K _s for recombinant JAKs	K _s = 0.68, 0.97, 0.24 and 4.4 nM for JAKs 1, 2, 3 and TyK2, respectively
D08A10337	Selectivity on JAK1/3 and JAK2/TyK2 from HPBC and HWB	IC ₅₀ s = 26-34 and 129 -501nM for JAK1/3 and JAK2/TyK2, respectively
D08A10338	Inhibition of STAT phosphorylation in HWB	IC ₅₀ s = 56, 406 and 1377 for JAK1/3, JAK1/2, and JAK2, respectively
113015	Effects on STAT phosphorylation in HWB	IC ₅₀ s for 54–406, 44-206 and 1377 nM for JAK1/3, 1/2, 1/TyK2 in various cells
083053	Selectivity for recombinant JAK3 and other kinases	IC ₅₀ s = 0.004 and > 1 μM for JAK3 and others
142305	Effect of HWB on JAK IC ₅₀ s in T225 cells	W/O WHB: IC ₅₀ s = 0.006 and 0.04 μM for JAK1/3 and JAK2/TyK2, respectively W/ WHB: IC ₅₀ s = 0.02 and 0.10 μM for JAK1/3 and JAK2/TyK2, respectively
153609	EC ₅₀ s in MHB	EC ₅₀ s = 273, 470 and 6656 nM for JAK1/3, JAK1/2, and GM-CSF, respectively

A, HPBC = human peripheral blood cells, HWB = human whole blood, MHB = mouse whole blood.

Selectivity of CP-690,550 on Recombinant JAK enzymes

CP-690,550 inhibited the activity of recombinant human JAK enzymes, but the IC₅₀s and K_s of CP-690,550 differed among JAK members. Currently, the JAK family is known to consist of 4 members: JAK1, JAK2, JAK3, and TyK2. The IC₅₀s and K_s of CP-690,550 were determined in a peptide mobility shift assay in vitro. Recombinant human JAK enzymes (i.e., JAK1, JAK2, JAK3, and TyK2) and their substrate peptides were incubated in media containing CP-690,550 (0.01 – 1000 nM).¹ The enzyme activity was determined by

¹ Reactions were carried out in a 384-well plate (Greiner) in 10-μL total volume. Reaction media contained 20mM HEPES, pH 7.4, 10mM magnesium chloride, 0.01% bovine serum albumin (BSA), 0.0005% Tween-20, ATP (see later), 2% dimethyl sulfoxide (DMSO) and 1μM peptide substrate.

the formation of phosphorylated substrate peptides. CP-690,550 caused concentration-dependent inhibition of JAK activities.

Table 2: IC₅₀s and K_is of CP-690,550 for JAKs

Enzyme	CP-690,550 (nM), n = 2 - 4	
	IC ₅₀ ± SEM	K _i ± SEM
JAK 1	3.2 ± 1.4	0.68 ± 0.12
JAK 2	4.1 ± 0.4	0.97 ± 0.03
JAK 3	1.6 ± 0.2	0.24 ± 0.03
TyK 2	34 ± 6	4.4 ± 0.3

Study D08AI0333 determined the IC₅₀s of CP-690,550 in recombinant JAK enzymes. CP-690,550 exhibited concentration-dependent inhibition of JAK activity for each member. Figure 1 provides examples of concentration-response relationship. See Table 2 (above) for the individual IC₅₀s of each JAK.

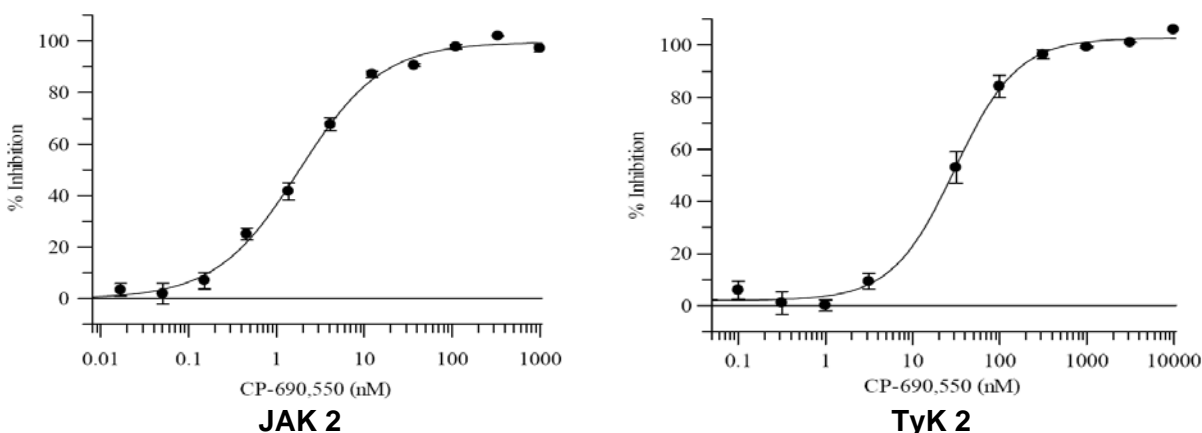


Figure 1: Concentration-response relationship of CP-690,550 concentrations and JAK activities

Study D08AI0334 determined the competitive inhibition constant (K_s) of CP-690,550 for individual JAK enzymes.² Recombinant human JAK enzymes and their substrates were incubated in media containing CP-690,550 and ATP. The enzyme activity was determined again by the formation of phosphorylated substrate peptides. Figure 2 shows the response relationship between concentrations of ATP and CP-690,550 in JAK1 activity. Other JAK enzymes behaved in similar manners. The K_s (Table 2) were similar (0.24 – 0.97 nM) for JAKs 1 - 3 while the K_i for TyK 2 (4 nM) was greater.

Peptide substrates were JAKtide for JAK2 and JAK3 and IRS-1 was for JAK1 and TyK 2, respectively. The other respective parameters for JAK 1, JAK2, JAK3, and Tyk2 were 20, 2, 1, and 7 nM in enzyme concentrations; 40, 4, 4, and 7 μM in ATP concentrations; and 240, 150, 90 and 60 minutes in the time of incubation. The assays were stopped at the specified times with 20 μL of 140 mM HEPES, 22.5 mM EDTA and 0.15% Coating Reagent 3. The report did not specify the temperate of the media.

² Reactions were carried out in a 384-well plate (Greiner) in 80-μL total volume. See Footnote 1 for the composition of the reaction media and peptide substrates except for the following: MgATP concentrations (8.2 – 2000 μM), CP-690,550 concentrations (0 – 6 nM) and enzyme concentrations (2 nM for JAK2, JAK3 and TyK2 and 30 nM for JAK 1, respectively). The concentrations of phosphorylated peptides were measured every 5 minutes for a 4-hr period. K_i was calculated using available software.

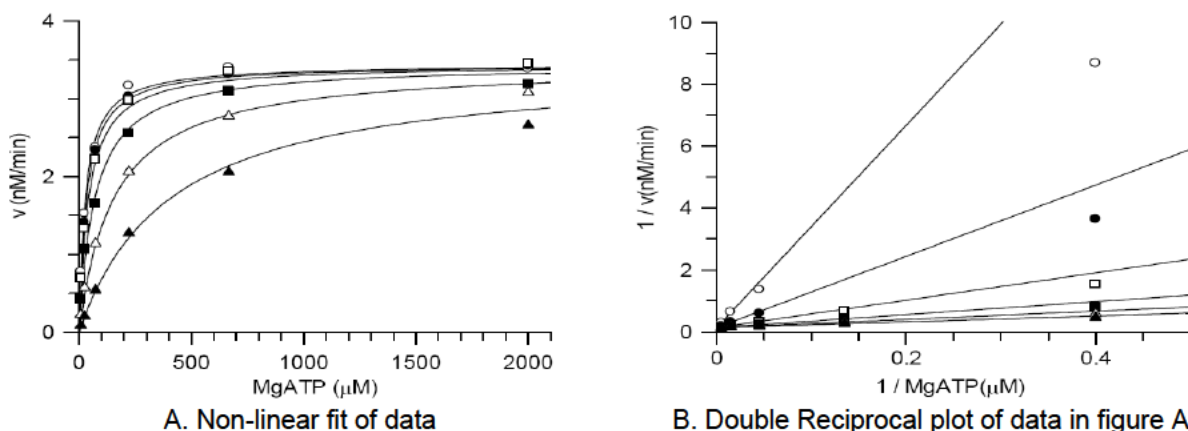


Figure 2: Competitive inhibition of JAK enzyme activity by CP-690,550. The concentrations of CP-690550 were 0 (○), 0.074 (●), 0.22 (□), 0.67 (■), 2 (Δ), and 6 nM (◻).

Effects of CP-690,550 on Cytokine Signaling in Cells in Vitro

JAK enzymes transmit cytokine signaling via heterodimers [e.g., JAK1/3, JAK1/2, etc]. Involvement of particular dimers is cytokine and cell-type dependent. Activation of JAK dimers resulted in STAT phosphorylation. The involvement of particular STAT was also cytokine and cell-type dependent. The involvement of JAK dimers in cytokine signaling were determined by measurements of cytokine-induced IFN γ production and STAT phosphorylation in whole blood and in peripheral blood mononuclear cells.

Study D08AI0337 evaluated the effect of pretreatment with CP-690,550 on IFN γ production induced by IL-2 and IL-12 in human whole blood (HWB) and human blood mononuclear cells (HBMC) in vitro.³ Results showed that CP-690,550 inhibited IFN γ production in both HWB and HBMC (Figure 3). The respective IC₅₀s for IL-2 (JAK1/3) and IL-12 (JAK2/TyK2)-induced IFN γ production was 26 ± 2 and 129 ± 36 in HBMC and 34 ± 6 nM and 501 ± 197 nM in HWB. The report states that the results show that CP-690,550 exhibits moderate selectivity for JAK1/3 over JAK2/TYK2 dependent signaling.

³ HWB or HBMC was incubated with CP-690,550 for one hour (at 37°C and 5% CO₂) followed by an 18-hour stimulation period. The stimulation was done by a combination of 1 μ g/mL mouse anti-human CD3 (b) (4), 0.01 μ g/mL mouse anti-human CD28 (b) (4) and 10 ng/mL IL-2 (b) (4) for JAK1/3 driven IFN γ ; and 1 μ g/mL mouse anti-human CD3 (b) (4), 0.01 μ g/mL mouse antihuman CD28 (b) (4) and 10 ng/mL IL-12 (b) (4) for JAK2/TYK2 driven IFN γ . After stimulation, inhibition of IFN γ production was assessed by (b) (4) technology.

JAK3 is associated with the common γ -chain of the receptors IL-2. Both JAK3 and JAK1 activity are necessary for this receptor's signaling. JAK2 and TyK2 play critical roles in the signaling of a number of cytokine receptors including IL-12.

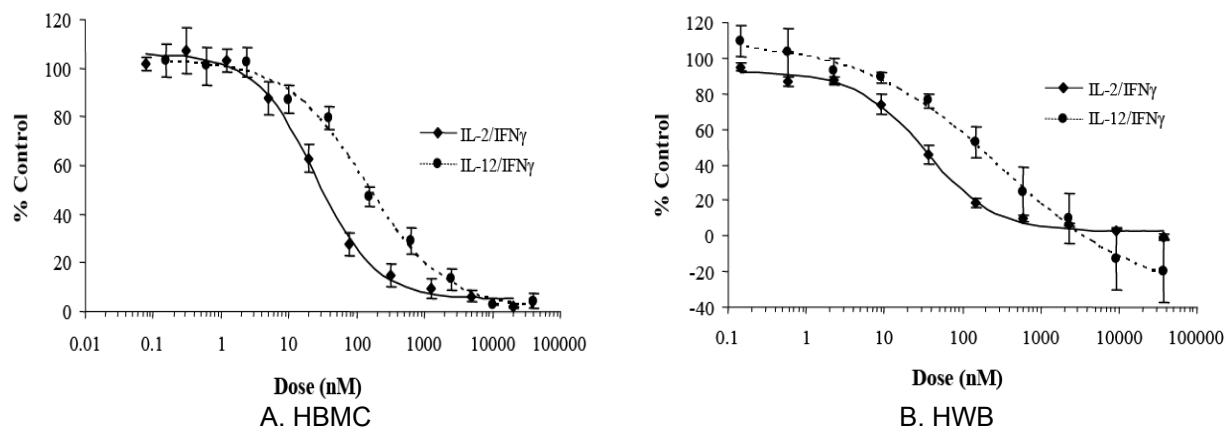


Figure 3: Inhibition of IL-2- and IL-12-induced IFN γ productions by CP-690,500: concentration-response relationship.

