# CENTER FOR DRUG EVALUATION AND RESEARCH

## APPROVAL PACKAGE FOR:

## APPLICATION NUMBER

## 21-743

# Pharmacology Review(s)

# MEMORANDUM

**Date:** October 13, 2004
**From:** John K. Leighton, Ph.D., DABT
Supervisory Pharmacologist, HFD-150
**To:** File for NDA #21-743
**Re:** Approvability for Pharmacology and Toxicology
Tarceva (erlotinib; OSI-774)

**Background:**

Erlotinib is a small molecule epidermal growth factor receptor (EGFR) inhibitor for the treatment of patients with non small-cell lung cancer. The sponsor has conducted a complete battery of toxicology studies, including safety pharmacology, genetic toxicology (ICH battery), general toxicology studies in rats and dogs, and reproductive toxicology (Segments I-III). These studies have been reviewed in detail by Dr. Benson. The recommended Pregnancy Category is "D", based on mechanism of action and findings (abortifacient, etc) for similar products previously reviewed and approved by the Division. Carcinogenicity studies have not been conducted and are not required for this indication, as per CDER practice and ICH guidance. These studies were summarized in the Executive Summary presented in her review. Also discussed in the Executive Summary are comments on the proposed label.

Dr. Benson noted that the pharmacology studies conducted by the sponsor are limited in breath and scope. Specificity of erlotinib as an EGFR inhibitor could not be ascertained due to the limited evaluation for inhibition of related thymidine kinases. These and other pharmacology studies would have been informative to the nonclinical safety assessment, but they generally do not impact on the approvable decision. No additional pharmacology studies by the sponsor are required as a condition of approval.

An impurity in the manufacturing process [ ] was present in approximately half the batches used in toxicology testing, including the long-term studies. It was not present in any batches used for genetic toxicity testing of erlotinib, but was separately tested in the Ames mutagenesis assay. If the Sponsor seeks indications for which carcinogenesis studies are necessary, then care should be taken to fully assess the genetic toxicity and carcinogenic potential of this impurity, if warranted by ICH guidance on impurities in new drug substances (Q3A).

**Recommendations:**

The pharmacology and toxicology data supports approval of this NDA. There are no outstanding issues that need to be addressed.

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

/s/
---
John Leighton
10/15/04 11:57:22 AM
PHARMACOLOGIST

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

# PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

| | |
|---|---|
| NDA NUMBER: | **21-743** |
| SERIAL NUMBER: | **001** |
| DATE RECEIVED BY CENTER: | **01/20/04** |
| PRODUCT: | **Tarceva** |
| INTENDED CLINICAL POPULATION: | **Locally advanced or metastatic NSCLC** |
| SPONSOR: | **OSI Pharmaceuticals** |
| DOCUMENTS REVIEWED: | **Module 4 – Nonclinical Study Reports** |
| REVIEW DIVISION: | **Division of Oncology Drug Products (HFD-150)** |
| PHARM/TOX REVIEWER: | **Kimberly A. Benson, Ph.D.** |
| PHARM/TOX SUPERVISOR: | **John K. Leighton, Ph.D.** |
| DIVISION DIRECTOR: | **Richard Pazdur, M.D.** |
| PROJECT MANAGER: | **Paul Zimmerman** |

Date of review submission to Division File System (DFS): 6 October 2004

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# *EXECUTIVE SUMMARY*

## I. Recommendations

A. Recommendation on approvability
Approvable. The non-clinical studies with oral erlotinib support the safety of its use in NSCLC.

B. Recommendation for nonclinical studies
The pharmacological studies have investigated the mechanism of action for erlotinib. Although the sponsor did not fully characterize the effects that erlotinib has on many other tyrosine kinases, it is not necessary that additional studies be conducted for approval. In addition, given the information that is currently known about both wild-type and mutant EGFR, it would be interesting to have a greater understanding on how erlotinib works against both types of receptors. Again, this is not something that would be required for approval of the drug, from a pharmacology/toxicology perspective.

The battery of nonclinical studies has sufficiently examined the toxicity of erlotinib in several animal models, identifying the target organs of toxicity. Mutagenicity, clastogenicity, effects on fertility and reproductive toxicology have all been addressed. Although the long-term dog study may have benefited from a dose higher than the final high dose tested, additional toxicology studies would not be needed nor likely shed any additional light on what is already known about the toxicology of erlotinib.

Carcinogenicity studies were not conducted with erlotinib and are not needed given the indication.

C. Recommendations on labeling

The sponsor proposed:
**Mechanism of Action**

C

J

We recommend:
**Mechanism of Action**
The mechanism of clinical antitumor action of erlotinib is not fully characterized. Erlotinib inhibits the intracellular phosphorylation of tyrosine kinase associated with the epidermal growth factor receptor (EGFR). Specificity of inhibition with regard to other tyrosine kinase receptors has not been fully characterized. EGFR is expressed on the cell surface of normal cells and cancer cells.

Reasoning:
The sponsor conducted studies in several tyrosine kinases, showing greater inhibition by erlotinib on the EGFR tyrosine kinase, relative to the kinases tested. However, erlotinib was never tested for its ability to inhibit many other kinases, including several kinases similar to EGFR (ErbB1) such as ErbB2, ErbB3 and ErbB4. At least 90 protein-tyrosine kinases and 43 tyrosine-kinase like proteins are now recognized [Roskoski, R., BBRC: 319(2004), pg. 1-11]. Additional studies with a battery of other tyrosine kinases may further characterize erlotinib's mechanism of action. However, these studies are not required for approval.

The sponsor proposed:
**Metabolism and Elimination**

[ ]

We recommend:

[ ]

Reasoning:
No data was presented by which a claim could be made regarding [ ]

The sponsor proposed:
**Carcinogenesis, Mutagenesis, Impairment of Fertility:**

[ ]

We recommend:
**Carcinogenesis, Mutagenesis, Impairment of Fertility:**
Erlotinib has not been tested for carcinogenic [ ]
Erlotinib has been tested for genotoxicity in a series of in vitro assays (bacterial mutation, human lymphocyte chromosome aberration, and mammalian cell mutation) and an in vivo mouse bone marrow micronucleus test. [ ]

Reasoning:
For continuity sake, because the heading lists carcinogenicity first. The information presented in the paragraph should be presented in the order listed in the heading.

The sponsor proposed:
**Pregnancy Category –**

Erlotinib has been shown to cause maternal toxicity with associated embryo/fetal lethality and abortion in rabbits when given at doses that result in plasma drug concentrations of approximately - imes those in humans (AUCs at 150 mg daily dose). [

]

No teratogenic effects were observed in rabbits or rats.

There are no adequate and well-controlled studies in pregnant women using TARCEVA. Women of childbearing potential – be advised to avoid pregnancy while on TARCEVA. Adequate contraceptive methods should be used during therapy, and for at least 2 weeks after completing therapy. Treatment should only be continued in pregnant women if the potential benefit to the mother outweighs the risk to the fetus. If TARCEVA is used during pregnancy, the patient should be apprised of the potential hazard to the fetus or potential risk for loss of the pregnancy.

We recommend:
**PREGNANCY CATEGORY D**
Erlotinib has been shown to cause maternal toxicity with associated embryo/fetal lethality and abortion in rabbits when given at doses that result in plasma drug concentrations of approximately 3 times those in humans (AUCs at 150 mg daily dose). [

]

No teratogenic effects were observed in rabbits or rats.

There are no adequate and well-controlled studies in pregnant women using TARCEVA. Women of childbearing potential should be advised to avoid pregnancy while on TARCEVA. Adequate contraceptive methods should be used during therapy, and for at least 2 weeks after completing therapy. Treatment should only be continued in pregnant women if the potential benefit to the mother outweighs the risk to the fetus. If TARCEVA is used during pregnancy, the patient should be apprised of the potential hazard to the fetus or potential risk for loss of the pregnancy.

Reasoning:
We recommend a pregnancy category D due to the embryo/fetal lethality that may be of consequence during the early stages of a pregnancy.

Comparison of the AUC(0-24) at the point that the rabbits aborted their litters, with the AUC(steady state) in the clinical trials, shows that the rabbit AUC is about 3 times that of the human. The next lower dose in the rabbit study has an AUC that is approximately equal to that in the human. Human = 41.3 h·μg/mL, rabbit higher dose = 162 h·μg/mL and rabbit lower dose = 31.6 h·μg/mL.

We recommend adding the embryo/fetal lethality seen in the rat Segment I study. Although this was not seen in the main Segment II study, it was seen in the Pilot Segment II study and the embryo/fetal lethality in the rat adds additional weight to the embryo/fetal lethality seen in the rabbit.

We also recommended the advice regarding women of childbearing years be changed from ' [redacted] ' to "should be advised".

## II. Summary of nonclinical findings

A. Brief overview of nonclinical findings:

The nonclinical findings have shown the target sites of toxicity with erlotinib to be gastrointestinal, hepatobiliary, renal, ocular and dermatological. Many of these toxicities are seen in the clinic and are direct effects of the pharmacology of erlotinib. Nonclinical studies showed minimal distribution of erlotinib across the blood-brain-barrier in the rat.

Erlotinib was not mutagenic or clastogenic in the *in vitro* and *in vivo* assays studied. Additionally, an impurity that may be present in the formulation of erlotinib also tested negative for mutagenicity and clastogenicity.

Erlotinib did not impair fertility when administered to either male or female rats prior to and during the mating time frame. It was not teratogenic in either the rat or the rabbit.

B. Pharmacologic activity

The pharmacological activity of erlotinib is inhibition of EGFR tyrosine kinase phosphorylation. Studies in cell lines and enzyme assays have both shown that erlotinib inhibits EGFR at concentrations significantly lower than those needed to inhibit *c-src* and *v-abl*. While erlotinib was more selective for EGFR tyrosine kinase than it was for several others tested, the sponsor did not run a comprehensive battery of tyrosine kinase assays. Several tyrosine kinases in the same family as EGFR were not tested. So while erlotinib appears rather selective for EGFR, insufficient data exist to make a definitive decision regarding selectivity.

Erlotinib showed efficacy in several mouse xenograft models. Inhibition of tumor growth and in some cases decreased tumor size, was seen with erlotinib administration to the mice injected with tumor cell lines. In combination therapy, suboptimal doses of gemcitabine or cisplatin and suboptimal doses of erlotinib were less toxic than the MTD doses of the drugs alone. A combination of the MTD doses of erlotinib and either of these chemotherapeutics proved to be far too toxic. The suboptimal combinations were

more efficacious at inhibiting tumor growth than the suboptimal single agent treatment alone. Combinations were, however, equally as efficacious as the MTD of the single agents, while being less toxic.

C. Nonclinical safety issues relevant to clinical use
Nonclinical pharmacokinetic studies with erlotinib have shown that the oral bioavailability ranges from 45-88%. Although there was a great degree of variability in the nonclinical models, the pharmacokinetics of erlotinib appear non-linear at doses up to 100 mg/kg, with a greater than dose-proportional increase seen in AUC as doses increase. At higher doses than 100 mg/kg, linear or less than dose-proportional increases in AUC are seen. Repeat-dose animal studies show a Tmax for erlotinib between 1-2 hrs. Organ distribution studies with $^{14}C$-labeled drug show that the radioactivity is essentially cleared from the animals by the 72 hr time point. Erlotinib has been shown to inhibit UDP-glucuronosyltransferase, the enzyme that conjugates bilirubin. Metabolism primarily involves the P450 isozymes CYP3A4 and CYP1A2, though extrahepatic isozymes may also be involved, including CYP1A1 and CYP1B1. Induction or inhibition of these enzymes does not appear to be occurring with erlotinib exposure. The primary excretion route of erlotinib is fecal, as mass balance studies show minimal recovery in the urine and the majority of the drug and metabolite recovery in the feces. Tissue distribution studies have shown erlotinib is widely distributed throughout the body, though there is little transport across the blood-brain-barrier. Protein binding of erlotinib was very similar across species, with mice, rats, dogs and humans showing 85-95% of erlotinib bound to plasma proteins. The metabolic profiles of erlotinib are very similar in the rat, dog and human.

The sponsor refers to the O-desmethylation metabolites OSI-413 and OSI-420 as active metabolites throughout the NDA. No data are presented to verify the efficacy of these main metabolites in EGFR inhibition. The presence of these metabolites has been noted in all the animals tested. Thus, the toxicity of these metabolites has sufficiently been investigated by the nature of their presence in the animals tested in the toxicology program. The genetic toxicity of the metabolites would also have been tested in the *in vivo* genetic toxicology studies.

The nonclinical safety issues seen in the toxicology program with erlotinib were mostly toxicities that could be suspected from a drug of this class. With tyrosine kinase inhibitors, rash has been one of the major toxicities, a toxicity that is speculated to be correlated to the efficacious activity of the drug, though that has yet to be proven.

Clinically, the DLT for erlotinib has been gastrointestinal. Doses higher than 150 mg/day have had to be discontinued due to diarrhea. Even the 150-mg dose has shown significant diarrhea problems, though treatment can usually continue with concomitant loperamide treatment.

Renal toxicity has been problematic in a few nonclinical reports. Upon necropsy, renal papillary necrosis and tubule dilatation were noted in the rat and dog. Few markers of this toxicity were noted during the execution of the study, however, making

determination of the potential for this problem in the clinic difficult. Urinalysis showed some decreased urine output, hematuria, and decreased urine pH in the dog. The renal function safety pharmacology study showed no erlotinib-related findings, however. In addition, to date no renal toxicity has been seen clinically.

The hepatobiliary system is another site of toxicity of erlotinib. The increased bilirubin is consistent across species in the nonclinical studies. Given that erlotinib is known to inhibit the enzyme that conjugates bilirubin, it is expected that increases in total bilirubin would be seen with erlotinib administration. Hepatic toxicity is of concern with erlotinib. The nonclinical studies have shown both consistent increases in total bilirubin along with increases in liver enzymes, an increase that became more prevalent and severe with repeated administration.

The ovaries have also exhibited toxicity of erlotinib. Following both IV and PO administration to rats, ovarian atrophy was seen during histopathology. No changes in fertility or mating indices were seen in the rat, however.

In the dog, ocular toxicity was severe at higher doses. These dogs had corneal ulcerations that were preceded by redness of palpabral and bulbar conjunctiva, lacrimation, purulent discharge, and protruding nictitating membranes. Attention should be paid to any ocular changes that may be indicative of a similar toxicity in the clinic. To date, one instance of corneal ulceration has been noted and it was believed that this was related to contact lens wearing. A possible exacerbation of erlotinib toxicity by the wearing of contact lenses can't be ruled out. Some patients have been advised to not wear their contact lenses while taking erlotinib.

The toxicities of erlotinib in nonclinical models are generally not severe and are expected as direct pharmacological actions of an EGFR tyrosine kinase inhibitor.

# *2.6 PHARMACOLOGY/TOXICOLOGY REVIEW*

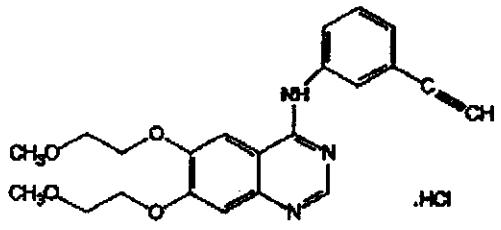
## 2.6.1 INTRODUCTION AND DRUG HISTORY

| | |
|---|---|
| **NDA number:** | **21-743** |
| **Review number:** | **1** |
| **Sequence number/date/type of submission:** | **01/20 January 2004/NDA** |
| **Information to sponsor:** | Yes ( ) No (X) |
| **Sponsor and/or agent:** | OSI Pharmaceuticals |
| **Manufacturer for drug substance:** | [redacted] |

| | |
|---|---|
| **Reviewer name:** | Kimberly A. Benson, Ph.D. |
| **Division name:** | Division of Oncology Drug Products |
| **HFD #:** | HFD-150 |
| **Review completion date:** | 27 September 2004 |

**Drug:**

| | |
|---|---|
| Trade name: | Tarceva |
| Generic name: | Erlotinib |
| Code name: | OSI-774; OSI-774-01; RO0508231; CP-358,774-01 |
| Chemical name: | N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)-4-quinazolinamine, monohydrochloride |
| CAS registry number: | |
| Molecular formula/molecular weight: | $C_{22}H_{23}N_3O_4 \cdot HCl$/429.9 (as hydrochloride salt) 393.4 (as free base) |
| Structure: | |



**Relevant INDs/NDAs/DMFs:** IND# 53,728

**Drug class:** Tyrosine kinase inhibitor

**Intended clinical population:** "TARCEVA is indicated for the treatment of patients with locally advanced or metastatic non-small cell lung cancer after failure of at least one prior chemotherapy regimen."

**Clinical formulation:**

| Tarceva® Tablet Composition – Tablet Core, Film Coat, And Printing Ink Solution | | | | | |
|---|---|---|---|---|---|
| | | | 25 mg | 100 mg | 150 mg |
| Component | Function | Quality Standard | mg/tablet | mg/tablet | mg/tablet |
| **Tablet Core** | | | | | |
| Erlotinib hydrochloride | Active Ingredient | Manufacturer | 27 | 109 | 163 |
| Lactose monohydrate | | NF | | | |
| Microcrystalline cellulose | | NF | | | |
| Sodium starch glycolate | | NF | | | |
| Sodium lauryl sulfate | | NF | | | |
| Magnesium stearate | | NF | | | |
| **Total Weight** | | | 100.00 | 300.00 | 450.00 |
| **Film Coat** | | | | | |
| Tablet core white | --- | --- | 100.00 | 300.00 | 450.00 |
| Purified water | | DMF Holder | | | |
| **Total Weight** | | | | | |
| **Printing Ink Solution** | | | | | |
| Tablet (Core + Film Coat) | | | | | |
| Orange[b] Product Identifier | Tablet Identifier | DMF Holder | | --- | --- |
| Gray[b] Product Identifier | Tablet Identifier | DMF Holder | --- | | --- |
| Maroon[b] Product Identifier | Tablet Identifier | DMF Holder | --- | --- | |
| **Total Weight** | | | Not significantly changed from film coated tablet | | |

[a] - Based on a drug substance potency of

[b] – Additional information relating to the qualitative and quantitative composition are incorporated by reference to DMF

**Route of administration**: Oral Tablets

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

**Studies reviewed within this submission:**

**PHARMACOLOGY**

**Primary Pharmacodynamics**

**OSI Study # I970176:** Induction of apoptosis and cell cycle arrest by CP 358,774, an inhibitor of epidermal growth factor receptor tyrosine kinase.
**OSI Study # M2003116:** The In vivo Cell Cycle Effects of Tarceva Using BrdU Incorporation Analysis in Human Tumor Xenografts.
**OSI Study # M990223:** Inhibition of epidermal growth factor receptor-associated tyrosine phosphorylation in human carcinomas with CP-358,774: dynamics of receptor inhibition in situ and antitumor effects in athymic mice.
**OSI Study # M2002314:** Evaluation of Tarceva™ in Combination with Gemcitabine or Cisplatin in Female CRL Nu/nu Mice Bearing the A549 Human NSCLC Xenograft Model.

**Secondary Pharmacodynamics**

**OSI Study # I960031:** General Pharmacology Evaluation of CP-358,774: Part 1.
**OSI Study # V2001140:** Evaluation of Actions on K+ Currents through Recombinant HERG Channels Expressed in HEK293 Cells.
**OSI Study # V2001141:** Evaluation of Actions on K+ Currents through Recombinant HERG Channels Expressed in CHO Cells.
**OSI Study # V2001151:** Evaluation of Effect on Cardiac Action Potential in Isolated Rabbit Purkinje Fibers.
**OSI Study # I960007:** General Pharmacology Evaluation of CP-358,774: Part 2.

**Safety Pharmacology**

**OSI Study # I960007:** General Pharmacology Evaluation of CP-358,774: Part 2.
**OSI Study # I960031:** General Pharmacology Evaluation of CP-358,774: Part 1.
**OSI Study # R2002124:** A Neurobehavioral Evaluation of OSI-774 Using a Functional Observation Battery in the Rat.
**OSI Study # R2002208:** A Pharmacologic Assessment of the Effect of OSI-774-01 on the Respiratory System of the Albino Rat.
**OSI Study # D2001142:** Evaluation of Effects on Blood Pressure, Heart Rate, and Electrocardiogram After Single Oral Administration to Conscious Dogs.

**Absorption**

**OSI Study # M2000138:** Plasma and Tumor Concentrations of CP-358,774 in Female Athymic Nude-nu Mice Administered Oral or Intraperitoneal Doses of CP-358,774.
**OSI Study # M2001148:** Evaluation of the Efficacy of the Roche EGFR Inhibitor Erlotinib (Tarceva, RO0508231) and Gefitinib (Iressa, RO332843) in the A549 Non-Small Cell Lung Cancer Xenograft Model.
**OSI Study # M2001152:** RO0508231 (Tarceva / OSI-774). Pharmacokinetic Monitoring in an Efficacy Study in the H460a Non-small Cell Lung Cancer Xenograft Model.
**OSI Study # R2002319:** RO0508231 (Tarceva/OSI-774): Pharmacokinetics in Female Fischer 344 Rats Following Single Oral Dose Administration of 20 and 100 mg/kg.
**OSI Study # D960008:** Pharmacokinetics of CP-358,774 in Dogs.

**Distribution**
**OSI Study # M960018:** Tissue Distribution of CP-358,774 in HN-5 Tumor-Bearing and Non Tumor-Bearing Athymic, nu/nu (nude), Female Mice.
**OSI Study # R2002318:** RO0508231: A Study of Distribution in the Rat by Quantitative Whole-Body Autoradiography.

**Protein Binding**
**OSI Study # I2001125:** Binding of CP-358,774 in Plasma.
**OSI Study # I960015:** Protein Binding of CP-373,420 in Mouse, Rat, Dog, and Human Plasma.

**Metabolism**
***In Vitro Metabolism***
**OSI Study # V950015:** Species Comparison of the Metabolic Half-life of EGFr Kinase Inhibitors in Rat, Dog and Human Liver Microsomes.
**OSI Study # V960014:** Identification of Metabolites of CP-358,774 in Rat and Human Liver Microsomes.
**OSI Study # V970162:** In vitro Metabolic Stability of EGFr Kinase Inhibitor CP-358,774 in Rat Liver and Lung Microsomes - Correlation with CYP1A1 Activity.

***In Vivo Metabolism***
**OSI Study # R960021:** Identification of Metabolites of CP-358,774 in Sprague-Dawley Rats Following Oral Administration of a Single 5 mg/kg Dose of [14C]-CP-358,774-01.
**OSI Study # D970157:** Metabolism and Excretion of CP-358,774 in Beagle Dogs After Oral Administration of a Single 5 mg/kg Dose of [14C]-CP-358,774-01.

**Excretion**
**OSI Study # R960020:** Mass Balance of CP-358,774 in Sprague-Dawley Rats Following Oral Administration of a Single 5 mg/kg Dose of [14C]-CP-358,774-01.
**OSI Study # R2002312:** RO0508231 (erlotinib): Excretion of Total Radioactivity in the Rat Following Oral and Intravenous Administration of [$_{14}$C]-RO0508231 (Tarceva; OSI-774).

**OSI Study # D970157:** Metabolism and Excretion of CP-358,774 in Beagle Dogs After Oral Administration of a Single 5 mg/kg Dose of [14C]-CP-358,774-01.

**TOXICOLOGY**

**Repeat-dose Toxicity**

**OSI Study # R980200:** 6-Month Oral Toxicity Study in Sprague-Dawley Rats.
**OSI Study # D980197:** 12-Month Oral Toxicity Study in Beagle Dogs.