Study D08AI0338 determined the CP-690,550 IC₅₀s in inhibiting IL-6, IL-15, or GM-CSF-induced STAT phosphorylation from human whole blood. The respective parameters in IL-6, IL-15, and GM-CSF groups were activities of JAK1/2, JAK1/3, and JAK2/2 JAK dimers. Activation of these dimers would result in phosphorylation of the down stream signaling: STAT3, STAT5 and STAT1. Results showed that IC₅₀s of 406 ± 68 , 56 ± 6 and 1377 ± 185 nM was for JAK1/2, JAK1/3, and JAK2/2 activities, respectively.

Study 113015 determined the CP-690,550 IC₅₀s in inhibiting a broader panel of cytokines from human whole blood. These cytokines included IL-2, IL-4, IL-7, IL-10, IL-15, IL-21, and IFN α . Table 3 (next page) lists cytokines, corresponding JAK dimers, the substrates (STAT) for the dimer, and IC₅₀s of CP-690,550 for inhibiting the dimer activity. The table also includes data from other studies (i.e., D08AI0337 and D08AI0338) for easy reference. Overall, the IC₅₀s ranged 25 – 111 nM, 54 – 406 nM, 44 – 206, and 1377 nM for combinations of JAK1/3, JAK1/2, and JAK1/TyK2, and JAK2/2, respectively. The overlap of IC₅₀s among the various dimers suggests little meaningful selectivity of CP-690,550 among the JAK subfamily members. This observation may have impact on the labeling review of the proposed Section 12.1 – Mechanism of Action. See the Labeling Review section for additional information.

CP-690-550 selectively inhibited JAK enzymes. Report 083053 determined the IC₅₀s of CP-690,550 on a panel of 79 kinases in vitro. JAK3 was used as the representative of the JAK family. Among them, 68 had IC₅₀s ≤ 10 μ M; four had IC₅₀s between 5 – 10 μ M; six had IC₅₀s between 1 – 5 μ M, while the IC₅₀ for JAK3 was significantly lower (0.004 μ M).⁴ The results indicate that CP-690,550 was a selective JAK inhibitor.

⁴ The kinases with IC₅₀s between 1 – 5 μ M were FYN (Proto-oncogen tyrosine kinase encoded by the FYN gene, 4.04 μ M), LCK (lymphocyte-specific protein tyrosine kinase, 3.71 μ M), MARK (serine/threonine protein kinase encoded by the MARK1 gene, 1.05 μ M), p160 ROCK (p160 Rho-dependent protein kinase, 1.07 μ M), PKA (Protein kinase A – nonspecific, 2.43 μ M), and PKC beta II (Protein kinase C-beta II, 4.71 μ M). The kinases with IC₅₀s between 5 – 10 μ M were ARK1 (aurora-related kinase 1), FGFR1 (fibroblast growth factor receptor 1), FLT3 (Fms-like tyrosine kinase receptor 3), and MAP3K9 (mitogen-activated protein kinase kinase kinase 9).

Table 3: IC₅₀s of CP-690,550 on JAK Dimers Involved in Cytokine Signaling

Dimer	Cytokine	Cell ^c	STAT ^a	IC ₅₀ (nM)	Report #
JAK1/3	IL-2	CD3+ T	5	28	113015
	IL-4	CD3+ T	6	50	113015
	IL-4	CD20+ B	6	111	113015
	IL-7	CD3+ T	3	38	113015
	IL-15	CD3+ T	5	30	113015
	IL-15	CD8+ T	5	56	D08AI0338
	IL-21	CD3+ T	3	25	113015
JAK1/2	IL-6	CD3+ T	1	54	113015
	IL-6	CD3+ T	3	367	113015
	IL-6	CD14+ M	1	406	D08AI0338
JAK1/TyK2	IFN γ	CD14+ M	1	178	113015
	IFN α	CD14+ M	1	148	113015
	IL-10	CD14+ M	3	206	113015
	IL-10	CD3+ T	3	141	113015
	IF α	CD3+ T	1	44	113015
JAK2/2	GM-CSF	CD14+ M	3	1377	D08AI0338
JAK2/TyK2	IL-12	PBMC	?	129	D08AI0337
	IL-12	WHB	?	501	D08AI0337

a. STAT (Signal transducer and activator of transcription) phosphorylated by JAK kinases when the enzyme is activated.

b. B, T, and M represent B and T lymphocytes and monocytes, respectively.

Study 153,609 determined the EC₅₀ values of plasma CP-690,550 concentration in inhibiting blood cell JAK activity in mouse. Male BALb1/J mice (4 – 5/dose) were given by oral gavage 0 – 50 mg/kg of CP-690,550. Blood JAK activities and plasma CP-690,550 levels were determined 1 hour after dosing. Results showed that JAK activities (induced by IL-6, IL-15, and GM-CSF) decreased when plasma CP-690,550 concentrations increased (Figure 4). The EC₅₀s for inhibiting JAK activities were 273 \pm 47, 470 \pm 85, and 6656 \pm 1243 nM for JAK1/3 (IL-15-induced), JAK1/2 (IL-6-induced) and JAK2 (GM-CSF-induced) activities, respectively.

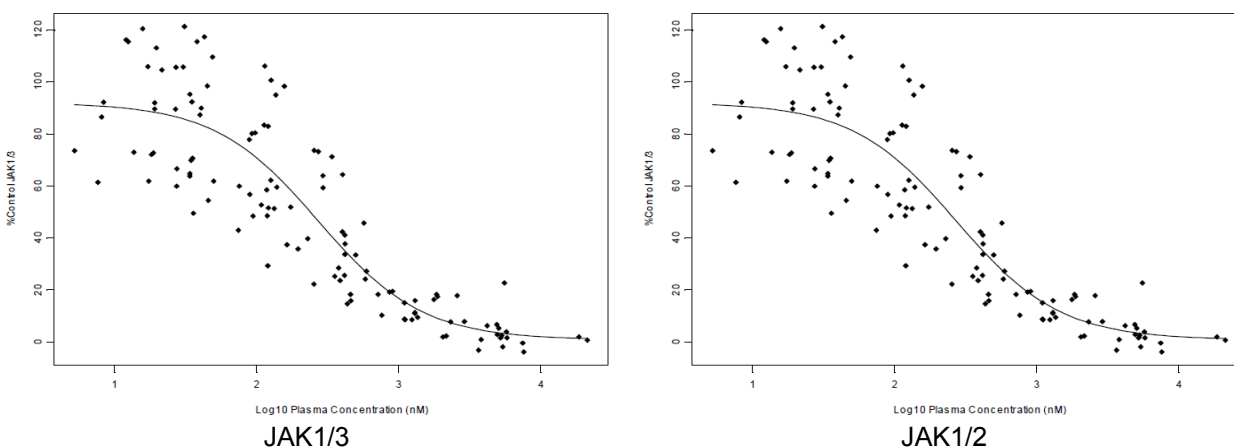


Figure 4: Plasma JAK activities as a function of CP-690,550 concentration in mice receiving oral CP-690,550 treatment

Pharmacological Activity in Vivo

Efficacy of CP-690,550 on arthritis was studied in animal models in vivo. Two animal models were used in the pharmacology program. They were a collagen-induced arthritis model in male DBA/1J mice (CIA model) and *Mycobacterium byturium*-induced arthritis model in female Lewis rats (AIA model). CP-690,550 attenuated the arthritis severity in both models in a dose-dependent manner.

Mice

Efficacy of CP-690,550 in treating arthritis was studied in the mouse CIA model. Arthritis in mice was induced by injection of two doses (named initial and challenging doses, respectively) of 50- μ g chick type-II collagen in complete Freund's adjuvant (CII) into the base of the tail. The challenge dose was given 20 days after the initial dose. The mice showed a full blown arthritis approximately 25 days after the challenge dose (45 days after the initial dose). The severity of the arthritis was assessed by measuring paw swelling, redness, deformity, and ankylosis on day 45. The mice were given single or repeat-dose (up to 7 days) of vehicle or CP-690,550 starting from day 45. The severity of paw arthritis was scored at various time points during or after the CP-690,550 treatment. Results showed that CP-690,550 prevented or attenuated arthritis and reduced plasma levels of some cytokines in a dose-dependent manner.

Report 141423 assessed the effect of single-dose CP-690,550 treatment on paw arthritis severity and cytokine levels in plasma and tissue paw. CP-690,550 doses were 0 (vehicle), 10, or 50 mg/kg/day.⁵ Arthritis severity and cytokine levels were determined for up to 48 hours post dosing. Table 4 summarizes the plasma CP-690,550 concentration and arthritis scores of the study. Plasma CP-690,550 concentrations rose dose-proportionally, but the CP-690,550 treatment had no effects on the arthritis scores.

Table 4: Plasma CP-690,550 Concentrations and Severity Scores after Single-Dose Treatment in Mice (Report 141423)

Treatment	Dose (mg/kg)	PK		Hours post-dose	Terminal Severity Score		n
		μ M	SEM		Mean	SEM	
Vehicle	-	-	-	0	4.14	0.32	7
		-	-	4	4.57	0.69	7
		-	-	12	4.71	0.61	7
		-	-	24	4.71	0.42	7
		-	-	48	4.57	0.57	7
CP-690,550	10	-	-	0	4.15	0.33	7
		0.1236	0.0143	4	4.00	0.93	6
		0.0038	0.0005	12	5.43	0.72	7
		0.0013	0.0013	24	4.00	0.69	7
		BLQ	BLQ	48	4.57	0.90	7
	50	-	-	0	4.25	0.34	7
		0.9133	0.1228	4	4.43	0.72	7
		0.0633	0.0373	12	4.71	0.78	7
		0.0003	0.0002	24	4.86	0.63	7
		BLQ	BLQ	48	5.57	0.43	7

⁵ The vehicle contains 0.5% methylcellulose and 0.025% Tween 20%.

Although CP-690,550 did not decrease the arthritis scores, it caused statistically significant reductions in some cytokine levels 4 – 12 hours after the dosing. The level of each cytokines returned to the pretreatment levels by 24 hours after dosing. Table 5 presents the plasma cytokine concentrations 4 hours post dosing. The 50 mg/kg group showed statistically significant reductions in plasma levels of IL-6, IP-10, KC, MCP-5 and MIG 4 hours post dosing; There were no statistically significant reductions in G-CSF, MCP-1, MIP-1 α , MIP-1 β levels, acute phase proteins SAA, SAP or PTX-3 levels. Only the reductions in the IP-10, MCP- 5 and MIG levels remained significant at 12 hours. All cytokine levels returned to the baseline 24 hours after dosing. The 10 mg/kg group showed statistically significant reductions in plasma cytokines IL-6, KC, and MCP-5 only at 4 hours post dosing.

Table 5: Plasma Cytokine Concentrations after Single Dose of CP-690,550 treatment

Time (h)	Group	pg/mL									
		G-CSF	IL-6	IP-10	KC	MCP-1	MCP-5	MIG	MIP-1 α	MIP-1 β	
4	Vehicle	1933.5	131.9	285.1	1345.0	142.5	443.3	510.4	51.1	41.1	Mean
		509.9	14.1	50.8	228.1	38.9	64.9	100.6	5.6	8.4	SEM
	10 mg/kg	1156.3	61.6	239.1	763.2	89.1	140.9	479.0	71.5	42.6	Mean
		317.1	14.7	19.7	210.3	16.1	38.0	65.5	6.9	2.8	SEM
		0.1202	0.0027	0.2429	0.0456	0.1292	0.0014	0.4030	0.0203	0.4387	p
	50 mg/kg	1073.4	25.7	184.2	449.3	96.4	156.8	299.0	68.1	34.7	Mean
		293.6	13.6	11.4	90.7	18.5	18.8	35.3	16.1	4.2	SEM
		0.0848	0.0001	0.0383	0.0017	0.1526	0.0006	0.0354	0.1690	0.2526	p

Report 165255 assessed the effect of CP-690,550 on plasma and tissue (paw) levels of cytokines and chemokines during a 7-day treatment period in CIA model. Arthritic mice were given 0 (vehicle) or 50 mg/kg/day CP-690,550 (bid) orally for 7 days. Arthritic scores of the paw and levels of a panel of cytokines and chemokines in the plasma and paw tissue were measured. The report contained results from 2 studies. Figure 5 shows that the CP-690,550 treatment resulted in lower arthritic scores in the paw.