**Genotoxicity**

***In Vitro***

**OSI Study # V950006:** Genetic Toxicology Report: Microbial Reverse Mutation Assays.
**OSI Study # V950007:** Genetic Toxicology Report: In Vitro Cytogenetic Assays.
**OSI Study # V950008:** Genetic Toxicology Report: Mammalian Mutation Assays.

***In Vivo***

**OSI Study # M950010:** Genetic Toxicology Report: In Vivo Mouse Micronucleus Assay.

**Reproductive and Developmental Toxicity**

***Fertility and Early Embryonic Development***

**OSI Study # R2003114:** RO0508231: Segment I (Tarceva): Oral Study of Male Fertility in the Rat.
**OSI Study # R2003115:** RO0508231: Segment I (Tarceva): Oral Study of Female Fertility and Early Embryonic Development in the Rat.

***Embryo-Fetal Development***

**OSI Study # R2002308:** Ro 50-8231/001 (Tarceva): Oral Pilot Study for Effects on Embryo-Fetal and Postnatal Development in the Rat.
**OSI Study # R2003113:** RO0508231: Segment II (Tarceva): Oral Study for Effect on Embryo-Fetal Development in the Rat.
**OSI Study # B2002281:** Oral (Stomach Tube) Developmental Toxicity Study of OSI 774 01 In Rabbits.

***Prenatal and Postnatal Development***

**OSI Study # R2002242:** An Oral Tarceva Maternal Function, and Pre- and Postnatal Development Study in Rats.

**Studies not reviewed within this submission:**

**PHARMACOLOGY**

**Primary Pharmacodynamics**

**OSI Study # M2003158:** Tarceva Combination Efficacy Studies in Solid Tumor Xenograft Models.

**PHARMACOKINETICS/TOXICOKINETICS**

**Analytic Methods and Validation Reports**

**OSI Study # A960032:** Validation Report: The Measurement of CP-358,774 and Metabolite CP-373,420 in Dog Plasma and Cross-Validated in Human, Rat and Mouse Plasma.

**OSI Study # A970163:** Assay Validation for CP-358,774 in Human Plasma and Cross Matrix Compatibility in Rat and Dog Plasma.

**OSI Study # A2002322:** Validation of a High Performance Liquid Chromatographic Mass Spectrometric Method for the Determination of OSI-774 and OSI-420 in Rat Plasma (Heparin) Specific to OSI Pharmaceuticals.

**OSI Study # A2002321:** Validation of a High Performance Liquid Chromatographic Mass Spectrometric Method for the Determination of OSI-774 and OSI-420 in Rabbit Plasma (Heparin) Specific to OSI Pharmaceuticals.

**OSI Study # A2001156:** Validation of a High Performance Liquid Chromatographic Mass Spectrometric Method for the Determination of OSI-774 and OSI-420 in Dog Plasma (Heparin) Specific to OSI Pharmaceuticals.

**OSI Study # A2002320:** Validation of a High Performance Liquid Chromatographic Mass Spectrometric Method for the Determination of OSI-774 and OSI-420 in Monkey Plasma (Heparin) Specific to OSI Pharmaceuticals.

**OSI Study # A2002323:** RO0508231 (Erlotinib): Validation of a High Performance Liquid Chromatographic Mass Spectrometric Method for the Determination of OSI-774 and OSI-420 in Mouse Plasma (EDTA) Specific to Hoffmann-La Roche Ltd.

**Absorption**

**OSI Study # M2001153:** RO0508231 (Tarceva/Erlotinib/OSI-774): Pharmacokinetics Following Single Oral Administration of 20 mg/kg and 100 mg/kg of RO0508231-001 to Nu/Nu Athymic Mice.

**OSI Study # M2001154:** RO0508231 (Tarceva/ Erlotinib/ OSI-774): Pharmacokinetics Following Single Oral Administration of 20 mg/kg and 100 mg/kg of RO0508231-001 to Nu-Nu Athymic Mice.

**OSI Study # M2001155:** RO0508231 (Erlotinib/ Tarceva TM / OSI-774): Pharmacokinetics Following Single Oral Administration of 20 and 100 mg/kg of RO0508231-001 to Nu-Nu Athymic Mice Using Captisol®.

**OSI Study # M2002315:** RO0508231 (Erlotinib/ Tarceva/OSI-774): Maximally Tolerated Dose (MTD) Study of the EGFR Inhibitor Erlotinib (Tarceva™, OSI-774) in Female Athymic Nu/Nu Mice Following a 2-week Administration in Captisol® Formulation.

**OSI Study # M2002316:** RO0508231 (Tarceva/OSI-774): Maximally Tolerated Dose (MTD) Study of the EGFR Inhibitor Erlotinib (Tarceva™, OSI-774) in Female Athymic Nu/Nu Mice Following a 2-Week Administration in Carboxymethylcellulose Formulation.

**OSI Study # R960019:** Pharmacokinetics of CP-358,774 in Sprague-Dawley Rats.

**OSI Study # R970165:** Pulmonary First-Pass Clearance of CP-358,774 in Sprague-Dawley Rats.

**OSI Study # D2002313:** RO0508231-001 (Tarceva/ OSI-774): Pharmacokinetics of RO0508231 and Active Metabolites in Fed Dogs After Oral Administration of 20 mg/kg Micronized Material Using Carboxymethylcellulose (CMC) and Hydroxyethylcellulose (HEC) Formulations in Cross-Over Design.
**OSI Study # D960009:** Pharmacokinetics in Beagle Dogs Administered Single IV Doses and PO Doses of the Mesylate and Hydrochloride Salts of CP-358,774 in Fed and Fasted States.
**OSI Study # P2001138:** Evaluation of the Pharmacokinetics Following a Single Administration of Two Intravenous and Two Oral Formulations of OSI-774-01 to Male Cynomolgus Monkeys: A 4-way Crossover Study.

**Metabolism**

***In Vitro Metabolism***

**OSI Study # V950014:** Identification of Metabolites of CP-358,774.
**OSI Study # V960013:** In vitro Metabolic Stability of CP-358,774 in Human and Rat Liver Microsomes - Correlation with CYP1A Activity.

***In Vivo Metabolism***

**OSI Study # R970167:** Determination of Plasma Concentrations of CP-358,774 and Total Radioactivity in Sprague-Dawley Rats After Oral Administration of [14C]-CP-358,774-01.
**OSI Study # D970158:** Determination of Plasma Concentrations of CP-358,774 and Total Radioactivity in Beagle Dogs After Oral Administration of [14C]-CP-358,774.

**TOXICOLOGY**

**Single-dose Toxicity**

**OSI Study # D2002239:** An Oral and Intravenous Toxicity Study of OSI-774-01 in Beagle Dogs.
**OSI Study # D950001:** Exploratory Toxicokinetic and Toleration Study in Beagle Dogs.
**OSI Study # D950004:** Exploratory Dose Escalation Study in Beagle Dogs.
**OSI Study # D2002261:** An Intravenous Toxicity Study of OSI-774-01 in Beagle Dogs.

**Repeat-dose Toxicity**

**OSI Study # M960003:** Oral 14-Day Exploratory Toxicity Study in Mice with CP-358,774-01.
**OSI Study # R950013:** Exploratory Toleration Study in Sprague-Dawley Rats.
**OSI Study # R2001119:** Preliminary Toxicity Study by Intravenous (Bolus) Administration to CD Rats for 2 Weeks.
**OSI Study # 2001121:** Toxicity Study by Intravenous (Slow bolus) Administration to CD Rats for 4 Weeks.
**OSI Study # D950002:** Exploratory Toleration Study in Beagle Dogs.
**OSI Study # D2001122:** Toxicity Study by Oral Gavage to Beagle Dogs for 28 Days.
**OSI Study # P970154:** Exploratory Toleration Oral Toxicity Study in Cynomolgus Monkeys.

***Embryo-Fetal Development***

**OSI Study # B2002229:** A Pilot (Non-GLP) Oral Embryo-Fetal Development Study of Tarceva in New Zealand White Rabbits.
**OSI Study # B2002296:** Exploratory Oral Tarceva Dose-Range-Finding PK and Embryo Survival Study in New Zealand White Rabbits.

**Local Tolerance**

**OSI Study # B980199:** A Single Dose Dermal Toxicity Study in Rabbits and a Single Dose Ocular Irritation Study in Rabbits.
**OSI Study # G980198:** Skin Sensitization Test (Guinea Pig Maximization Test).
**OSI Study # V2001128:** Ro 50-8231/001: Phototoxicity Study (In Vitro).
**OSI Study # D2001122:** Toxicity Study by Oral Gavage to Beagle Dogs for 28 Days.
**OSI Study # R2001127:** Ro 50-8231/001 (Epidermal Growth Factor Receptor Antagonist): Study of Acute Phototoxicity in the Hairless Rat.

**Other Toxicity Studies**

***Mechanistic Studies***

**OSI Study # R970156:** Exploratory Toleration Study in Sprague-Dawley Rats.
**OSI Study # V2001117:** Inhibition Kinetics of OSI 774 with Bilirubin UGT and the Inhibitory Effect of OSI 774 on Other Human UGT Isoforms.
**OSI Study # V2001131:** Results of OSI774 Interactions With Bilirubin Glucuronidation in vitro.
**OSI Study # V2001133:** Preliminary Assessment of In vitro Glucuronidation of OSI 420 and Inhibition of Bilirubin UDP-Glucuronosyltransferase by OSI-420.
**OSI Study # V2001134:** The Inhibition of Cynomolgus Monkey Liver Microsomal Glucuronidation of Bilirubin by OSI-774.

***Impurities***

**OSI Study # V2003148:** RO0729853-001: Chromosome Aberration Test with Human Peripheral Blood Lymphocytes
**OSI Study # V2003149:** RO0729853-001: Bacterial Reverse Mutation Test (Ames Test).

**Studies reviewed in original IND**

**Single-dose Toxicity**

**OSI Study # I950009:** Single-Dose Oral and Intravenous Toxicity Studies In Mice and Rats.

**Repeat-dose Toxicity**

**OSI Study # R960005:** One Month Oral Toxicity Study In Sprague-Dawley Rats.
**OSI Study # D950003:** Exploratory Toleration Study in Beagle Dogs.
**OSI Study # D960001:** One Month Oral Toxicity Study In Beagle Dogs.

## 2.6.2 PHARMACOLOGY

### 2.6.2.1 Brief summary

General pharmacology studies were conducted to determine the mechanism of action of erlotinib. Using *in vitro* and *in vivo* assays, the effects of erlotinib were examined on enzyme activity, cell lines and in xenograft models with the athymic mouse. The inhibition of EGFR by erlotinib was compared to that seen with *c-src* and *v-abl* and significantly larger concentrations of erlotinib were needed to inhibit the other enzymes when compared to the inhibition of EGFR. The specificity for erlotinib for the inhibition of recombinant EGFR kinase domain was also compared to insulin receptor kinase domain and IGF-IR kinase domain, with far greater inhibition seen with the EGFR. Erlotinib and its effects on DNA synthesis inhibition were compared when the cells were incubated with EGF, PDGF, bFGF, IGF-I showed greater inhibition of the EGF stimulated DNA synthesis. While these studies do show a significant effect of erlotinib on the EGFR kinase and significant specificity, there are many more tyrosine kinases that were not tested. A true claim of specificity for EGFR can not be made when other kinases within the same kinase family, such as ErbB2, ErbB3 and ErbB4, were not investigated. Despite the information regarding correlations between EGFR mutations and the efficacy of other tyrosine-kinase inhibitors, no studies have been conducted to examine differences in effects of erlotinib on the wild-type or mutated EGFR.

*In vivo* studies have used the athymic mouse xenograft model with several tumor cell lines injected. Cell lines included HN5, A431, H460a, A549, MDA-MB-468 and DiFi. Assays that were used included examination of the BrdU incorporation and flow cytometric analysis of cell cycle distribution of an erlotinib-treated HN5 cell line to investigate the effects of erlotinib on the cell cycle. This and *in vitro* studies have led to the belief that erlotinib exerts its effects via a partial $G_1$ cell cycle arrest. Xenograft models with several human tumor cell lines have shown that erlotinib not only inhibits EGFR-associated tyrosine phosphorylation but that it arrested tumor growth and slightly decreased tumor volume.

Several *in vivo* studies were conducted using erlotinib in combination with known chemotherapeutic drugs, gemcitabine or cisplatin. When the compounds were combined at their MTDs, the toxicity was significant and animals were found moribund. When the two drugs were combined at sub-optimal doses, 1/4 MTD, the toxicity was less than that seen with the chemotherapeutic at MTD and the tumor growth inhibition was comparable.

Secondary pharmacology studies have shown that erlotinib has low affinity for peripheral benzodiazepine, adenosine $A_1$, and μ-opiate receptors, with $IC_{50}$ concentrations over 100-fold greater than that needed for EGFR inhibition. Additional studies showed some reduction in basal beating of the isolated guinea pig atria preparation and a reduction in histamine-induced contraction of the isolated guinea pig ileum. Erlotinib also blocked the potassium currents through recombinant HERG channels. In the rabbit Purkinje

fibers, erlotinib had no effect on action potential duration, amplitude, maximum upstroke velocity, or resting membrane potential. Erlotinib was neither proconvulsant or anticonvulsant in the pentylenetetrazole-induced convulsion test in mice. Problems related to these study endpoints have not been observed clinically.

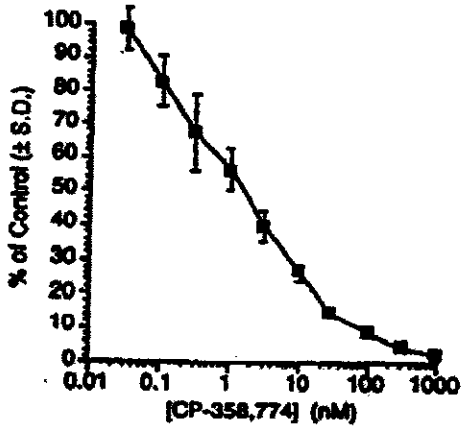
Safety pharmacology studies showed erlotinib to be a relatively safe compound. There was no effect on cardiovascular, renal, central nervous or respiratory systems. The only effect that was seen was a dose-dependent inhibition of gastric emptying in the rat.

### 2.6.2.2 Primary pharmacodynamics

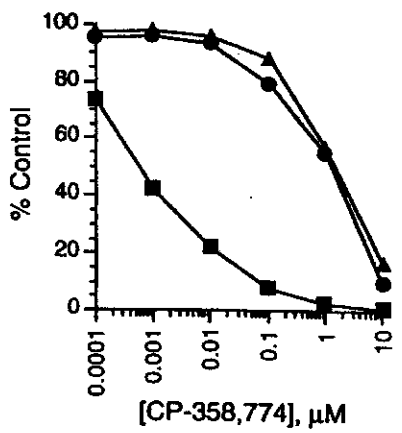
Mechanism of action:

**Report #I970176** - Induction of apoptosis and cell cycle arrest by CP 358,774, an inhibitor of epidermal growth factor receptor tyrosine kinase. Module 4.2.1.1

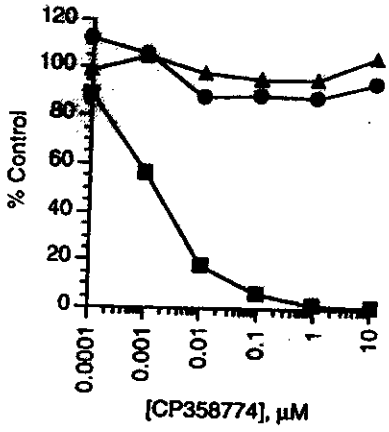
CP-358,774 was tested for activity in the EGFR kinase assay and was shown to inhibit the purified kinase as seen in the following figure, taken from a published report submitted by the sponsor.




Additional assays were run comparing the inhibition by CP-358,774 of native human EGFR with its inhibition of the *v-abl* and *c-Src* tyrosine kinases. A second set of assays designed to examine the specificity of CP-358,774 compared its inhibitory effects on recombinant EGFR kinase domain (squares) to insulin receptor kinase domain and IGF-IR kinase domain. Again, greater specificity is seen for the EGFR kinase than the other two kinase domains tested.




| | IC50, µM |
|---|---|
| EGFR | 0.0006 |
| src | 1.3 |
| abl | 1.5 |




| | IC50, µM |
|---|---|
| EGFR | 0.001 |
| IGF-1R | > 10 |
| IR | > 10 |

When EGF is added to cells expressing EGFR, there is rapid autophosphorylation of the EGFR on tyrosine residues. This provides an informative assay for the inhibition of EGFR tyrosine kinase activity in intact cells. A Western blot showed an $IC_{50}$ of 20nM for CP-358,774 when added to HN5 human head and neck tumor cells, a cell line that expresses high levels of EGFR. Similar results were seen in DiFi human colon cancer cells and MDA-MB-468 human breast cancer cells.

The adapter protein SHC is an endogenous substrate phosphorylated by EGFR tyrosine kinase. The addition of CP-358,774 blocks this phosphorylation while not blocking the insulin-induced phosphorylation of IRS-1, an insulin receptor tyrosine kinase substrate.

An *in vivo* model of EGFR inhibition consists of administration of EGF to the mouse, which will produce autophosphorylation of EGFR in liver and other tissues. In the liver of athymic mice, 10 mg/kg of CP-358,774 yielded a 54% inhibition of the tyrosine phosphorylation of EGFR following administration of EGF, and 93% inhibition following pretreatment with 100 mg/kg/ of CP-358,774. In human HN5 tumors in athymic mice, pretreatment with 10, 25, and 100 mg/kg of CP-358,774 1 hr before administration of EGF produced a 61%, 89% and 100% inhibition of EGF-induced autophosphorylation of tumor EGFR, respectively.

Both DiFi human colon tumor cell lines an HN5 human head and neck tumor cell lines express high levels of EGFR. The addition of CP-358,774 to either cell lines led to a significant decrease in cell proliferation, though was not cytotoxic as it did not decrease

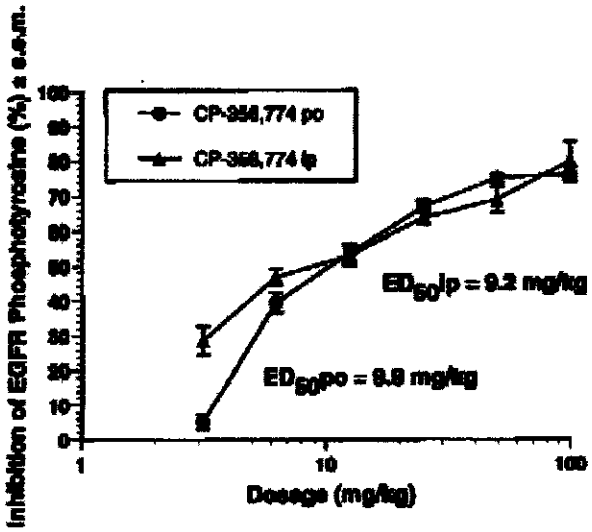
the cell viability. Additional studies showed that the decrease in proliferation was due to a partial $G_1$ cell cycle arrest.

In addition to the cell stasis effect, it was speculated that CP-358,775 would also lead to apoptosis in tumor cell lines. Several methods of determining apoptosis induction supported this hypothesis. The exact mechanism by which CP-358,774 led to a 3.5 fold increase in apoptotic cells, but not in the percent of necrotic cells, is not known. An inhibition of cell growth, coupled with an increase in cell death, is an important combination of drug effects and could prove to be very effective if this is seen clinically.

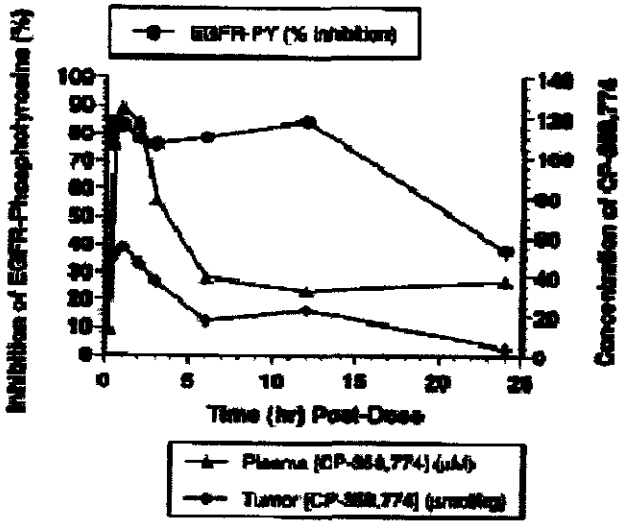
Drug activity related to proposed indication:

**Report # M990223:** Inhibition of epidermal growth factor receptor-associated tyrosine phosphorylation in human carcinomas with CP-358,774: dynamics of receptor inhibition in situ and antitumor effects in athymic mice. Module 4.2.1.1

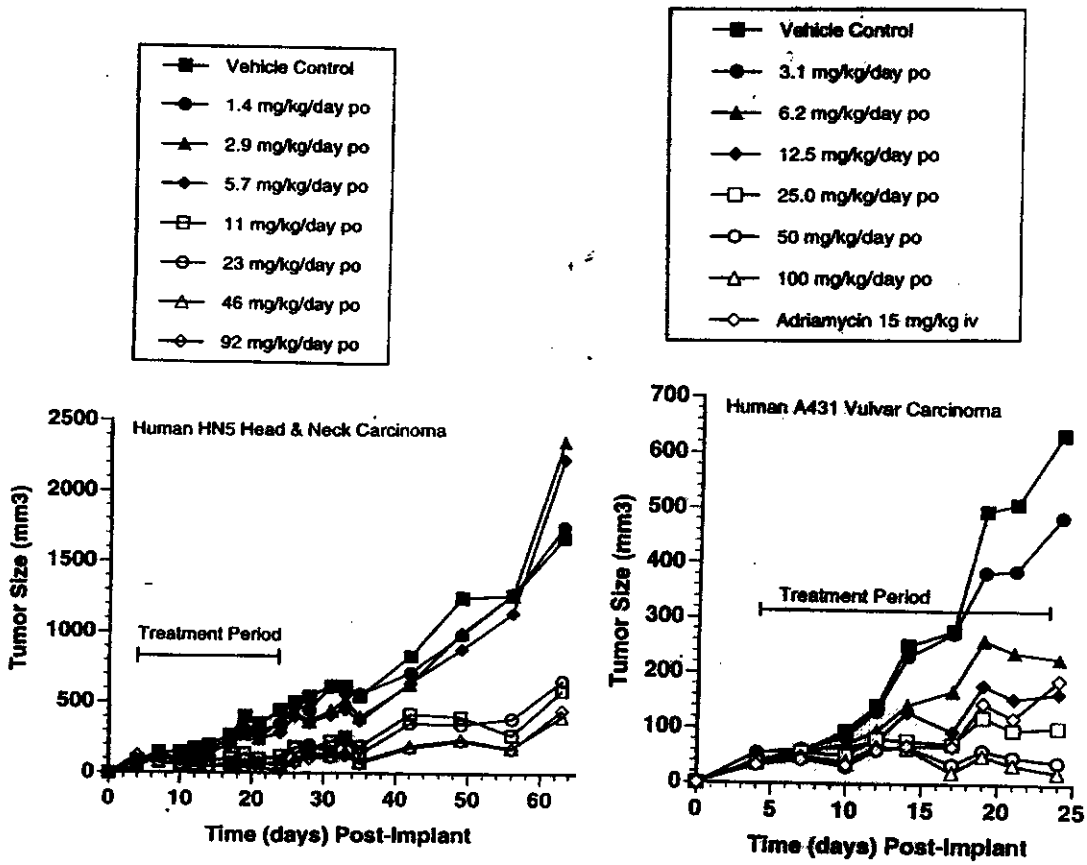
An assay was designed to quantify EGFR-specific tyrosine phosphorylation using human tumor tissues implanted subcutaneously into athymic mice. Athymic mice were implanted with HN5 cells. Cells were harvested from the mice after PO or IP treatment with CP-358,774 (usually 1 hr after treatment). The cells were flash frozen and homogenized to prevent further phosphorylation. A double-antibody ELISA was used to determine the EGFR tyrosine phosphorylation. The figure below, taken from a publication by the sponsor, shows that following both IP and PO administration of CP-358,774, significant decreases in EGFR-associated phosphotyrosine was seen, with similar $ED_{50}$s for the two routes of administration. The plasma concentration associated with the PO $ED_{50}$ of 9.9 mg/kg was 8 μM ($EC_{50}$ = 8 μM). As other studies have shown that in the mouse, CP-358,774 is 95% plasma protein bound that would yield a free plasma level $EC_{50}$ of 0.4 μM of CP-358,774.