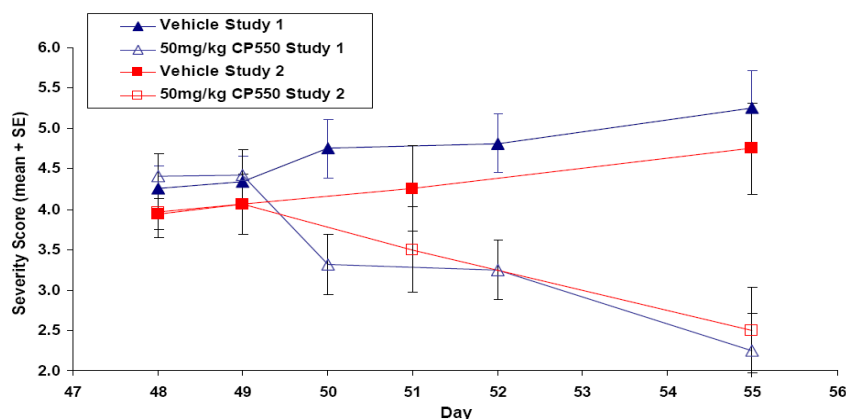


Figure 5: Time course of arthritis severity score with and without 7-day CP-690,550 treatment in mice. The graph shows two independent and identical studies (Studies 1 and 2). CP-690,550 treatment started on Day 48.

The CP-690,550 treatment generally resulted in decreases in the level of some cytokines and chemokines (Table 6). Those with significant decreases (< 50%) in blood levels on treatment day 7 (Day 55 in the table) included IL-5, IL-7, IL-13, IL-1 β , KC, MCP1, RANTES, and TNF α . Smaller changes were observed in remaining parameters in plasma and all parameter in paw tissues. IL-1 α levels actually went up in both plasma and paw tissues on day 55.

Table 6: Mean Plasma Cytokine Concentrations with or without CP-690,550 Treatment in Mice

Treatment	Day	pg/mL Plasma										
		G-CSF	GM-CSF	IFN- γ	IL-10	IL-12	IL-13	IL-15	IL-17	IL-1 α	IL-1 β	IL-2
Vehicle	48	690.9	8.4	3.2	3.0	8.9	429.7	31.4	1.6	42.5	3.2	1.9
CP-690,550		375.4	6.3	1.6	2.6	16.3	466.3	22.5	1.9	19.7	2.1	1.6
Vehicle	49	382.4	15.0	5.5	3.2	25.0	416.9	27.8	3.3	42.9	8.8	6.5
CP-690,550		336.9	7.1	5.6	1.8	9.3	310.6	46.8	1.6	19.0	2.9	1.6
Vehicle	52	341.4	6.5	2.3	5.5	13.6	453.6	18.7	5.1	23.3	4.4	2.6
CP-690,550		511.0	3.2	2.6	1.8	7.8	221.3	16.8	2.3	24.3	3.9	1.6
Vehicle	55	599.4	7.8	2.4	2.1	13.0	507.6	24.7	5.3	20.8	25.2	3.0
CP-690,550		450.1	3.4	2.1	1.6	15.4	245.2	17.1	4.3	27.5	6.7	2.4
Naive		242.7	11.9	2.4	3.8	6.8	279.6	12.6	5.2	6.4	5.7	1.9
Treatment	Day	pg/mL Plasma										
		IL-4	IL-5	IL-6	IL-7	IL-9	IP-10	KC	MCP-1	MIP-1 α	RANTES	TNF- α
Vehicle	48	1.6	9.7	29.6	3.6	8.0	178.1	79.1	59.7	61.4	22.1	7.3
CP-690,550		1.6	6.4	10.2	2.3	8.0	94.6	34.3	35.8	55.5	12.3	5.8
Vehicle	49	2.3	5.9	22.2	2.9	13.5	172.3	81.3	38.3	154.4	33.5	7.8
CP-690,550		1.6	3.9	6.9	12.4	8.0	116.7	33.3	26.6	69.4	10.1	5.4
Vehicle	52	2.9	19.7	6.9	1.9	8.0	139.2	24.6	43.2	67.2	40.6	5.6
CP-690,550		1.6	4.5	7.2	1.6	8.0	118.4	39.4	14.2	54.1	9.1	4.7
Vehicle	55	1.6	8.7	13.6	9.4	8.0	218.0	58.4	67.7	81.7	20.7	19.2
CP-690,550		1.6	1.6	9.6	1.6	8.0	118.3	17.7	21.0	71.0	10.3	4.9
Normal		1.6	1.6	4.3	1.8	8.0	43.9	7.3	9.0	40.3	48.0	1.8

Report 151443 studied the efficacy of CP-690,550 in preventing arthritis from occurring. Mice were immunized with CII as described previously. These mice were given daily dose of CP-690,550 subcutaneously or orally during the period of day 22 to 55. The CP-690,550 doses ranged 0 - 15 mg/kg/day by subcutaneous injection and 0 - 200 mg/kg/day by oral gavage. The oral gavage dose was given either twice daily (bid) or once a day (qd). The report contains results of a total of 6 studies. JAK modeling assessed the phosphorylation of STAT1 and STAT5 in CD8⁺/CD3⁺ T cells obtained from the whole blood. The phosphorylation of STAT1 was induced by IL-6 while STAT 5 by IL-15 or GM-CSF. Table 7 presents the design of each study and the key endpoints of the study.

Table 7: Study Designs of Report 151443

Study	ROA	CP-669,550 (mg/kg/day)	Prednisolone (mg/kg/day)	Endpoints
1	SC	0, 1.5, 5, 15	-	Arthritis severity score (ASS), plasma drug levels on day 42, 49, and 56
	PO	0, 100 (50, bid)	20 (10, bid)	
2	PO	0, 1, 3, 10, 30, 100, bid	20 (10, bid)	Plasma drug levels on day 26
	PO	0, 100 qd	-	
3	PO	0, 0.5, 5, 50, bid	20 (10, bid)	Plasma drug levels on days 25 and 26, JAK modulations on day 56
4 & 5	PO	0, 1, 3, 10, 30, 100, bid	20 (10, bid)	Plasma drug levels and JAK modulations on days 42 and 49
6	PO	0, 1, 3, 10, 30, 100, bid	20 (10, bid)	Paw arthritis scores on days 29, 34, 48 and 55

Results generally showed dose-dependent increases in plasma CP-690,550 concentrations regardless of the route of administration. There were also dose-dependent decreases in paw arthritic scores. Table 8 presents the summary of arthritis scores of Study 6 as an example. The efficacy was shown as early as day 26.

Table 8: Summary of Efficacy (Arthritis Scores) Results of Study 6

Treatment Group	Dose (mg/kg)	Average Severity Score \pm SEM					% Incidence
		Day 29	Day 34	Day 43	Day 48	Day 55	
Vehicle BID	-	0.4 \pm 0.3	1.3 \pm 0.4	3.9 \pm 0.6	4.2 \pm 0.6	4.7 \pm 0.6	93
CP-690,550 BID	1	0.5 \pm 0.3	1.1 \pm 0.3	3.3 \pm 0.5	4.1 \pm 0.4	4.9 \pm 0.3	100
	10	0.4 \pm 0.2	0.6 \pm 0.3	1.1 \pm 0.4	2.3 \pm 0.5	3.4 \pm 0.5	87
	30	0.1 \pm 0.1	0.3 \pm 0.2	1.2 \pm 0.4	1.5 \pm 0.4	1.5 \pm 0.4	67
	100	0.2 \pm 0.1	0.1 \pm 0.1	0.1 \pm 0.1	0.3 \pm 0.2	0.2 \pm 0.1	13
Prednisolone BID	10	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0
Normal	-	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0

In the JAK modeling experiments, CP-690,550 generally inhibited JAK1 and JAK1/3 activities in dose-dependent manners; the drug, however, did not show any inhibition of the JAK2 (GM-CSF-induced) activity. Study 3 showed that the ED50s were 4.81 and 3.3 mg/kg/day for JAK1 and JAK1/3 activities while the EC50s were 0.19 μ M and 0.13 μ M for JAK1/2 and JAK1/3, respectively. Table 9 presents the overall summary results of the PK/PD assessment in Report 151443. The mean AUC50 was 0.49 – 0.85 and 0.99 – 2.03 μ g.h/mL for twice and once daily dosing, respectively.

Table 9: Summary of the PK/PD Assessment (C_{ave}) Results in Report 151443.

Dosing Paradigm	24h AUC ₅₀ ($\mu\text{g}\cdot\text{h}/\text{mL}$)	AUC ₅₀ 95% CI	r ²	Dose (mg/kg)	C _{max} / C _{min} ($\mu\text{g}/\text{mL}$)	C _{ave} ($\mu\text{g}/\text{mL}$)
Study 2-BID PO	0.49	0 - 1.19	0.839	4.3	0.07/ 0.002	0.02
Study 6-BID PO (Blinded)	0.85	0.58 - 1.13	0.991	12.8	0.123/ 0.003	0.036
Study 4-QD PO	2.03	0 - 4.35	0.859	40.5	0.668/ 0.00006	0.085
Study 5-QD PO	0.99	0 - 2.28	0.731	33.5	0.356/ 0.00001	0.04
Study 1-SC Pump	-	-	-	-	-	0.014

a. AUC₅₀ = Estimated AUC corresponding to ED₅₀, C_{ave} = mean steady state plasma concentration.

Report 150736 modeled the therapeutic properties of CP-690,550 in CIA model and humans. The report analyzed data collected from previously completed in vitro and in vivo studies. Mouse in vivo data were from studies of subcutaneous infusion and oral administration (QD and BID). Human in vivo data were from various clinical modeling data. Analysis of human data was based on the clinical trial which suggests that the ED₅₀ was 2 mg, bid. The report concluded that the JAK1/3 dimer played the key role in the CP-690,550 efficacy because the plasma AUCs in both mice and humans provided higher and longer coverage (multiple folds) for the concentration that was required to inhibit JAK1/3 rather than JAK2, based on the in vitro IC₅₀s of these dimers. It is unclear at the present time that such a conclusion can be drawn.

Rats

Efficacy of CP-690,550 in treating arthritis was studied in the AIA model in female rats. Arthritis in rats was induced by intradermal tail injections of 2.5-mg heat-killed *M. byturium* (three injection sites around the base of the tail, 50 $\mu\text{L}/\text{site}$, 15 mg *M. byturium*/mL squalene oil). The rats showed full blown arthritis approximately 15 days after the treatment. The rats were given single or repeat-dose (up to 10 days) of vehicle or CP-690,550 starting from day 15. The severity of the arthritis was measured by the average of paw volume in a water-filled plethysmometer. The arthritis severity was scored at various time points before, during, or after the CP-690,550 treatment. Results showed that CP-690,550 prevented/attenuated arthritis and reduced plasma levels of some cytokines in a dose-dependent manner.

Report 141170 studied the dose-response of CP-690,550 efficacy after single-doses or a 7-day treatment in AIA rats. One group of AIA rats was dosed with 6.2-mg/kg/day CP-690,550 orally for 7 days, with the first dose being given on day 16. Additional groups of rats were given 0, 10 or 2 mg/kg of the drug on day 16 only. Paw volumes were assessed on days 16, 20 and 23. Cytokine and mRNA levels in the plasma and paw tissues were determined in subsets of rats on days 16, 17, 20, and 23. A number of cytokines were determined in the paw tissue while only IL-6, IL-17 and $\alpha 2$ -microglobulin (A2M) were measured in the plasma. Figure 7 presents the paw volume between the normal, AIA rats (vehicle) and CP-690,550 (6.2 mg/kg/day for 7 days) treatment groups.