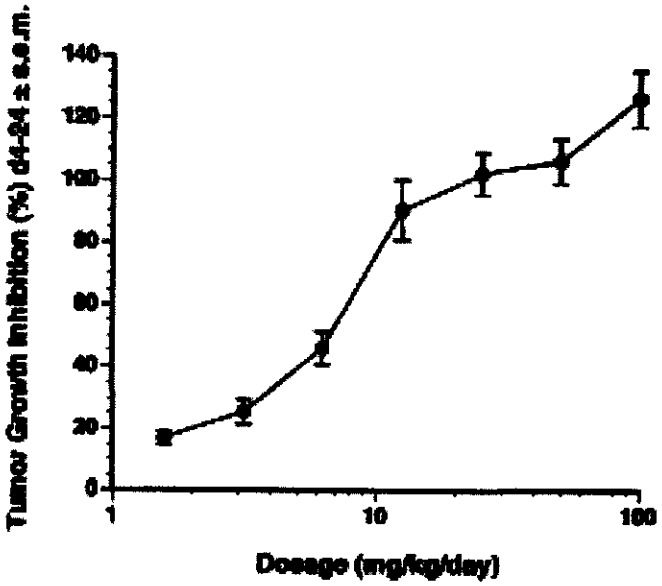
Additional studies were conducted to determine the duration of the inhibitory action of CP-358,774. The following graph shows the inhibition of EGFR-phosphotyrosine along with the plasma and tumor concentrations of CP-358,774, following administration of 92 mg/kg PO of CP-358,774 to athymic mice with HN5 xenografts. For 12 hrs, a significant inhibition of EGFR phosphotyrosine was seen (75-85%) and reductions at 24 hr after CP-358,774 administration were still measurable (25-40%). Similar results as 12 hrs were seen following IP administration, but no reduction was detectable at the 24-hr time point.



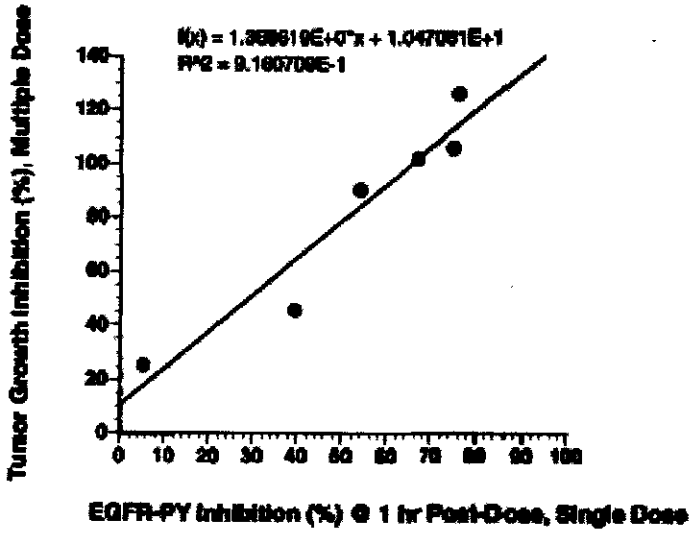
The antitumor effects of CP-358,774 were also evaluated in the athymic mouse, with xenografts of either HN5 cells or A431 carcinomas. In both cell lines, decreased tumor volumes were seen during daily treatment with CP-358,774. The following two graphs show the results in the HN5 tumors and then the A431 tumors.



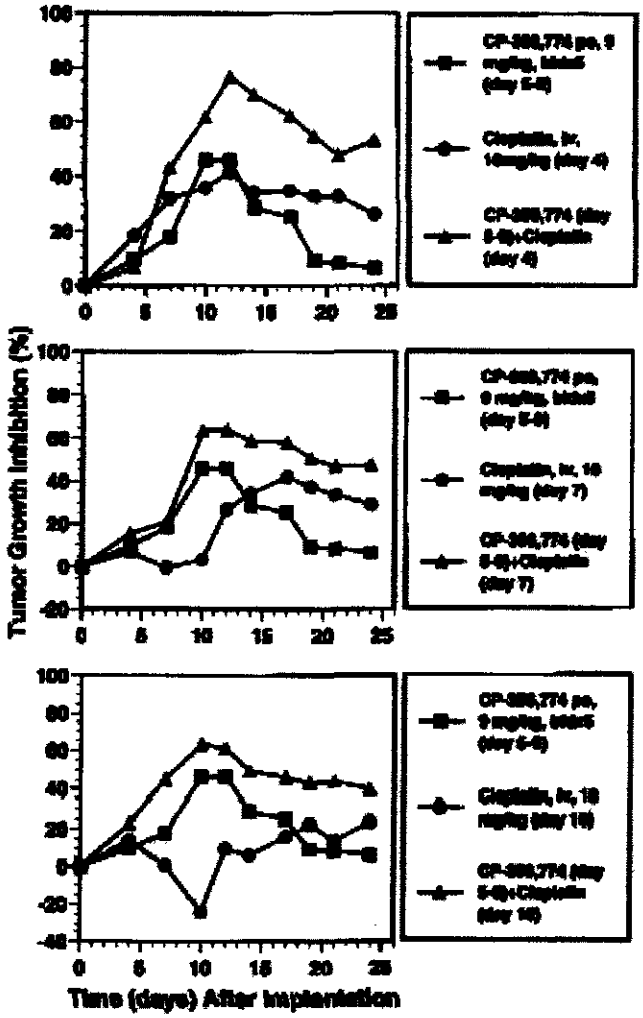
Tumor growth inhibition in the HN5 carcinoma xenograft is dose-related during the course of treatment, as is seen in the following graph. From the data presented below, the PO $ED_{50}$ was calculated to be 9.2 mg/kg/day.




Data from the HN5 tumor inhibition studies were also used to examine the correlation between the inhibition of EGFR phosphotyrosine and the inhibition of tumor volume. Tumor size was measured in millimeters across two diameters using calipers, three times a week. Tumor volume was calculated using the formula tumor volume = (length x $[width]^2$)/2. This comparison of the pharmacodynamic effect with the therapeutic effect yielded a strong correlation ($r^2 = 0.92$), suggesting that the therapeutic effect (tumor volume reduction during 20 days of CP-358,774 dosing) is strongly related to the pharmacodynamic effect (EGFR phosphotyrosine inhibition 1hr after single dose administration). The following graph shows this correlation.



The HN5 mouse model was also used to examine the effects of combination therapy. The $ED_{50}$ dose of CP-358,774 was administered after, concurrently or before, respectively in the graphs below, the administration of the MTD of cisplatin, as determined in a preliminary study. Following all three scenarios, more tumor inhibition was seen with the combination of drugs than was seen in the single treatment alone, as seen in the sponsor's figure below. The graphs below show that the addition of CP-358,774 to cisplatin was equally effective whether it was added to the regimen before, during or after the 5-day administration of cisplatin.




**Report # M2002314:** Evaluation of Tarceva™ in Combination with Gemcitabine or Cisplatin in Female CRL Nu/nu Mice Bearing the A549 Human NSCLC Xenograft Model. Module 4.2.1.1

Nude mice with xenografts of the human non-small cell lung cancer (NSCLC) cell line A549 were dosed with Tarceva in combination with either gemcitabine or cisplatin. This

cell line is a slower growing NSCLC in vivo model that is usually less sensitive to cytotoxic agents than other NSCLC cell lines such as the H460a line. When the mice were dosed with the MTD of both drugs in the combination, as determined in preliminary studies with naïve nude mice, significant toxicity was seen, including lethality. The MTDs of the drugs alone showed significant tumor growth inhibition. The combinations were too toxic and all the mice either died or were sacrificed moribund. Additional groups of mice were dosed with the combination of erlotinib and either gemcitabine or cisplatin, but at doses 1/4 the MTD, without significant differences in toxicity from the single agents, as measured by loss of body weight. From the two graphs presented by the sponsor, it is evident that the combinations of sub-optimal doses of erlotinib and either cisplatin or gemcitabine are more efficacious than the sub-optimal doses of the drugs alone in decreasing the tumor volume. However, the sub-optimal doses in combination do not decrease the tumor volume significantly more than that seen with the MTD of the drugs as single agents.

**Figure 4 Efficacy Graph of Experiment 524**

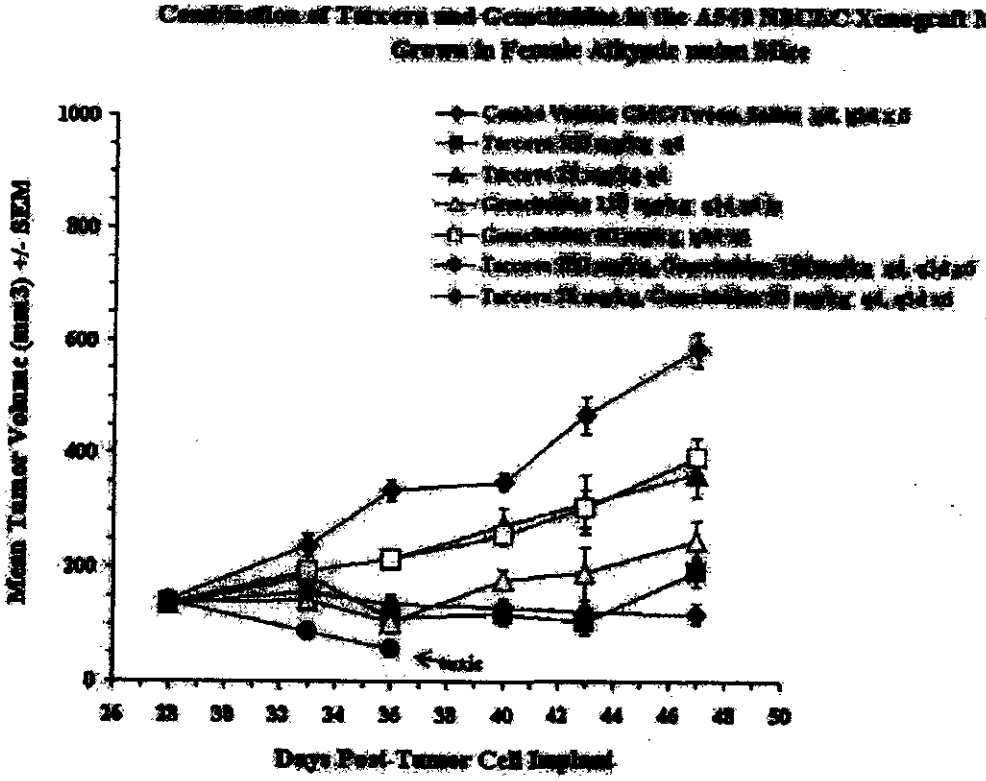
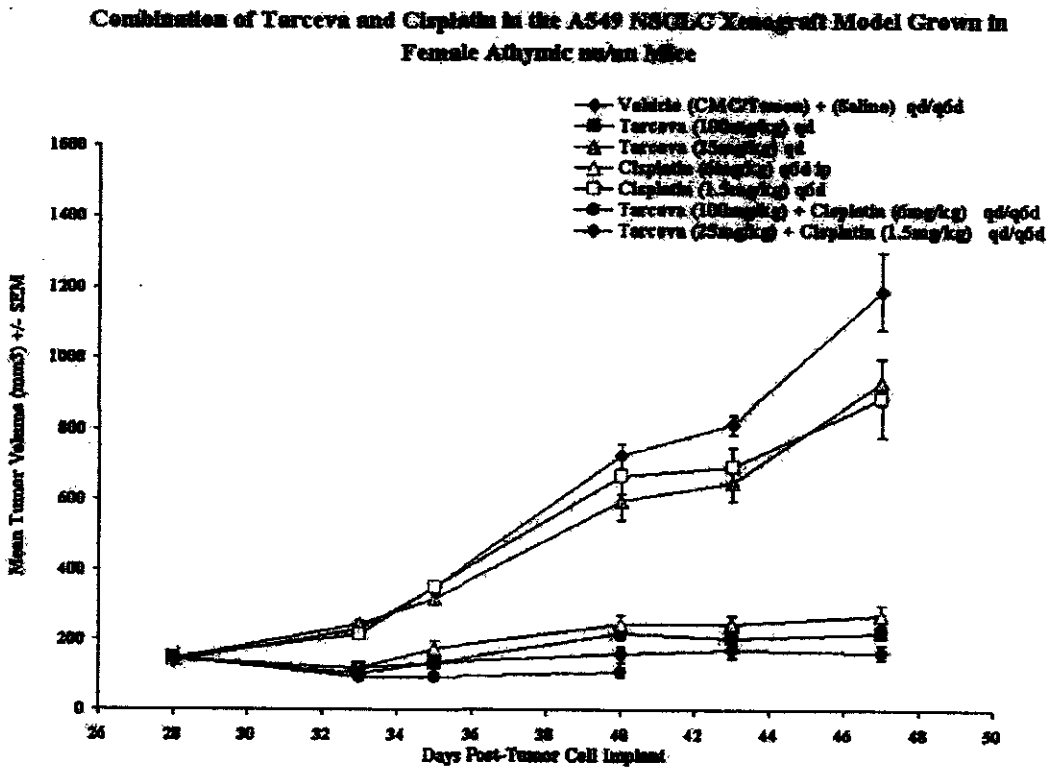