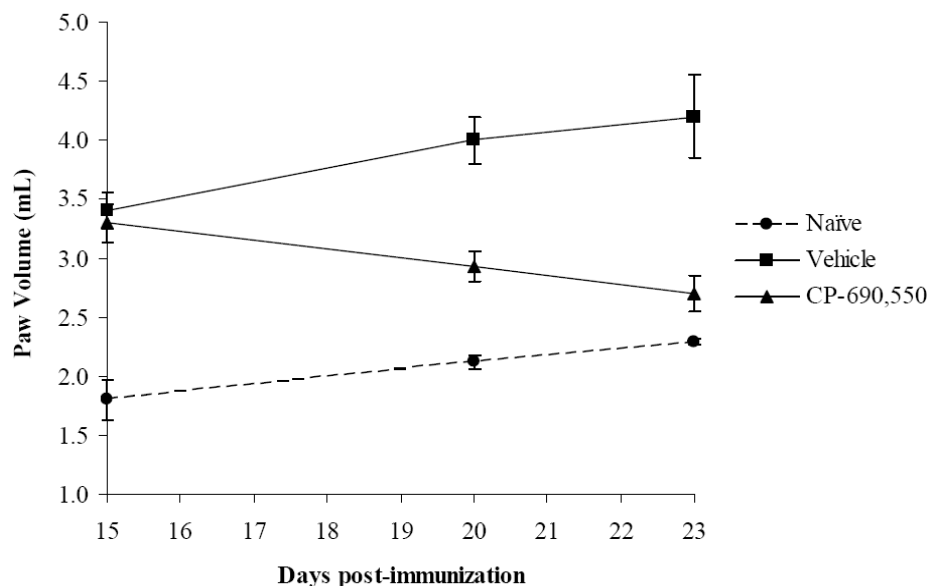


Figure 6: Paw volume vs treatment time in AIA rats. The CP-690,550 dose was 6.2 mg/kg/day from day 16 to day 22.

Table 10 summarizes concentrations of selected cytokines in the plasma and paw tissues on day 23. CP-690,550 treatment (at 6.2 mg/kg/day for 7 days) resulted in statistically significant decreases in plasma levels of IL-6, IL-17 and A2M, but only IL-6 level in the paw tissue.

Table 10: Cytokine Concentrations in the Plasma and Paw Tissue on Day 23

Tissue	Group	IL-6	IL-17	A2M ^a
Plasma (pg/mL)	Vehicle	79.8 ± 16.5	56.5 ± 13.6	6915.9 ± 817.3
	CP-690	15.3 ± 3.8 ***	20.0 ± 6.6 **	1714.2 ± 495.4 ****
Paw (pg/mg protein)	Vehicle	26.5 ± 6.8	1.5 ± 0.8	-
	CP-690	0.8 ± 0.1***	0.8 ± 0.1	-

a. A2M = α2-macroglobulin.

Table 11 presents a complete profile of the cytokines in the paw tissue measured in the study. CP-690,550 treatment (at 6.2 mg/kg/day for 7 days) resulted in statistically significant decreases in all parameters measured except for IL-17.

Table 11: Effect of CP-690,550 (6.2 mg/kg) on Cytokine Concentrations in Paw Tissues

	Day	Treatment	pg/mg paw protein							
			IL-6	Gro/KC	IL-17	MCP-1	MIP-1α	VEGF	Leptin	RANKL
Mean	Naive		1.7	4.9	1.7	3.4	1.7	32.5	391.0	9.1
	16	Vehicle	29.3	153.5	3.1	18.5	8.7	21.5	132.6	48.4
		CP-690,550	1.1	157.0	1.0	16.0	10.8	10.6	119.6	45.8
	17	Vehicle	67.2	119.9	3.6	15.9	13.0	9.6	103.3	86.5
		CP-690,550	37.3	90.7	1.1	9.9	10.1	13.5	130.8	44.5
	20	Vehicle	12.2	85.0	10.5	18.5	11.1	1.5	60.7	55.4
		CP-690,550	1.2	34.9	1.2	2.3	6.5	10.2	117.3	28.9
	23	Vehicle	26.5	88.5	1.5	17.3	9.5	2.5	64.5	44.2
CP-690,550		0.8	9.1	0.8	1.8	2.0	16.2	140.0	14.0	
SEM	Naive		0.2	1.7	0.2	1.8	0.2	3.8	28.0	0.9
	16	Vehicle	9.0	57.5	1.2	4.9	4.5	7.4	30.6	23.1
		CP-690,550	0.2	37.5	0.2	4.2	3.8	6.4	29.7	14.1
	17	Vehicle	23.6	46.3	1.7	5.2	4.1	3.4	22.4	21.8
		CP-690,550	23.5	36.7	0.1	4.6	3.2	3.7	21.2	13.2
	20	Vehicle	5.1	29.6	6.2	4.3	2.4	0.7	15.8	12.1
		CP-690,550	0.1	10.8	0.1	1.1	2.2	4.7	22.3	4.7
	23	Vehicle	6.8	44.2	0.8	6.8	3.8	1.4	13.1	11.6
CP-690,550		0.1	6.2	0.1	0.6	1.1	4.2	35.3	3.4	
p-value (vs vehicle)	day 16		0.0043	0.4801	0.0758	0.3542	0.3604	0.1438	0.3840	0.4622
	day 17		0.1944	0.3147	0.0956	0.2048	0.2966	0.2256	0.1960	0.0682
	day 20		0.0249	0.0689	0.0800	0.0016	0.0932	0.0445	0.0304	0.0324
	day 23		0.0009	0.0401	0.1432	0.0160	0.0324	0.0070	0.0435	0.0106

Report 160531 showed that CP-590,550 treatment inhibited paw tissue inflammation and significant reduction in bone destruction. In this study, CP-690,550 treatment (6.2 mg/kg/day up to 7 days) was started on day 16. Paw tissue inflammation and bone structure were evaluated by microscopic examinations at 4, 96 and 178 hours after the first dose. Figure 7 presents the summary data of the study.

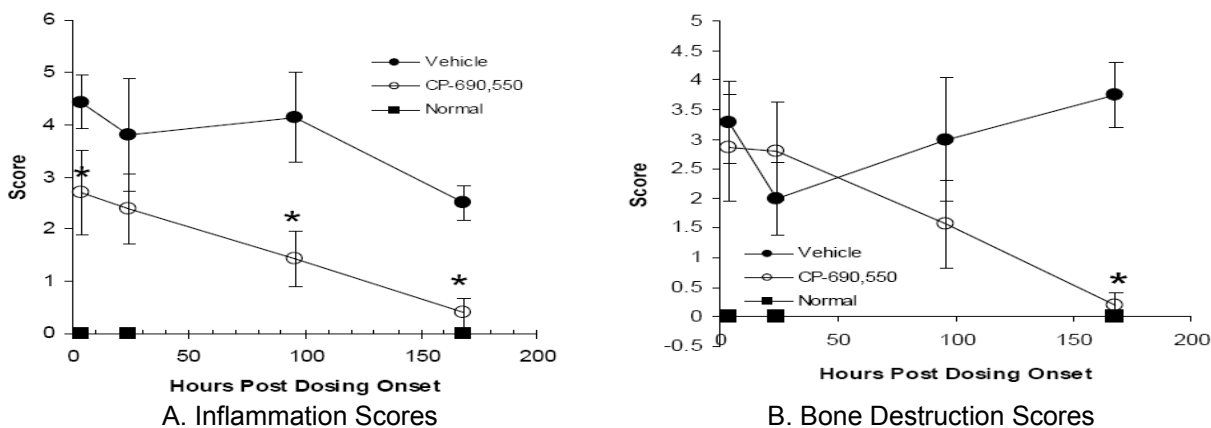


Figure 7: Time course of tissue inflammation and bone destruction by histological evaluation. The CP690,550 dose was 6.2 mg/kg/day by oral gavage. Time 0 indicates the first CP-690,550 dose.

Report 112613 investigated the transcriptional profiling of inflammatory cytokines and chemokines in AIA model. Arthritic rats were given 0 (vehicle) or 6.2 mg/kg/day CP-690,550 orally for 7 days. Additional rats which did not have arthritis (normal in Table 10) served as references. A transcription profile of a cytokine panel, STAT1 activation markers, macrophage surface markers, and surface markers of B and T cells in the paw tissue were measured. Results showed that the adjuvant treatment resulted in significant elevations of the gene expression levels of inflammatory mediators. Table 12 presents the cytokine mRNA levels after the vehicle and CP-690,550 treatment on days 0, 1, 4 and 7 of the treatment. Similar trends were observed in other parameters.

Table 12: Changes in cytokine mRNA levels in paw tissue without (vehicle) and with CP-690,550 treatment in AIA rats.^a

Major Cytokines	Agilent ProbeID	Rat AIA Fold Change							
		Vehicle vs Normal				CP-690550 vs Vehicle			
		0.17 Day	1 Day	4 Day	7 Day	0.17 Day	1 Day	4 Day	7 Day
Il6	A_44_P371339	60.1	90.8	29.4	35.8	-12.5	-4.4	-6.6	-15.6
Ccl17	A_44_P409840	4.4	4.2	4.8	4.5	-4.8	1.1	-3.9	-5.2
Il19	A_44_P229338	6.4	4.1	2.5	2.9	-8.5	-4.8	-4.2	-6.4
Ccl20	A_43_P11985	122.6	190.7	132.3	100.0	-9.4	-4.8	-8.3	-30.6
Ccl7	A_44_P1022002	8.8	12.3	10.0	11.4	-6.0	-3.6	-6.0	-10.2
Ccl12	A_44_P1057055	11.2	18.2	18.9	22.0	-5.7	1.1	-6.6	-13.4
Cxcl6	A_44_P270366	110.0	213.8	259.7	233.8	-3.1	-7.2	-5.9	-18.7
Mmp19	A_44_P480817	5.3	6.6	8.1	6.0	-3.1	1.2	-3.5	-4.0
Cxcl3	A_44_P363116	15.4	26.9	29.1	22.2	-3.2	-4.9	-4.4	-10.5
Tnfsf11	A_43_P13048	6.2	15.3	8.8	10.3	-3.9	-3.6	-4.6	-6.0
Il11	A_44_P278445	6.4	13.1	10.7	9.8	-3.6	1.0	-3.6	-5.8
Cxcl9	A_44_P1043157	7.3	7.1	5.5	6.1	-3.7	-3.7	-4.0	-5.0
Ccl9	A_44_P412940	12.3	18.9	25.7	23.0	1.2	-3.1	-3.2	-4.6
Cxcl1	A_42_P473398	15.0	20.3	13.6	12.5	-4.3	-5.0	-3.9	-9.2
Il1b	A_43_P14911	3.5	3.9	3.6	4.7	-3.5	-3.3	-3.6	-5.2
Ccl3	A_42_P714311	5.8	6.6	8.9	7.4	1.1	-3.3	-3.5	-7.0
Mmp12	A_44_P555271	26.6	43.5	55.6	59.8	1.1	-3.1	-3.1	-7.7
Il17a	A_44_P144602	3.8	5.8	12.5	9.4	-3.5	1.2	-3.8	-7.4
Cxcl2	A_44_P515197	4.1	6.4	5.6	7.4	-3.6	-3.1	-3.8	-7.7
Mmp8	A_43_P12170	18.0	12.3	11.6	16.1	1.0	1.6	1.0	1.1
Adams3	A_44_P342279	32.9	29.3	22.4	18.3	1.1	-3.0	1.9	-3.4

a. Normal in the table refers to rats that did not receive any *M. Butyrium* treatment.

Figure 6 CP-690,550 presents the individual values of signal values for three inflammatory marker genes (IL-1B, TNF and IL-17a) in normal rats (normal) and AIA rats with (CP-550) and without CP-690,550 (vehicle) treatments on day 7. The signal intensity for each marker in the normal rats was relatively low. The arthritic rats receiving no CP-690,550 (vehicle) showed marked elevations of each of the markers. CP-690,550 treatment resulted in decreases (or return to background levels) for each marker.

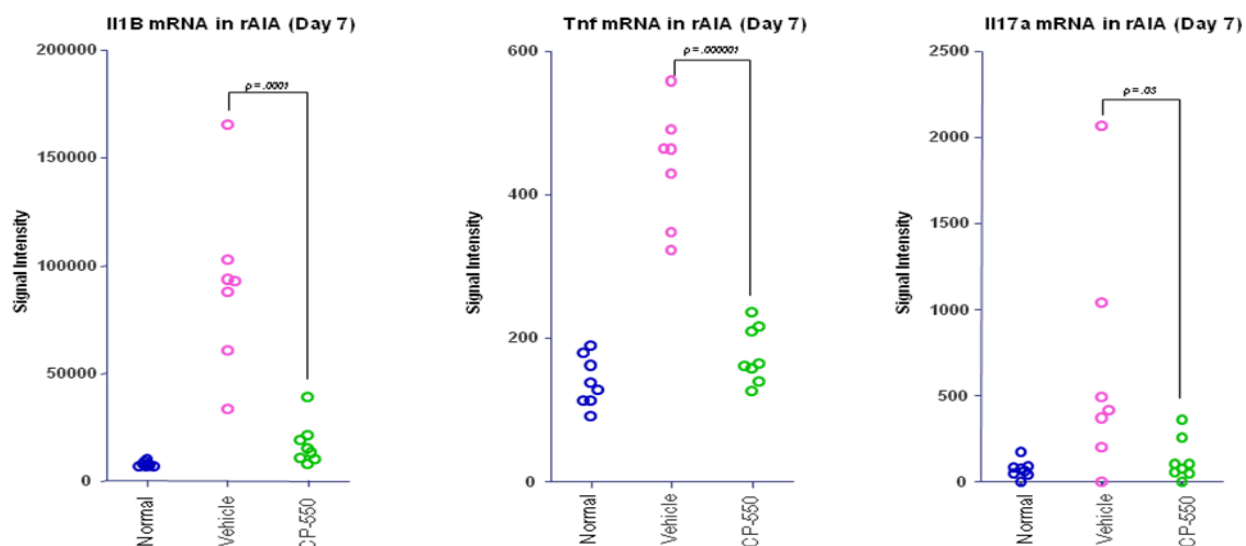


Figure 8: Tissue (paw) mRNA levels of IL-1B, TNF and IL17a 7 days after CP-690,550 treatment in normal and arthritic rats.

Report 100214 evaluated the efficacy of 10-day oral CP-690,550 treatment and the dose-response for such treatment. The first dose was given on day 11 (in contrast to day 15 in other studies). CP-690,550 doses ranged from 0.06 mg/kg/day to 60 mg/kg/day, bid or qd. Efficacy was determined by paw volume. Also measured were the plasma drug levels, peripheral blood neutrophil counts (PBNC), and some cytokines (IL-6, IL-17, and A2M). Results showed that CP-690,550 treatment resulted in dose-dependent decreases in paw volume and PBNC. The ED₅₀s for bid and qd were < 1 and 0.66 mg/kg/day, respectively. These doses corresponded to AUC_{50s_{0-24hr}} of 0.162 and 0.392 $\mu\text{g}\cdot\text{h/mL}$, respectively.

Report 1002743 evaluated the effect of dose and frequency of administration on the CP-690,550 in the AIA model. Arthritic rats were dosed with 0 – 18.5 mg/kg/day of CP-690,550 for a week. The frequency of CP-690,550 treatment was twice a day (bid), once a day (qd), or once every other day (eqd). Efficacy was determined by paw volume. Also measured were the plasma drug levels, and peripheral blood neutrophil counts (PBNC). Results showed that CP-690,550 treatment resulted in dose-dependent decreases in paw volume and PBNC. The ED₅₀s were 0.7, 0.9 and 16.6 mg/kg for bid, qd, and eqd, respectively.

4.2 Secondary Pharmacodynamics

CP-690,550 exhibited no significant binding or activity to receptors, ion channels, enzymes and transducers. Reports 7570532 and 7571347 determined the binding of CP-690,550 (at 10 μM) to a broad panel of receptors, ion channels, enzymes, and transducers from human, rat, mouse, hamster and pig using the CEREP wide ligand profile. The receptors included those for adenosine, adrenergic, angiotensin, and so on. Ion channels included those of Ca^{++} , Na^{+} , K^{+} , Cl^{-} , and transporters. Table 12 presents a partial list of receptors and ion channels screened for ligand binding. Enzymes included Abl kinase, ACE, acetyl cholinesterase, ATPase, CaMK2 α , caspase-3, carbonic anhydrase II, COX2, FLT-1 kinase, Lyn A Kinase, MMP-9, p38a kinase, PDE2, PDE3, PDE4, PDE6, PDE11, and ZAP70 kinase, cannabinoids (CBs), cholecystokinin (CCKs). Significant inhibition (>50%) was observed

only for the MT₃ receptor and VEGFR1, CaMK2 α , and LynAKinase. The IC₅₀, however, was at least 10 times the C_{max} levels in patients receiving therapeutic dose of the drug.⁶

Table 13: Receptors and Ion Channels Screened for CP-690,550 Binding^a

Receptors	Ion Channels	Uptake sites
Adenosine (A ₁ , A _{2a} , A ₃)	Calcium channels:	Dopamine
Adrenergic (α 1, α 2 non-selective; β 1, β 2)	L-type (verapamil, diltiazem or dihydropyridine sites)	GABA
Angiotensin-II (AT ₁ , AT ₂)	N-type	5-Hydroxytryptamine
Benzodiazepine	HERG K ⁺ channel	Norepinephrine
Bradykinin (B ₁ , B ₂)		Choline
Dopamine (D ₁ , D ₂ , D ₃ , D ₄)		
GABA (non-selective)		
Glutamate (AMPA, kainate, NMDA)		
Glucocorticoid		
Histamine (H ₁ , H ₂ , H ₃)		
5-Hydroxytryptamine (5HT _{1A} , 5HT _{2A} , 5HT _{2C} , 5HT ₃ , 5HT ₄ , 5HT ₇)		
Melanocortin (MC ₄)		
Muscarinic (M ₁ , M ₂ , M ₃ , M ₄)		
Nicotinic (neuronal, muscle)		
Opiate (Delta, Kappa, Mu)		
Glucocorticoid		
Platelet activation factor (PAF)		
Tachykinin (NK ₁)		
Thyroid hormone (TH)		
Vasopressin (V ₁ , V ₂)		

a. Extracted from a nonclinical review completed by Dr. Hamid R. Anouzadeh on June 24, 2005 in IND 70903.

4.3 Safety Pharmacology

Safety pharmacology of CP-690,550 was evaluated in vitro or vivo. The in vitro studies evaluated the effect of the drug on hERG channel current in HEK-293 cells using the patch clamp technique and the effect on action potential in canine Purkinje Fibers. Table 14 summarizes the assay conditions and results of the in vitro assays. Briefly, CP-690,550 did not affect the hERG channel current or action potential.

Table 14: In Vitro Safety Pharmacology Studies of CP-690,550

In Vitro					
Type of Test	Test Cells/Tissues	Test Concentrations	Results	GLP Compliance	Study Number
hERG Patch Clamp	HEK-293 cells stably expressing hERG channels	10 μ M (3120 ng/mL)	Inhibits hERG current by 6.4% at 10 μ M.	No	48879-104
hERG Patch Clamp	HEK-293 cells stably expressing hERG channels	0, 10, 30, 100 μ M (3120, 9372, 31240 ng/mL)	Inhibits hERG current by 0.8% at 10 μ M, 3.6 % at 30 μ M, and 17.8% at 100 μ M vs 0.3% at 0 μ M (vehicle control) ^a IC ₅₀ estimated to be >100 μ M	Yes	110106.QHJ
Cardiac Action Potential	Canine purkinje fibers	0.1, 1, 10 μ M (31.2, 312, 3120 ng/mL)	No significant effect on resting membrane potential, action potential amplitude, maximal depolarization velocity, or action potential duration at any concentration tested.	No	CP690550-10/CG/001/00

GLP = Good Laboratory Practice; hERG = Human ether 'a-go-go-related gene; HEK = Human embryonic kidney; NA = Not applicable; IC₅₀ = Inhibitory concentration.

^a Results at each test concentration were compared to control.

⁶ MT₃ = Melatonin receptor 3, VEGFR1 = vascular endothelial growth factor receptor 1, CaMK2 α = calmodulin dependent protein kinase 2 α , and LynAKinase = Lyn A kinase. The Ki value for the was 5.2 μ M, 3.7 μ M, 12 μ M, and 2.3 μ M For the MT3 receptor, VEGFR1, CaMK2 α and LynA Kinase enzymes, respectively. The exposure margins for these enzymes are 23-fold, 16-fold, 53-fold, and 10-fold, respectively, based on the human unbound C_{max} of 0.22 μ M (71 ng/mL).

The in vivo safety pharmacology studies were completed to evaluate the effect of CP-690,550 on the CNS, cardiovascular and respiratory systems and urine excretion in mice, rats, and monkeys. Table 15 summarizes the design and results of the in vivo studies.

Table 15: In Vivo Safety Pharmacology Studies of CP-690,550

In Vivo								
Organ Systems Evaluated	Species/ Strain	Method of Administration	Doses ^b (mg/kg)	Number/ Sex/Group	Cmax (ng/mL)	Results	GLP Compliance	Study Number
Central Nervous System	Mouse/CD-1	Oral Gavage	3.2-32 100 320 1000	3 M	≤709 4800 14900 >14900	No noticeable effect ↓ in spontaneous locomotor activity, hunched to flattened posture, splayed hind limbs, ↑ eye closure, and vocalization ↓ in spontaneous locomotor activity, hunched posture, flattened posture, vocalization, twitches upon movement, splayed hind limbs, and mild seizures; ↓ in body tone, toe pinch, tail pinch and corneal responses and exploratory behavior; 1/3 mice showed ↓ in respiration, tremors, loss of righting reflex, and death. ↑ in intensity of symptoms above + seizures and death	No	NA
Pro/Anticonvulsive Activity	Mouse/ CD-1	IP	3.2-32	4 M	≤709	No significant effect	No	NA
Cardiopulmonary	Rat/Sprague-Dawley	Oral Gavage	10 100	4 M	971 8630	No significant effect ↓ in mean arterial pressure by 37 mm Hg and ↑ in heart rate by 100 bpm	No	NA
Renal	Rat/Sprague-Dawley	Oral Gavage	3-10 100	12 M	≤971 8630	No significant effect Potassium excretion elevated by 104%	No	NA
Gastrointestinal Transit	Rat/Sprague-Dawley	Oral Gavage	10 30-100	5-6 M	971 ≤8630	No significant effect Inhibition in gastric emptying by 18%; ↓ in mean geometric center by 33%	No	NA
Cardiovascular	Monkey/ Cynomolgus	Oral Gavage	100 300	4 M ^c	3000 3310	Emesis in 1 of 4 monkeys Emesis, salivation, ↑ in heart rate at 2 to 3 hours after dose; no significant effect on blood pressure, cardiac rhythm and QT interval	No	745-03432
Cardiovascular Blood Pressure Systolic (SBP) Diastolic (DBP) Mean (MBP) Heart Rate (HR) Body Temperature (BT) Activity (ACT) Toxicokinetics Dose (mg/kg/day) Day 1	Rat/Sprague-Dawley	Oral Gavage 10 mL/kg	10 30 75	8F ^d	≥10 mg/kg: Dose dependent ↓ in MBP, SBP, DBP (5 to 17 mmHg) 0-2 hours postdose on Days 1, 3, and 5; Dose dependent ↑ HR (+30 to 67 bpm) 0 to 2 hours postdose on Days 1 and/or 5; ↓ BT (up to -0.37 °C) 8-12 hours postdose; no change in activity 30 and 75 mg/kg: ↓ HR (28 to 39 bpm) at 8 to 12 hours postdose on Days 1 and 5.	No	11GR001	
		0	10	30	75			
		NC ^e	2560	4760	5080			
		NC ^e	9850	26900	43400			

C_{max} = Maximum (peak) observed drug concentration; GLP = Good Laboratory Practice; M = Male; NA = Not applicable; QT = Time from the beginning of the QRS complex to the end of the T wave in the electrocardiogram; bpm = Beats per minute; NC = Not collected; AUC(0-24) = AUC from time 0 to 24 hours postdose.

^a Single dose unless specified otherwise.

^c Randomized cross-over study design.

^d 5-day repeat-dose per dose group.

^e Samples not collected from control animals.