**Figure 5 Efficacy of Experiment 539**




**Report # M2003116:** The In vivo Cell Cycle Effects of Tarceva Using BrdU Incorporation Analysis in Human Tumor Xenografts. Module 4.2.1.1

The sponsor submitted a study report on the effects of single dose erlotinib administration on the incorporation of 5-bromodeoxyuridine (BrdU) in human tumor xenografts in nude mice. The mice were dosed by either the IP or PO route with 150 mg/kg erlotinib, then administered 10 mg/kg BrdU 2 hr before harvesting of the tumors. The tumors were fixed and stained for BrdU incorporation. The incorporation of BrdU was used as a marker for the S phase of the cell cycle. The sponsor's tables show that BrdU incorporation peaked at anywhere from 4-48 hrs after treatment. The PO route showed more consistent results than the IP route did, which was attributed to precipitate that was evident in the peritoneum of these mice, and would likely lead to lower drug exposure due to lower drug bioavailability. Variability in bioavailability of erlotinib would lead to variability in the results obtained. In the PO route the BrdU levels returned to predose levels by a maximum of 72 hrs. The effects of erlotinib on cell cycle arrest last for upwards of 48-72 hrs following PO administration. This may be important in future

studies that seek to combine Tarceva with other drugs that may exert their effects on other phases of the cell cycle.

**Table 1: Summary of BrdU Labeling Index of Gut and Tumor Tissue with Tarceva Dosed IP**

| Xenograft Model | BrdU Nadir (hours) | BrdU Return (hours) |
|---|---|---|
| KB (tumor) | 72 | ND |
| KB (gut) | 8 | 24 |
| H460 (tumor) | 8–24 | 48 |
| H460 (gut) | 24 | 48 |
| SW48 (tumor) | 48–72 | ND |
| SW48 (gut) | 8 | 24 |
| A549 (tumor) | 72 | ND |
| A549 (gut) | 24 | 48 |

**Table 2: Summary of BrdU Labeling Index of Gut and Tumor Tissue with Tarceva Dosed PO**

| Xenograft Model | BrdU Nadir (hours) | BrdU Return (hours) |
|---|---|---|
| KB (tumor) | 12–48 | 72 |
| KB (gut) | 12 | 48 |
| H460 (tumor) | 12 | 48 |
| H460 (gut) | 12 | 48 |
| SW48 (tumor) | 4 | 8 |
| SW48 (gut) | 8 | 48 |
| A549 (tumor) | 48 | 72 |
| A549 (gut) | 24 | 48 |
| GEO (tumor) | 24 | 48 |
| GEO (gut) | 12 | 48 |

### 2.6.2.3 Secondary pharmacodynamics

**OSI Study # I960031:** General Pharmacology Evaluation of CP-358,774: Part 1. Module 4.2.1.2

CP-358,774 was studied for its interaction with a wide variety of neurotransmitter receptors, regulatory binding sites, opiod receptors, neurotransmitter uptake sites, and brain/gut peptides. The only positive results noted were very low affinity for binding with peripheral benzodiazepine, adenosine-1 and μ-opiate receptors. These were all at concentrations of CP-358,774 far greater than that needed to yield the pharmacodynamic effect of EGFR kinase inhibition.

| | |
|---|---|
| Peripheral benzodiazepine | 2.5 |
| Adenosine-1 | 6.8 |
| μ-opiate | 7.0 |
| Inhibition of EGFR kinase | 0.02-0.15 |

A battery of tests were conducted to determine the effects of CP-358,774 on isolated tissues such as guinea pig aorta, guinea pig atria, guinea pig ileum, guinea pig ileal longitudinal muscle and rat uterus.

- No effect was seen of CP-358,774 on norepinephrine-stimulated contraction of guinea pig aorta at concentrations up to 10 μM.
- Up to concentrations of 1 μM of CP-358,774 did not affect the basal beating or histamine-induced increases in beating rate of the guinea pig right atria. At 10 μM of CP-358,774, the basal beating rate decreased by 10%, but the histamine-stimulated rate was unaffected.
- At 10 μM contraction of the guinea pig ileum was inhibited by 42%. Additional studies were done to determine if this was due to an inhibition of $Ca^{+2}$-mediated contraction.
- CP-358,774 produced a 32% inhibition in $Ca^{+2}$-mediated contraction of the guinea pig ileal longitudinal muscle at a concentration of 100 μM. This result does not explain the inhibition of histamine-stimulated guinea pig ileum contractions.
- No effect was seen of concentrations of CP-358,774 up to 10 μM on oxytocin-induced contraction of the rat uterus.

**OSI Study # V2001140:** Evaluation of Actions on K+ Currents through Recombinant HERG Channels Expressed in HEK293 Cells. Module 4.2.1.2
**OSI Study # V2001141:** Evaluation of Actions on K+ Currents through Recombinant HERG Channels Expressed in CHO Cells. Module 4.2.1.2
**OSI Study # V2001151:** Evaluation of Effect on Cardiac Action Potential in Isolated Rabbit Purkinje Fibers. Module 4.2.1.2

These three studies were conducted to examine the effects of CP-358,774 on hERG K+ channels and the potential for induction of cardiac arrhythmias. Blocking of repolarizing currents through herg K+ channels is known to lead to QT prolongation. The first two studies did show that CP-358,774 blocked hERG K+ channels in HEK293 and CHO cells. In the HEK293 cells, CP-358,774 has an $IC_{50} \approx 7$ μM and $IC_{20} \approx 0.6$ μM assuming an absence of current run-down, a partial block and non-unitary slope to the concentration-block relationship. Assuming some current run-down, a partial block and a unitary slope to the concentration-block relationship, there is an $IC_{50} \approx 2.8$ μM and $IC_{20} \approx 0.7$ μM. In the CHO assay, no $IC_{50}$ could be determined as the maximal block did not reach a level of 50%. The $IC_{20}$ is approximately 2.9 μM. Using isolated rabbit Purkinje fibers, no effect was seen on action potential duration, repolarization, action potential amplitude, and maximum upstroke velocity or resting membrane potential.

**OSI Study # I960007:** General Pharmacology Evaluation of CP-358,774: Part 2. Module 4.2.1.2

CP-358,774 was studied for its effects as a proconvulsant/anticonvulsant in mice, on renal function in rats, on gastrointestinal transit in rats, cardiopulmonary function in rats and cardiovascular function in conscious dogs.

| Treatment | | n | % Gastric Emptying | Geometric Center |
|---|---|---|---|---|
| Vehicle | 0.5% MC | 13 | 74.4 ± 2.1 | 3.66 ± 0.17 |
| CP-358,774 | 0.5 mg/kg | 6 | 76.7 ± 3.0 | 3.61 ± 0.19 |
| | 1 mg/kg | 6 | 70.8 ± 1.8 | 3.49 ± 0.07 |
| | 5 mg/kg | 12 | *58.4 ± 2.4 | *2.44 ± 0.14 |
| | 10 mg/kg | 7 | *44.5 ± 3.2 | *1.84 ± 0.14 |
| | 50 mg/kg | 7 | *23.0 ± 1.7 | *0.90 ± 0.07 |

*p<0.05 compared to vehicle group by Dunnett's multiple comparison test.

- In the rat, dosing at levels that would achieve plasma levels of CP-358,774 twice that projected to be clinically efficacious (3 μg/mL) led to no biologically significant effect on renal function, blood gases, blood pH, mean arterial pressure or heart rate.
- Studies in the rat did show a decrease in gastric emptying. The table below shows the effect of several PO doses of CP-358,774 on gastric emptying. Similar results were seen with IP administration, showing that it is not due to the topical exposure of the intestinal mucosa to CP-358,774. The 10-mg/kg PO dose has been shown to yield a plasma level equal to the proposed clinically efficacious plasma levels.
- CP-358,774 was neither proconvulsant or anticonvulsant in the mouse at doses that achieved a plasma level that was 5 times the efficacious plasma concentration of 3 μg/mL, based on in vivo inhibition of EGFR autophosphorylation).
- The mean plasma levels achieved in the dog cardiovascular study were highly variable and a dose of 50 mg/kg, that was expected to achieve comparable plasma levels to the efficacious level of 3 μg/mL, yielded a level of 1.4 μg/mL. A higher dose of 100 mg/kg was added and the mean plasma level in this group was actually lower, at 0.7 μg/mL. No cardiovascular effects were seen in the conscious dogs with plasma levels of 16-85% of the efficacious 3 μg/mL level. One dog had a plasma level of 7 μg/mL and also showed no cardiovascular effects of CP-358,774.

### 2.6.2.4 Safety pharmacology

Neurological effects:
Previously reviewed studies **OSI Study # I960031** and **OSI Study # I960007** showed CP-358,774 to have little to no interaction at a multitude of neurotransmitter receptors and also showed it was neither proconvulsant or anticonvulsant in the mouse. An additional neurological effects study was conducted using the FOB in rats.

**OSI Study # R2002124:** A Neurobehavioral Evaluation of OSI-774 Using a Functional Observation Battery in the Rat. Module 4.2.1.3

Female rats were administered OSI-774-01 by oral gavage at doses of 50, 225, and 1000 mg/kg, single administration on Day 1. The functional observational battery (FOB) was conducted pre-dose, 1 and 24 hr after dosing to assess the acute neurotoxicity of OSI-774-01. The only significant effect seen was a slight decrease in mean body temperature 1 hr after dosing.

| | 50 mg/kg | 225 mg/kg | 1000 mg/kg |
|---|---|---|---|
| Body Temperature Differences | ↓ 1% | ↓ 2%* | ↓ 2%** |

* - Significantly different from control; ($p<0.05$)
** - Significantly different from control; ($p<0.01$)

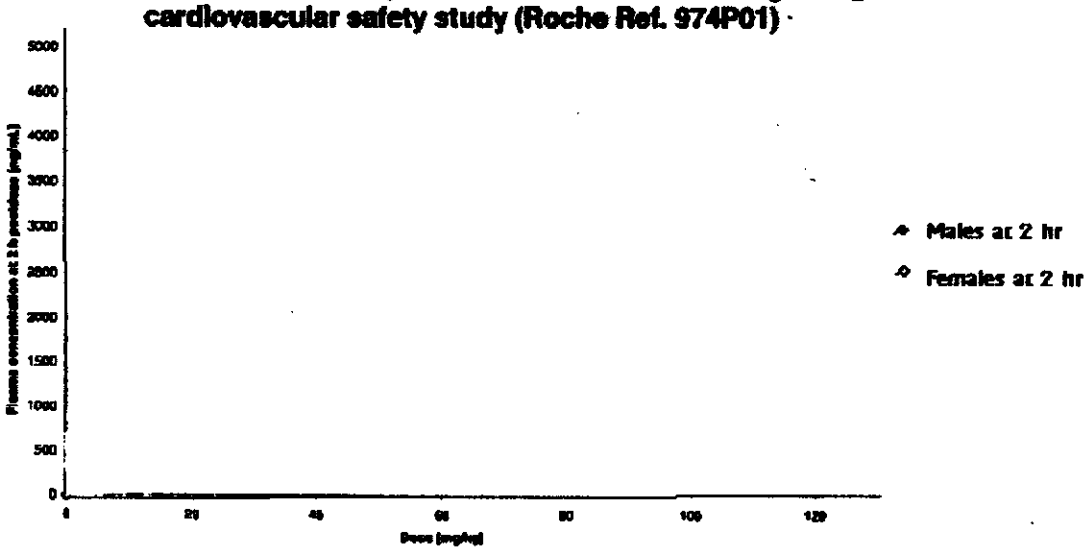
Cardiovascular effects:
Previously reviewed studies **OSI Study # I960031** and **OSI Study # I960007** showed no effects of CP-358,774 on guinea pig aorta and atrial contractions or on dog cardiovascular functions. An additional telemetry study was conducted in the conscious dog.

**OSI Study # D2001142:** Evaluation of Effects on Blood Pressure, Heart Rate, and Electrocardiogram after Single Oral Administration to Conscious Dogs. Module 4.2.1.3

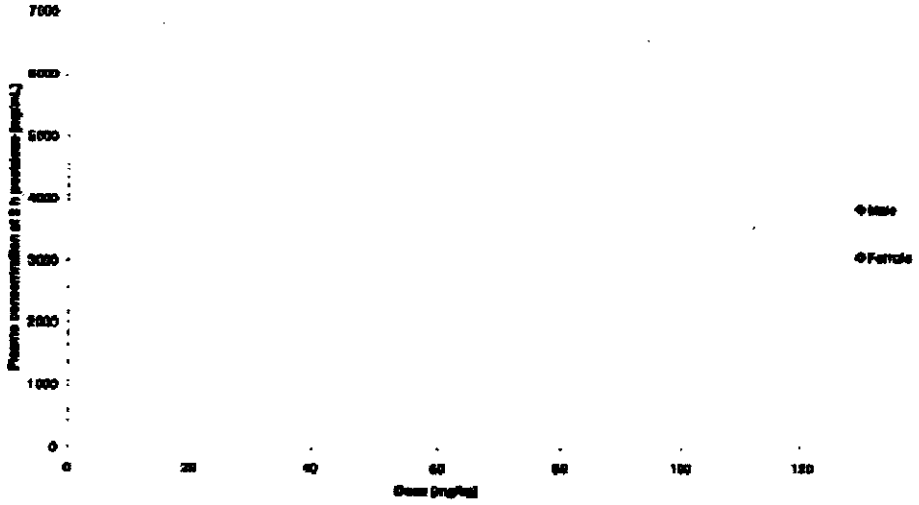
Conscious dogs were treated with RO0508231 (OSI-774) PO, single doses of 10, 20, 50 or 100 mg/kg and blood pressure, heart rate and ECGs were evaluated with implanted telemetric transmitters. Measurements began 24 hrs prior to drug administration and continued for 24 hrs post-administration in 3 dogs of each gender. At 2 hrs after drug treatment, blood was taken from these dogs for determination of plasma drug levels. Given the fairly rapid absorption of RO0508231 following oral administration, electrocardiogram measurements were conducted during a phase when the dogs would have had high plasma concentrations of the drug, though perhaps not have reached the Cmax yet. Cmax has been shown in other studies with the dog to be achieved at approximately 1.5 hrs. Additionally, the plasma concentrations have been quite variable in the dogs, making the data obtained less informative. No treatment-related effect was seen on any of the cardiovascular parameters measured. The toxicokinetics showed that while there was a large variability in plasma concentration in the dogs, the mean levels were comparable to the plasma concentration efficacious in vivo in preclinical studies (3 μg/mL). The following graph from the sponsor shows that at the highest dose of 100 mg/kg, the plasma levels in 3 male and 3 female dogs are close to the— μg/mL range.

The second graph shows individual data from the 6 dogs, showing the variability seen in plasma concentration among these animals.

**Dose-dependence of RO0508231 at 2 h postdose after oral administration of 10, 20, 50, and 100 mg/kg RO0508231-001 (Tarceva / OSI-774) to 3 male and 3 female Beagle dogs in a cardiovascular safety study (Roche Ref. 974P01)**




**Individual plasma concentrations of RO0508231 at 2 h postdose after oral administration of 10, 20, 50, and 100 mg/kg RO0508231-001 (Tarceva / OSI-774) to 3 male and 3 female Beagle dogs in a cardiovascular safety study (Roche Ref. 974P01)**



Pulmonary effects:
Previously reviewed study **OSI Study # I960007,** reviewed above, showed no effect of CP-358,774 on cardiopulmonary function in the rat.

An additional study examined the pharmacological effect of OSI-774-01 (CP-358,774) on the respiratory system of the albino rat.

**OSI Study # R2002208:** A Pharmacologic Assessment of the Effect of OSI-774-01 on the Respiratory System of the Albino Rat. Module 4.2.1.3

Albino rats were given OSI-774-01 PO, single dose administration, at doses of 50, 225, and 1000 mg/kg. The rats were put in 'head out' plethysmographs and ventilatory parameters were measure for approximate 15 minute periods at time points of pre-dose, 2, 6, and 24 hours post-dose. Tidal volume, respiratory rate and derived minute volume were measured and compared to those of the control rats. No treatment-related effects on these parameters were noted.

Renal effects:
Previously reviewed study **OSI Study # I960007**, reviewed above, showed no effect of CP-358,774 on renal function in the rat.

Gastrointestinal effects:
Previously reviewed studies **OSI Study # I960031** and **OSI Study # I960007**, both reviewed above, have shown that CP-358,774 can inhibit the histamine-stimulated contractions of the guinea pig ileum. Additionally, CP-358,774 inhibited the gastric emptying in rats.

Abuse liability:
Abuse liability was not directly studied. Studies in previously reviewed report **OSI Study # I960007**, reviewed above, looked at the effect of CP-358,774 on a large range of receptors. The results showed there to be only a very weak interaction with peripheral benzodiazepine and μ-opiate receptors, and no interaction at a large number of other receptors. These results would not be consistent with a drug with abuse potential.

Other:
No other safety pharmacology studies were conducted

**2.6.2.5 Pharmacodynamic drug interactions**
None

## 2.6.3 PHARMACOLOGY TABULATED SUMMARY

| Assay Systems | $IC_{50}$ ng/ml |
|---|---|
| Inhibition of Purified EGFR Tyrosine Kinase | 0.79 |
| Inhibition of Cellular EGFR – Western Blot of HN5 Cells | 7.9 |
| Inhibition of HN5 Cell Proliferation in Cell Culture | 9.8 |
| Plasma Concentration at $ED_{50}$ for ↓ of Tumor EGFR Phosphotyrosine | 3,100 |
| Inhibition of HN5 Tumor Growth | 670 |
| Efficacy Mouse Data ($EC_{50}$) | 8,000 |
| Efficacy Mouse Data ($EC_{50}$) – Free Plasma Levels Assuming 95% Protein Bound | 400 |

## 2.6.4 PHARMACOKINETICS/TOXICOKINETICS

### 2.6.4.1 Brief summary

The pharmacokinetic behavior of erlotinib was examined in all the nonclinical species tested for toxicity. Great variability was seen within studies, among varying studies in the same species and across the different species. As the doses increased in the animals, proportional increases were usually seen in AUC. As the doses increased, around the 100-mg/kg range, PO, the AUC was supraportional to the increase in dose, indicative of a saturation of the clearance of the drug. Even higher doses however, show less than dose-proportional increases in AUC and Cmax calculated. This effect can be seen when there is limitations on the rate of absorption of a drug. In the rat, there was a definite gender difference in drug exposure, with higher AUCs in the female than the male. This correlated with the increased toxicity usually noted in female rats.

Tissue distribution studies show that erlotinib distributes quickly and to nearly all tissues. The cerebellum and cerebrum levels were considerably lower than that seen in the pituitary, indicative of limited distribution of erlotinib across the blood-brain-barrier. Excretion of erlotinib was primarily by the biliary route, into the feces, with predominantly metabolites and not the parent compound being retrieved in the feces. Some metabolites are also present in the urine, but >70% of the drug is accounted for in the feces.

The metabolic profile of erlotinib is very similar across species; rat, dog, and human. Three major metabolic pathways have been determined, with the O-demethylated metabolites being the main metabolites found in the plasma. Metabolism appears to involve the P450 isozymes CYP3A4 and CYP1A2, though extrahepatic metabolism via CYP3A4 in the intestine, CYP1A1 in the lung and CYP1B1 in tumor tissue may also contribute to erlotinib metabolism. The main metabolites identified have been OSI-413

and OSI-420 and they are purported to be pharmacologically active. These have been measured in both the pharmacology/pharmacokinetics studies and the toxicology studies. Under the study conditions tested, it is believed that CYP3A4 and CYP 3A5 are primarily responsible for the metabolism to OSI-413 or OSI-420.

**2.6.4.2 Methods of Analysis**

[see under individual study reviews]

**2.6.4.3 Absorption**

**OSI Study # M2000138:** Plasma and Tumor Concentrations of CP-358,774 in Female Athymic Nude-nu Mice Administered Oral or Intraperitoneal Doses of CP-358,774. Module 4.2.2.2

Pharmacokinetics data were obtained from several studies in the athymic mouse examining the inhibition of EGFR phosphorylation and anti-tumor effects of CP-358,774. The figure below shows that the PO route achieved and sustained higher plasma and tumor concentrations of CP-358,774 than the IP route. It is speculated that the acidic pH of the gastrointestinal tract is more conducive to keeping the drug in suspension and therefore increasing its bioavailability. Studies using the IP route have noted drug precipitate in the peritoneal cavity upon necropsy, impacting the bioavailability of the drug by the IP route. In addition to plasma and tumor levels of CP-358,774, phosphotyrosine levels were also measured. At 12 hrs, both routes of administration showed a 70-85% reduction in phosphotyrosine levels from pre-dose levels. By the 24-hr time point, there was still a 40% decrease in phosphotyrosine levels in the PO treated animals but no reduction seen in the IP group, when compared to pre-dose levels. The continued pharmacodynamic effect while the plasma and tumor levels of CP-358,774 are sufficiently low, has led to speculation of an active metabolite.