Briefly, CP-690,550 at ≥ 100 mg/kg (PO) affected the CNS and cardiovascular systems and renal excretion. The CNS effects included decreases in spontaneous activity and signs of general toxicity in mice. The cardiovascular effects included decreases in blood pressures (\downarrow ~37 mmHg) and increases in heart rate (\uparrow 100 bpm) in rats. Increases in renal potassium excretion (\uparrow 104%) occurred in rats. Changes in blood pressure (\downarrow 5 mmHg) and heart rate (\uparrow 30 bpm) were also observed at 10 mg/kg in rats. The mean AUC at 10 mg/kg was 9.85 $\mu\text{g}\cdot\text{h}/\text{mL}$ in rats.

11 Summary and Evaluation

Tofacitinib is an inhibitor of the Janus associated kinases (JAK) family. Tofacitinib possesses anti-inflammatory property by disrupting the cytokine signaling pathway which requires the activation of JAKs. Inhibition of JAK activity results in decreases in the production and releases of cytokines and other inflammatory mediators.

The JAK is a family of 4 tyrosine kinases: JAK1, JAK2, JAK3, and TyK2. Among them, JAK1, JAK2 and TyK2 are ubiquitously expressed and associated with numerous types of cytokine receptors and JAK3 is preferentially expressed in hematopoietic cells. The JAK transmit cytokine signals via heterodimers: JAK1/3, JAK1/2, JAK2/2, JAK1/TyK2, or JAK2/TyK2. The involvement of particular dimers was cytokine and cell type-dependent. Generally, the JAK1/3 dimer is involved in ILs-2, 4, 10, 15, and 21 signaling; the JAK1/2 dimer is for IL-6 signaling; and the JAK1/TyK2 dimer is for IL-10, IFN α and IFN γ signaling. However, there was no clear definition regarding the involvement of particular dimers.

CP-690,550 inhibited the activity of individual recombinant JAK and dimers in vitro, but its IC50s varied significantly among them. The recombinant individual JAKs had the lowest IC50s (i.e., 3.2, 4.1, 1.6 and 34 nM for recombinant JAK1, JAK2, JAK3, and TyK2, respectively). The dimers had significantly higher IC50s (20- 1377 nM). Briefly, the IC50s range for dimers was 25 – 111 nM, 54 – 406 nM, 44 – 206, 100 – 501 nM for JAK1/3, JAK1/2, JAK1/TyK2, and JAK2/TyK2 dimers, respectively (Table 16). The IC50s among JAK1/3, JAK1/2, JAK1/TyK2 and JAK2/TyK2 apparently overlap. These overlaps may have implications in the labeling review regarding the Mechanism of Action section. See LABELING REVIEW section for additional discussions.

Table 16: Mean IC50s of CP-690,550 for Inhibiting JAK Dimer Activities

Cells	Mean IC50 (nM)					Study No.
	JAK1/3	JAK1/2	JAK2/2	JAK1/TyK2	JAK2/TyK2	
Human whole blood	34				501	D08AI0337
HBMC ^a	26				129	D08AI0337
Human whole blood	56	406	1377			D08AI0338
Human whole blood	25 - 111	54 - 178		44 - 206		113015
T-225 cell	6				40	142305
T-225 + HWB	20				100	142305

a. HBMC = human blood mononuclear cells, HWB = human whole blood.

CP-690,550 was effective in attenuating arthritis in animal models in vivo. Arthritis models in rats and mice that were used to study the efficacy of the drug, were described in detail in

Section 4.1. Briefly, repeat-dose CP-690,550 treatment attenuated paw arthritis scores (swelling, edema and paw volumes) in both arthritis models. The efficacy was dose, frequency of administration, and the treatment-duration dependent. For example, the ED50 for a 7-day treatment duration was 0.7, 0.9 and 16.6 mg/kg/day after bid, qd, and every other day treatment schedules. CP-690550 treatment also resulted in decreases in the plasma levels of a number of cytokines.

Secondary pharmacology studies were completed to evaluate the binding of CP-690,550 to other receptors, ion channels and transporters, and the inhibitory effects of on other kinase activities. CP-690,550 did not show any significant binding to these receptors, ion channels and transporters. The drug did not show any significant effects on these kinases.

Safety pharmacology studies were completed to evaluate the effect of CP-690,550 on hERG channel current and on action potential in Purkinje fibers in vitro and the effects of the drug on the CNS, respiratory and cardiovascular systems and gastrointestinal tract. CP-690,500 did not affect the hERG channel current or action potential. CP-690,550 at ≥ 100 mg/kg (PO) affected the CNS and cardiovascular systems and renal excretion. The CNS effects included decreases in spontaneous activity and signs of general toxicity in mice. The cardiovascular effects included decreases in blood pressures (\downarrow ~37 mmHg) and increases in heart rate (\uparrow 100 bpm) in rats. Increases in renal potassium excretion (\uparrow 104%) occurred in rats. Changes in blood pressure (\downarrow 5 mmHg) and heart rate (\uparrow 30 bpm) were also observed at 10 mg/kg in rats. The mean AUC at 10 mg/kg was 9.85 $\mu\text{g}\cdot\text{h}/\text{mL}$ in rats.

LABELING REVIEW

This section evaluates the proposed text for Section 12.1 Mechanism of Action. The review presents the text proposed by the applicant and recommended by the nonclinical review team in a sequential order. Rationale for the recommended edits is provided later. Pfizer proposed the following for Section 12.1:

(b) (4)



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/s/

LUQI PEI
05/14/2012

TIMOTHY W ROBISON
05/14/2012
I concur

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

Chemistry Consult

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	203214
Supporting document/s:	SD-000 (NDA, and interim study report) SD-9 (final study report)
Applicant's letter date:	Oct 21. 2011, Feb 16 2012
CDER stamp date:	Oct 21. 2011, Feb 16, 2012
Product:	Tofacitinib
Indication:	Rheumatoid Arthritis
Applicant:	Pfizer Inc.
Review Division:	Division of Pulmonary, Allergy and Rheumatology Products
Reviewer:	L. Steven Leshin, D.V.M., Ph.D.
Supervisor/Team Leader:	Molly Topper, Ph.D.
Division Director:	Badrul Chowdhury, M.D., Ph.D.
Project Manager:	Philantha Bowen

Background

On March 16 2012, following review of the CMC submitted information concerning drug (b) (4) impurities and degradants, a CMC-orientated IR letter was communicated to the applicant. The following recommendation associated with the PharmTox assessment of impurities was conveyed:

11. Revise the acceptance criterion for each of the following drug (b) (4) specified impurities to not more than (b) (4) as you have not provided sufficient individual data to qualify these at a higher level from a toxicological perspective: (b) (4)
(b) (4) Alternatively, you may provide supporting toxicological data.

The issue raised with the request pertains to toxicological support for the drug (b) (4) impurity specifications. For these 3 drug (b) (4) impurities, (b) (4) (b) (4), the proposed specification is (b) (4).

The tables and data in the toxicology studies, with the exception below, indicated the levels were "not assessed." Also there were no separate studies of these individual drug (b) (4) impurities. Therefore, no degree of safety could be associated with these specifications.

The applicant responded indicating the juvenile monkey toxicological study (Report 09GR248) submitted as an interim report in the NDA submission with a final report submitted to the NDA Feb 16 2012 did assess the level of 3 of the drug (b) (4) impurities, (b) (4), but not (b) (4).

This exposure margin for these 3 impurities was approximately (b) (4) above the maximally recommended human dose human exposure if they were present at the proposed specification limit of (b) (4) as presented in the table below. There are no independent assessments of the effects of these impurities and they presumably have been present in previous toxicology studies although their levels were not assessed in previous studies. Thus, it is not possible to separate the toxicities associated with the impurities from that of the API, however, no new toxicities were evident in the supportive juvenile monkey study. Therefore; the proposed specification limits are acceptable.

Impurity	Acceptance Criteria	Levels at the Max Clinical Daily Dose (expected AUC exposure, ng-h/mL)	Juvenile Monkey with 26 week recovery (Report 2501-010)	Expected Impurity Exposure in the Monkey [†] (ng-h/mL)	Expected Impurity Exposure in the Human (ng-h/mL) [‡]	Exposure Margin [‡]
Drug	(b) (4)	Impurity	(b) (4)			

Recommendation

The (b) (4) proposed specification is acceptable from a toxicology perspective.

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/s/

LAWRENCE S LESHIN
04/16/2012

MOLLY E TOPPER
04/16/2012
I concur.

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

PRODUCT QUALITY CONSULT

Application number:	203214
Supporting document/s:	SD-1
Applicant's letter date:	Oct 21, 2011
CDER stamp date:	Oct 21, 2011
Product:	tofacitinib
Indication:	Rheumatoid Arthritis
Applicant:	Pfizer Inc.
Review Division:	Division of Pulmonary, Allergy and Rheumatology Products
Reviewer:	L. Steven Leshin, D.V.M., Ph.D.
Supervisor/Team Leader:	Molly Topper, Ph.D.
Division Director:	Badrul Chowdhury, M.D., Ph.D.
Project Manager:	Philantha Bowen

BACKGROUND

CP-690,550 (tofacitinib) is a new molecular entity, small molecule kinase inhibitor being developed as an immunosuppressive for the treatment of rheumatoid arthritis. (b) (4)

The drug is formulated for oral administration as 5 and 10 mg tablets. The maximal proposed dose would be 10 mg, b.i.d. Separate consults from ONDQA were submitted for a toxicological analysis of the acceptance criteria for impurities in the drug (b) (4) (DARRTS Nov 28, 2011) and in the drug (b) (4) (DARRTS Dec 12, 2011). All of the drug (b) (4) impurities contain the same (b) (4) moiety, a structural alert for mutagenicity, which is also present in the active pharmaceutical ingredient CP-690,550. There were no previous toxicological consults for this drug substance or product during the IND phase of this NDA or for the other IND indications.

Drug (b) (4)

The drug (b) (4) has two specified degradation products (b) (4). Both have acceptance criteria of NMT (b) (4). The structures of these compounds are presented in the Appendix. The Applicant's justification for the proposed acceptance criteria is as follows:

(b) (4)

(b) (4)

The acceptance criteria of (b) (4) established for these specified impurities are consistent with the qualification threshold in ICH-Q3A(R2) for a maximum daily dose of 10 mg – 100 mg.

Drug (b) (4)

There are 8 impurities of the drug (b) (4). The structures of these impurities are presented in the Appendix. They all contain the

same (b) (4) structural alert moiety that is in the structure of the active pharmaceutical ingredient CP-690,550. Note that (b) (4) is also an impurity of the drug (b) (4) as a degradant. (b) (4) contains a similar (b) (4) structural alert function but with a (b) (4). Impurity (b) (4) has a (b) (4). Impurity (b) (4) contains a second structural alert function, (b) (4).

The Applicant's proposed acceptance specifications for the drug (b) (4) impurities are listed in the following table:

Impurity	Acceptance Criteria	Level at the Max Daily Dose
(b) (4)		

The Applicant claims in S.4.5 of Module 3 that “the limits of specified impurities are justified based on inclusion in toxicological studies and the absence of structurally alerting functional groups when screened through DEREK software.”

Genetic Toxicology

CP-690,550 (tofacitinib) was not mutagenic as determined by the Bacterial Reverse Mutation (Ames) assay, and not clastogenic as determined by an *in vitro* chromosome aberration assay with human lymphocytes, and an *in vivo* rat micronucleus assay. A positive result occurred in the *in vitro* chromosome aberration assay with human lymphocytes in the presence of enhanced levels of metabolic enzymes. CP-690,550 caused a statistically significant increase in chromosome aberrations in cultured human lymphocytes (Report 01-2063-10) in the 3-hour test with metabolic activation, but not in the absence of the addition of induced liver enzymes. The dose at which the positive response occurred, $\geq 1700 \mu\text{g/mL}$, corresponded to 48% mitotic suppression. The results suggest a metabolite may have clastogenic activity however a follow-up *in vitro* CHO-HGPRT assay was negative, as was an *in vivo* rat hepatocyte unscheduled DNA synthesis assay. Considering a weight of evidence approach, the API was determined to be negative for genetic toxicity.

The amounts of impurities in the assays for genetic toxicity assessments are indicated in the table below. All 5 studies were conducted with lot 43798-2-1H, with (b) (4) CP-690,550 purity. The (b) (4) were present at a level exceeding the proposed specifications for the drug substance, but levels of 4 of the impurities were not determined for this lot. None of the levels of the impurities were at sufficient levels to adequately qualify them in these genotoxicity assays.

Impurity	Acceptance Criteria	Levels at the Max Daily Dose	Impurity levels in Genetic Toxicology Assays*
(b) (4)			(b) (4)

NA Not assessed

* from Applicant's Table 2.6.7.4

Amount of Impurity in Genetic Toxicology Assays

Impurity	Impurity levels in Genetic Toxicology Assays*	Microbial Reverse Mutation Assay (mcg/plate) and CHO cells HGPRT gene (mcg/mL)	In Vitro Chromosomal Aberration, Human Lymphocytes (mcg/mL)	In Vivo Rat Micro- nucleus (mg/kg)
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(b) (4)

Genetic Toxicology Studies

Cells or Species	Concentrations or Doses	Findings
Mutagenic Assays		
Microbial Reverse Mutation Assay (Report 01-2063-11)		
<i>S.typhimurium</i> <i>E. coli</i>	0.010-5000 µg/plate	Negative
Mammalian Mutation Assays (Report 01-2063-16)		
(HGPRT) gene locus, in Chinese hamster ovary K1-BH4 cells	16-5000 µg/mL with and without S9 Substantial cytotoxicity at 950, 1000, and 1100 µg/mL with average day relative cell survivals of 43%, 29%, and 17%, respectively	Negative
Clastogenic Assays		
In Vitro Cytogenetic Assays (Report 01-2063-101)		
human lymphocytes, in vitro	3-hour test: without S9: 236, 393, 960, 1200, and 3000 with S9: 403, 1540, 1920, 2400, and 3000 mcg/mL 2nd test: 1600, 1700, 1800, 1900, and 2000 mcg/mL 24-hour test without S9: 25.1, 41.8, 116, 540, and 900 mcg/mL	Negative without S9 Positive with metabolic activation (+S9) in the 3 hour test at cytotoxic concentrations that induce approximately ~48% mitotic suppression or greater.
Rodent Micronucleus Assay (Report 01-2063-12)		
rat, bone marrow cells	62.5, 125, 250 mg/kg, oral The mean total Cmax and AUC(0-tlast) values (males + females combined) for CP-690,550 on Day 3 in rats given 250 mg/kg were 8480 ng/mL and 51700 ng-h/mL, respectively.	Negative
In Vivo/In Vitro Unscheduled DNA Synthesis (Report 01-2063-17)		
rat, male hepatocytes	125, 250, 500 mg/kg single dose Livers collected at 2-4hr and 14-16 hr after dosing	Negative

General Toxicology

In the two pivotal nonclinical CP-690,550 toxicology studies, a 6-month study in rats and a 9-month study in monkeys, NOAELs could not be determined as adverse effects were

noted in each of the lowest doses tested, described below. The major effects were toxicities associated with suppression of the immune and hematopoietic systems evidenced by suppression of myeloid and erythroid bone marrow production, reduced or atrophied lymphoid organs, and reductions in circulating red and white blood cells.

Consistent with its known pharmacology (immune suppression), CP-690,550 was associated with a dose-dependent decrease in white blood cell counts. In rats, this decrease was primarily due to decreases in lymphocytes which occurred earlier at higher doses, but was seen at 1 mg/kg and above (females only) by week 13. Slight to moderate lymphoid depletion of the bone marrow was seen at 10 mg/kg and above in males and females and at 1 mg/kg (in a single female). RBC parameters also decreased in a dose and duration of treatment dependent manner. At necropsy a dose-dependent decrease in splenic weight was noted in all treated rats. Similar signs of immune suppression were observed in the 9-month monkey study. Decreases in lymphocytes, lymphocyte subsets, and NK cells were observed at all doses, whereas the effects on RBC parameters occurred at ≥ 2 mg/kg/day. In both studies, the effects on lymphocytes, lymphocyte subsets, and the immune system, were consistent with the intended JAK1/3 pharmacologic activity and decreases in RBC parameters were consistent with JAK2 inhibition. Female monkeys at the lowest dose (0.5 mg/kg/day) had decreased lymphocyte counts by Day 91. Tumors (lymphomas) developed in 3 monkeys. In the shorter 4-week study, some monkeys developed signs of bacterial and viral infections.

RATS

In the 6-week (Studies 01-2063-06) and 6-month (Study 7743) rat studies, Sprague-Dawley rats were administered oral doses of CP-690,550 at 0, 1, 10, or 100 mg/kg/day. In the 6-week study, the major treatment-related hematologic findings included decreases in leukocyte parameters as well as a time-dependent decrease in red blood cell count, hemoglobin, and hematocrit. Macroscopic changes were observed in the thymus and spleen of animals in the 100 mg/kg dose group, and histopathological changes included lymphoid depletion in lymphoid tissues, thymus, spleen, mesenteric lymph node, and bone marrow. A NOAEL was not identified in the study since adverse hematological changes were noted at the lowest dose level examined, 1 mg/kg, but histopathology was not conducted in this group.

In the 6-month study, dose-dependent decreases in total white blood cell counts that were primarily due to decreases in lymphocyte counts were seen at doses ≥ 10 mg/kg/day at weeks 4, 13, and 26 (males and females) and at ≥ 1 mg/kg/day during weeks 13 and 26 (females only). In males, slight decreases in red blood cell parameters (RBC, Hb, Hct) were seen at 100 mg/kg/day at week 13 and then at doses ≥ 10 mg/kg/day by week 26. In females, mild time-dependent decreases in RBC parameters were observed at 100 mg/kg/day at weeks 4, 13, and 26; minimal decreases in those parameters were also seen at 10 mg/kg/day at weeks 4 and 13. Dose-related decreases in CD3+, CD4+, CD8a+, CD45RA+, and CD161a+ lymphocyte sub-populations were observed which paralleled the decreases in total lymphocyte counts at

weeks 14 and 26 in both sexes. At necropsy, a dose-dependent decrease in splenic weight was noted in rats receiving ≥ 1 mg/kg/day. Relative thymus weights were decreased at the 100 mg/kg/day level and relative liver weights were increased at 100 mg/kg/day.

Atrophy of the lymph nodes (ileofemoral, inguinal, mesenteric), spleen, and thymus was observed microscopically, primarily in males and females receiving 100 mg/kg/day and in females at 10 mg/kg/day. Atrophy of the gut-associated lymphoid tissues was only noted in males and females at the 100 mg/kg/day group. In the spleen, lymphoid atrophy was accompanied by a relative decrease in the number of hematopoietic cells in the red pulp, primarily in rats given 100 mg/kg/day. The change was described as minimal to slight decreased extramedullary hematopoiesis and was observed in 7/15 males and 14/15 females of the 100 mg/kg/day group. In the lungs alveolar histiocytosis and interstitial inflammation was noted in males given ≥ 10 mg/kg/day and females of the 100 mg/kg/day.

In both studies, changes observed were consistent with the intended inhibition of JAK1/3 pharmacologic activity (decreases in circulating lymphocytes and moderate lymphoid depletion in spleen, thymus, mesenteric lymph node, and the bone marrow) and with side effects consistent with JAK2 inhibition (decreases in RBC parameters [red blood cells, hemoglobin, and hematocrit] and reticulocytes). The observed effects were dose- and time-dependent and were generally reversed in the 4-week recovery group from the 6-week study. There were no recovery groups in the 6 month study.

At 1 mg/kg/day, treatment-related effects were limited to hematological changes in females and an increased relative splenic weight in both males and females. As such a NOAEL could not be determined.

CYNOMOLGUS MONKEYS

In the 4-week toxicity study (Study 01-2063-09) cynomolgus monkeys were administered total daily oral doses of 0, 10, 50, or 100 mg/kg/day (divided TID, ~ 7 hours apart). Additional animals from the control and 50 mg/kg/day groups were allowed to recover for 4 weeks at the end of the dosing phase of the study. At ≥ 10 mg/kg/day observations included decreases in lymphocytes, lymphocyte subsets, natural killer (NK) cells, and hemoglobin. Treatment-related findings at ≥ 50 mg/kg/day consisted of death, decreased activity, decreased RBC parameters, and granulocytic depletion in the bone marrow, active bacterial (streptococcus and staphylococcus) and/or viral (cynomolgus polyoma and herpes virus), infections in multiple organs (heart, kidney, gastrointestinal tract, buccal cavity and skin), probably secondary to immunosuppression. Except for the presence of infections, the observed effects increased in severity with dose and were generally reversed in the recovery group from the 4-week study.

In the 39-week study (Study 2003-0301), cynomolgus monkeys were administered daily doses of 0, 0.5, 2, or 10 mg/kg/day (0.25, 1, or 5 mg/kg/dose BID) by oral gavage. All

animals in the 0 (control), 0.5, and 2 mg/kg/day dose groups and all male monkeys of the 10 mg/kg/day dose group survived until study termination. One female at 10 mg/kg/day (#30) was euthanized in a moribund condition on day 214. Ulceration/erosions in the stomach, associated with an infiltrative lymphoma, resulted in hemorrhage into the upper gastrointestinal tract and were responsible for the moribund condition.

Male and female monkeys of the 10 mg/kg/day dose group had mildly decreased RBC parameters (RBC count, Hb, Hct) starting on Days 27/28 (89 to 94% of control mean) and continued until the end of the study (80 to 90% of control mean). Minimal decreases in RBC parameters (92 to 93% of control mean) were observed for female monkeys at the 2 mg/kg/day on Day 266. Decreased mean total lymphocyte numbers (57 to 74% of control mean) were observed for female monkeys of the 2 and 10 mg/kg/day dose groups on Days 28, 91, 182, and 266. Female monkeys of the 0.5 mg/kg/day dose group had decreased lymphocyte numbers (74 to 82% of control mean) on Days 91, 182, and 266. Male monkeys of the 10 mg/kg/day dose group had decreased (57 to 77% of control mean) lymphocyte numbers on Days 27, 181, and 265. There were no changes in group mean clinical chemistry values that were considered to be related to treatment with CP-690,550.

Treatment-related gross necropsy observations were limited to three monkeys in the 10 mg/kg/day group (females: # 30, #32; male: #15). Female monkey #30 had increased red discolored fluid in the abdominal cavity and multiple organs were encompassed by firm tissue. Organs that appeared enlarged included the right adrenal, kidneys, and spleen. Male monkey #15 had enlarged bronchial and mesenteric lymph nodes and three tan circumscribed nodules in the liver. Female monkey #32 had an enlarged lymph node which correlated microscopically with lymphocyte hyperplasia. Differences in organ weights among groups were minimal or sporadic and were not attributed to treatment with CP-690,550. However, absolute and relative weight of epididymides and testes at all doses and ovaries at 10 mg/kg/day were decreased in treated animals compared to vehicle controls.

Three monkeys of the 10 mg/kg/dose group (#30, #32 and #15) had lymphomas and multiple monkeys of all treated groups had lymphocyte hyperplasia in lymphoid tissue. Two of the 3 lymphomas were B cell lymphomas and positive for lymphocryptovirus (LCV) by immunohistochemical (EBNA-2) and in situ hybridization (EBER-1) staining. One of the 3 monkeys had a lymphoma in the peri-thymic fat that was determined to be a T cell lymphoma based on immunohistochemical staining.

Male and female monkeys in the 10 mg/kg/day group had erythroid hyperplasia in the bone marrow (sternum) and a mild increase in immature erythroid stages (left shift) in the bone marrow smear. These observations correlated with mildly increased group mean absolute reticulocyte numbers on Days 265/266 for male and female monkeys at 10 mg/kg/day. Additional sternum marrow changes included erythroid hyperplasia for a control male and a male at 0.5 mg/kg/day, but were considered to be incidental because

there were no correlative changes in the marrow smear and peripheral blood erythroid parameters were within normal ranges.

NOAELs were not obtained in the repeated dose toxicology studies due to the immunosuppressive findings that were generally dose related in severity culminating in bacterial or viral infections. This precluded delineating between a nonadverse and adverse pharmacological effect.

The amounts of impurities in the repeated dosing toxicology assessments are indicated in the table below. None of the levels of the impurities were at sufficient levels to adequately to qualify them in these toxicity studies.

Impurities in the Repeated Dose Toxicology Studies [expressed as % and (mcg/day[#]) for the maximum administered dose]

Impurity	Acceptance Criteria	Levels at the Max Clinical Daily Dose	Rat	Monkey	Rat	Monkey
(b) (4)			6-week with 1-month recovery	1-month with 1-month recovery	6-month	39-week
			43798-2-1H*		54422-88-1F*	
	(b) (4)					

NA Not assessed; ND = Not detected;

* from Applicant's Table 2.6.7.4

based on body weight of 350 g for the rat and 4 kg for the monkey, the approximate average of male and female weights in the high dose groups at the end of each study (maximal dose of the 6-week and 6-month rat studies was 100/mg/kg/day, maximal dose of the 1-month monkey study was 100/mg/kg/day and of the 9-month monkey study was 10 mg/kg/day).

Carcinogenicity

Carcinogenicity studies consisted of a 6-month study in CB6F1/Jic-Tg(rasH2) transgenic mice and a 2-year study in Sprague-Dawley (SD) rats.

Mice were treated with oral doses of 25, 75 and 200 mg/kg/day. There was no evidence of CP-690,550-related carcinogenicity, although malignancies were present in the positive control male and female groups (lymphosarcomas, squamous cell tumors of the stomach, and keratoacanthomas (female mice only).

In the 2-year rat carcinogenicity study, the oral doses were 10, 30 and 75 mg/kg/day. In females the high dose was lowered from 100 to 75 mg/kg/day during month 4 of the study because of the early deaths that were due to infection (*Clostridium piliforme*, Tyzzer's Disease). In males there were CP-690,550-related leydig (interstitial cell) tumors in the testis. In females, there were CP-690,550-related thymomas and hibernomas. These findings were confirmed as drug-related by the Executive Carcinogenicity Assessment Committee (ECAC) (March 6, 2012 meeting).

Based on expected impurity exposure (assuming similar ADME and PK parameters between parent compound and the impurity/degradant), the amount of impurity exposure in the rat carcinogenicity study far exceeds, (b) (4), the impurity/degradant exposure of patients at the maximal clinical dose (refer to the Table below). Considering these systemic exposure ratios, the ECAC agreed that the impurities were sufficiently qualified at the proposed specifications.

Impurities in the Carcinogenicity Studies [expressed as % and (mcg/day[#]) for the maximum administered dose]

Impurity	Acceptance Criteria	Levels at the Max Clinical Daily Dose (expected AUC exposure, ng-h/mL)	Mouse, transgenic, 6 month	Rat, 2 year	Expected Impurity Exposure in the Rat [†] (ng-h/mL)	Exposure Margin [‡]
(b) (4)						
Lot			E010008412*	E010006488*		
CP-690,550		10 mg, bid (b) (4)	(b) (4)	(b) (4)		
Impurity						
(b) (4)						

NA Not assessed

* from Applicant's Table 2.6.7.4

based on body weight of 25 g for the mouse and 500 g for the rat, (approximate average of male and female weights in the high dose groups at the end of each study (maximal dose for the mouse study was 200 mg/kg/day CP-690,550 and for the rat study was 75 mg/kg/day CP-690,550).

(b) (4)

‡ [REDACTED] (b) (4)

QSAR Analysis

The structures of the 9 compounds comprising the drug product and drug substance impurities were submitted to the FDA Computational Toxicology group for genetic toxicology analysis. The results of the analysis by 4 different software programs are presented in the table below. Overall, a positive prediction for genetic toxicity was found for 1 impurity in the drug [REDACTED] (b) (4)

[REDACTED] (b) (4) compound, [REDACTED] (b) (4) There was one drug [REDACTED] (b) (4) for which no determination could be made due to inadequate representation of chemical features in the database.

**Summary Table: Overall Calls for Predicting
ICH S2 Battery Genetox Tests**

Chem. No.	Chemical Name	Salm. Mut.	E. coli Mut.
1	[REDACTED] (b) (4)	-	+
2		-	NC
3		-	NC
4		-	NC
5		-	NC
6		+	+
7		-	NC
8		-	NC

The predicted toxicological activities are scored as follows in all tables:

+	Positive
Eqv	Equivocal/marginally active
-	Negative
NSA	No structural alerts identified by DFW
NC	No call can be made because the chemical's structural features are not adequately represented in the model (poor coverage)
N/A	No model available for this endpoint with this software

The specific results from analysis by the 4 different program models are presented below for the two positive overall results, [REDACTED] (b) (4)

Chemical 1: (b) (4)

Genetic Toxicity for Predicting ICH S2 Battery

Software	<i>Salmonella</i> Mutagenicity	<i>E. coli</i> Mutagenicity
Derek Nexus	NSA	NSA
Leadscope	-	+
MC4PC	-	-
SciQSAR	-	N/A
Overall Prediction	-	+

Chemical 2: (b) (4)

Genetic Toxicity for Predicting ICH S2 Battery

Software	<i>Salmonella</i> Mutagenicity	<i>E. coli</i> Mutagenicity
Derek Nexus	NSA	NSA
Leadscope	-	NC
MC4PC	-	NC
SciQSAR	-	N/A
Overall Prediction	-	NC

CONCLUSIONS

The toxicities in the drug substance appear to be due to the expected pharmacodynamic immunosuppressive effects of the drug substance. The contribution of the impurities or degradants to the toxicological profile of CP-690,550 is not known, as none of the impurities or degradants has been toxicologically characterized independent of each other or the API.

Both drug product impurities/degradants, (b) (4), contain the same (b) (4) structural alert that is also present in the API, CP-690,550. There are no genetic toxicological data that adequately qualifies the drug (b) (4) impurities and degradants for safety. However, a battery of genetic toxicology tests of the API indicated it was not genotoxic. Considering the API contains the same structural alerts as the two drug (b) (4) impurities, by extension, these impurities are considered qualified (Draft Guidance for Industry: Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches, Dec 2008).

Due to the lack of specific toxicological information for the drug (b) (4) impurities/degradants, QSAR analysis was also employed to aid in predicting their potential toxicity.

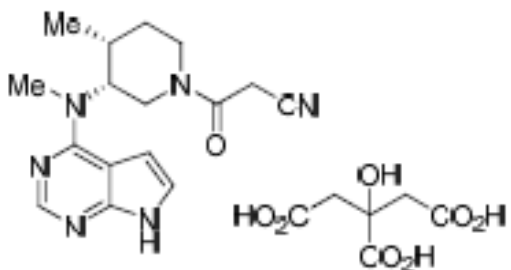
For (b) (4), QSAR analysis was negative for predicting mutagenicity in the Salmonella assay, with insufficient information for a determination in the E. coli assay. Since the (b) (4) moiety is also present in the API which was negative in all the standard tests for genotoxicity, the weight of evidence indicates that (b) (4) is not genotoxic. Therefore, it should be controlled at levels commensurate with ICH-S2B(R2), which is 0.5%, the same level as proposed by the Sponsor.

(b) (4) is present in the drug (b) (4) and as a degradant in the drug (b) (4). QSAR analysis was negative for predicting mutagenicity in the Salmonella assay, but positive in the E. coli assay for predicting mutagenicity. The rat carcinogenicity study indicated API (CP-690,550)-related positive malignancies, but the mouse rasH2 carcinogenicity study was negative. The levels of this compound in the carcinogenicity studies (b) (4) were below the proposed specification limit (b) (4). Given this situation where potentially positive genotoxicity results were negative in a carcinogenicity assay (rasH2 mouse) highly sensitive to mutation-inducing carcinogenic mechanisms, it would seem reasonable to conclude that the amount of (b) (4) in that assay is also not carcinogenic. However, since the rat carcinogenic assay with (b) (4) was positive for neoplasms, a CP-703058 effect cannot be separated from the effect of the API, or even the other impurities in the drug (b) (4). Therefore, an additional comparison was performed of the expected exposure of (b) (4) in the rat based on (b) (4) of the parent drug, with human exposure of the proposed specification based on maximal dose pharmacokinetics. There was a (b) (4) exposure margin in the carcinogenicity study for (b) (4) indicating that the proposed specification of (b) (4) would be toxicologically acceptable.

Discussion with the ECAC on March 6 2012 led to the understanding that since the drug (b) (4) impurity/degradants contain the same structural alert as parent CP-690,550 which was negative in a battery of genetic toxicology assays, therefore the impurities/degradants are also not genotoxic. Also, based on the findings in the carcinogenicity studies, and the estimated exposure of these impurities in those studies, compared with the potential exposure in patients at the maximal recommended dose, there is at least a (b) (4) exposure margin for the impurities/degradants. Whether the impurities/degradants contribute to the neoplastic findings is not known, but the proposed specification level of these impurities/degradants are adequately qualified, being incorporated within the toxicological profile of the API.

RECOMMENDATIONS

- 1) The proposed specification of (b) (4) are acceptable.
- 2) Reduce the impurities in the drug (b) (4) to meet ICH-Q3A(R2) or as low as feasibly possible.

APPENDIX:**Structure of the Drug Product API: CP-690,550 (tofacitinib)****CP-690,550-10** (citrate salt, from Applicant's Figure 2.3.S.1-1)

Molecular Formula: $C_{16}H_{20}N_6O \cdot C_6H_5O_7$ (citrate salt)

Molecular Weight: 504.49 Daltons (citrate salt)

Structure of Impurities in the Drug (b) (4)

Structures of Impurities in the Drug (b) (4)
(from Applicant's Table 2.3.S.3-1)

Identity	Structure	Origin
(b) (4)		(b) (4)

(b) (4)

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/s/

LAWRENCE S LESHIN
03/09/2012

MOLLY E TOPPER
03/09/2012
I concur.

NDA Pharmacology/Toxicology Fileability Check List

NDA **203214**

Product: Tofacitinib

Applicant: Pfizer

Date of submission: Oct 21, 2011

Date of Fileability meeting: Fri, Dec 2, 2011

Date of Filing Review: Dec 5, 2011

Information to Sponsor: Yes () No (X)

(add information to Sponsor at the end of this template)

Filing Checklist: (insert written elaboration where appropriate)

		Yes	No	NA
1	On its face, is the Pharm/Tox section of the NDA organized in a manner to allow substantive review?	X		
2	On its face, is the Pharm/Tox section of the NDA legible for review?	X		
3	Are final reports of all required and requested preclinical studies submitted in this NDA?	X		
	Pharmacology	X		
	ADME	X		
	Toxicology (duration, route of administration and species specified)	X		
	Acute	X		
	Subchronic and Chronic studies	X		
	Mutagenicity studies	X		
	Carcinogenicity studies	X		
	Reproductive studies	X		
	Special studies (Impurity)	X		
	Others	X		
4	<p>If the formulation to be marketed is different from the formulation used in the toxicology studies, is repeating or bridging the studies necessary?</p> <p>If no, state why not:</p> <p>If yes, has the applicant made an appropriate effort to repeat the studies using the to-be-marketed-product, to bridge the studies or to explain why such repetition or bridging should not be required?</p>			X

5	Are the proposed preclinical labeling sections (carcinogenesis, mutagenesis and impairment of fertility, pregnancy category and overdosage) appropriate (including human dose multiples expressed in either mg/m ² or comparative systemic exposure levels) and in accordance with 201.57?	X		
6	Has the applicant submitted all special studies/data requested by the Division prior to the submission including but not limited to pre-NDA discussion?	X		
7	On its face, does the route of administration used in the pivotal toxicity studies appear to be the same as the intended clinical route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
8	Has the applicant submitted a statement(s) that all of the toxicity studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
9	Has the applicant submitted any studies or data to address any impurity or extractable issues (if any)? Consult from CMC to address in drug substance and drug product, structural alerts and specifications for impurities, Applicant claims specifications are covered by previous toxicology studies and DEREK tox software testing.	X		
10	Are there any outstanding preclinical issues? If yes, identify those below:		X	
11	From a preclinical perspective, is this NDA fileable? If no, state below why it is not:	X		
12	Should any additional information/data be requested?		X	

Additional Comments: none

Information to the sponsor: None

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

/s/

LAWRENCE S LESHIN
12/05/2011

MOLLY E TOPPER
12/05/2011
I concur.