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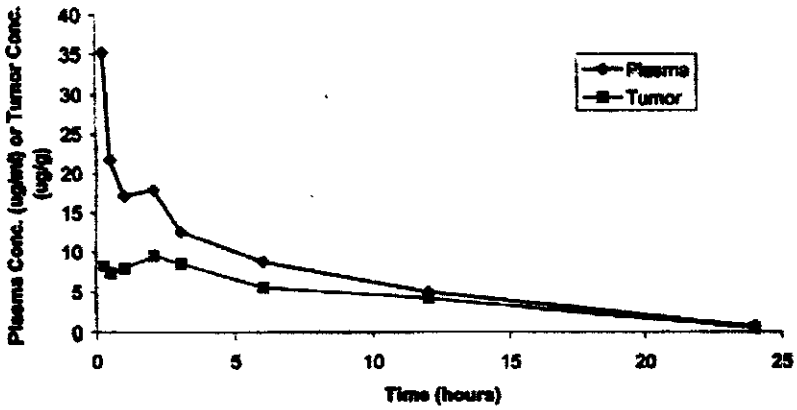
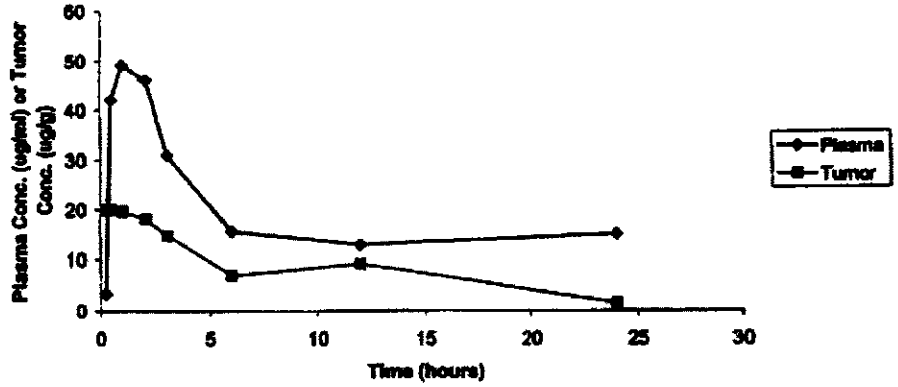
A. Mean Plasma Concentrations (µg/ml) and Mean Tumor Concentrations (µg/g) of CP-358,774 in Mice Administered a Single 100 mg/kg IP Dose




B. Mean Plasma Concentrations (µg/ml) and Mean Tumor Concentrations (µg/g) of CP-358,774 in Mice Administered a Single 100 mg/kg PO Dose



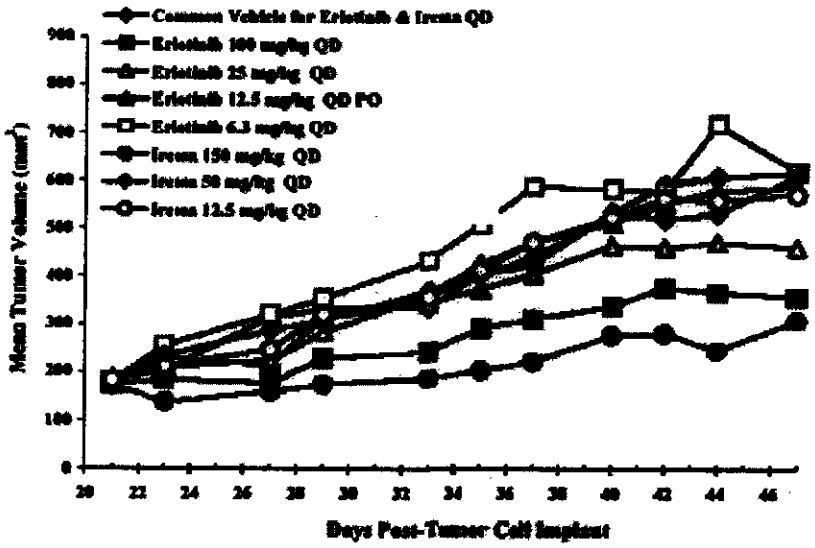

**OSI Study # M2001148:** Evaluation of the Efficacy of the Roche EGFR Inhibitor Erlotinib (Tarceva, RO0508231) and Gefitinib (Iressa, RO332843) in the A549 Non-Small Cell Lung Cancer Xenograft Model.

This study was conducted to look at toxicity, systemic exposure and anti-tumor efficacy of erlotinib and gefitinib in the subcutaneous A549 NSCLC tumor model in athymic mice. Doses of erlotinib (PO) tested are shown in the table and chart below. Dosing began at Day 21 after tumor implantation and continued for approximately 24 days, with animals euthanized on Day 47. The following table and sponsor's graph show the efficacy of erlotinib and gefitinib in reducing the mean tumor volume in this model. Toxicity at the higher doses of both drugs exhibited as a reddening and crusting of the muzzle, a well-documented toxicity that may be due to the high expression of EGFR in the skin. The toxicity was less pronounced in the 100-mg/kg/day erlotinib group (5/10

mice) than it was in the 150-mg/kg/day gefitinib group (10/10). The efficacy, however, was essentially equivalent between 100 mg/mg/day of erlotinib and 150 mg/kg/day of gefitinib in this model system.

| | 6.3 mg/kg/day | 12.5 mg/kg/day | 25 mg/kg/day | 100 mg/kg/day | 150 mg/kg/day Gefitinib |
|---|---|---|---|---|---|
| **Tumor Volume Change from Mean** | 0% | ↓ 7% | ↓ 37% | ↓ 57% | ↓ 69% |

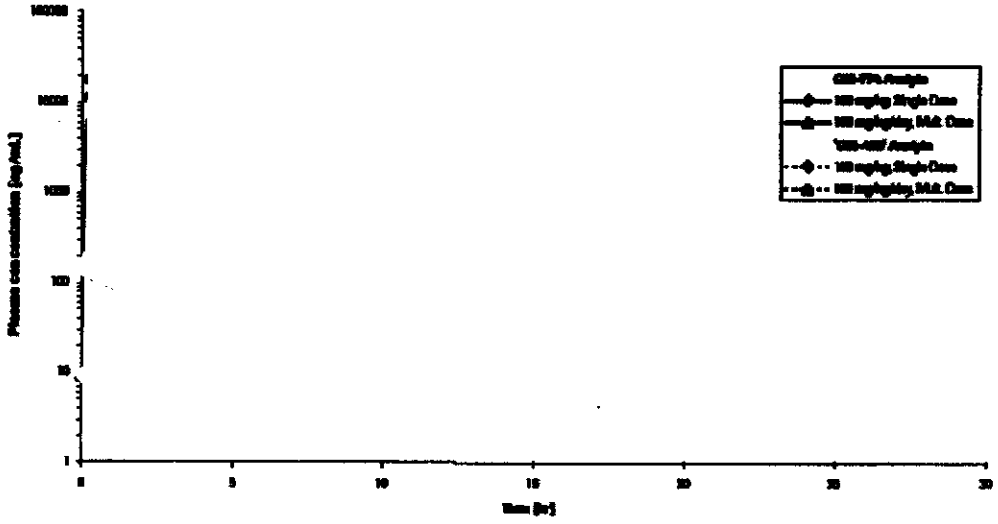
**Efficacy study of erlotinib (RO50-8231) & gefitinib (RO0332843) in A549, non-small cell lung carcinoma xenograft grown in female athymic nu/nu mice**




Plasma concentrations of erlotinib (RO0508231) were monitored during dosing with 12.5 and 100 mg/kg/day multiple doses and for a single dose of 100 mg/kg. The combined sum of the main metabolites (OSI-420 and OSI-413) was measured as well. The sponsor's graph, shown below, depicts both single and multiple dosing at 100 mg/kg and the plasma concentration of both the parent compound and the combined active desmethylation metabolites. The data show that oral administration of erlotinib leads to a rapid absorption of the drug, with the maximum plasma concentration reached within the first hour after dosing. A plateau was seen at around the 2-hr time point in the parent drug concentration after multiple dosing. A similar plasma concentration-time profile to

the parent compound was seen in the metabolites measured, with the ratio of the main metabolites to the parent compound ranging from 31-59%.

**Comparison of plasma concentration-time profiles of RO0508231 and main active desmethyl metabolites (sum of OSI-413 and OSI-420) from composite data following single dose and multiple oral administration of 100 mg/kg RO0508231-001 over 3 weeks to nu-nu athymic mice (Study No. ONC EFF 474)**





The sponsor refers to OSI-413 and OSI-420 as active metabolites. In the summary of pharmacology studies, the sponsor shows these data. While this would support the assumption that the two metabolites are pharmacologically active, the actual studies where these data were obtained were never presented for review.

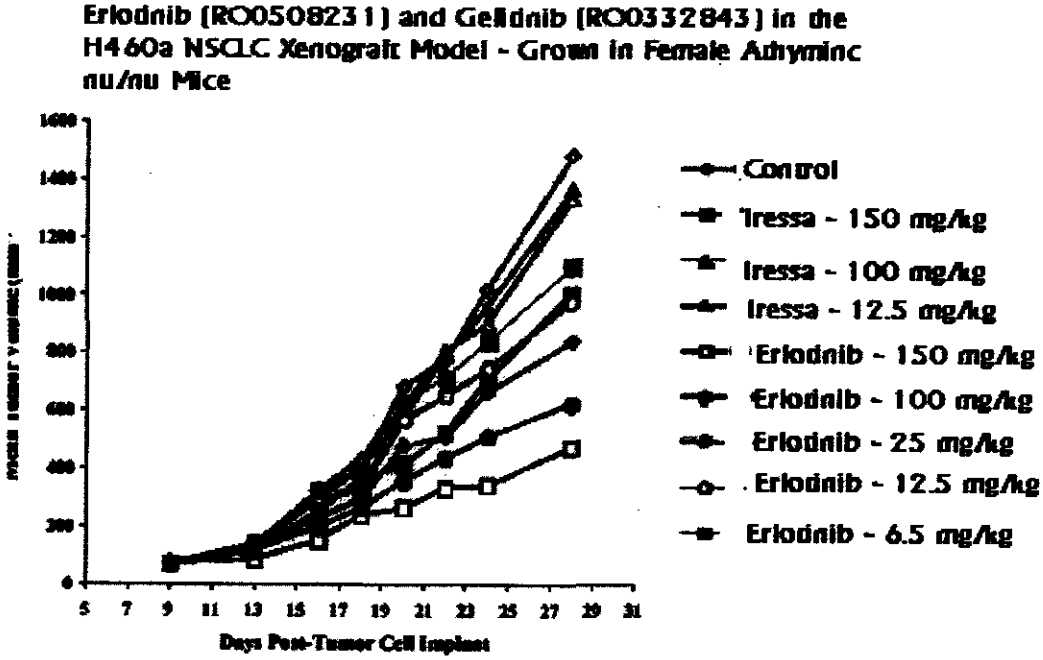
| | Inhibition of EGFR Tyrosine Kinase Activity: $IC_{50}$ | Inhibition of Cellular EGFR Tyrosine Kinase Activity: $IC_{50}$ |
|---|---|---|
| **Erlotinib** | 2 nM | 20 nM |
| **OSI-420** | 2.5 nM | 14 nM |
| **OSI-413** | 1.4 nM | 8 nM |

The table below shows that both Cmax and AUC-last are higher after a single dose of 100 mg/kg than after 21 days of daily administration at the same dose level.

| | Cmax ng/mL | AUC-last h·ng/mL |
|---|---|---|
| 12.5 mg/kg/day – Day 21 of Multiple Dosing | | 14500 |
| 100 mg/kg/day – Day 21 of Multiple Dosing | | 129000 |
| 100 mg/kg – Single Dose | | 177000 |

**OSI Study # M2001152:** RO0508231 (Tarceva / OSI-774). Pharmacokinetic Monitoring in an Efficacy Study in the H460a Non-small Cell Lung Cancer Xenograft Model. Module 4.2.2.2

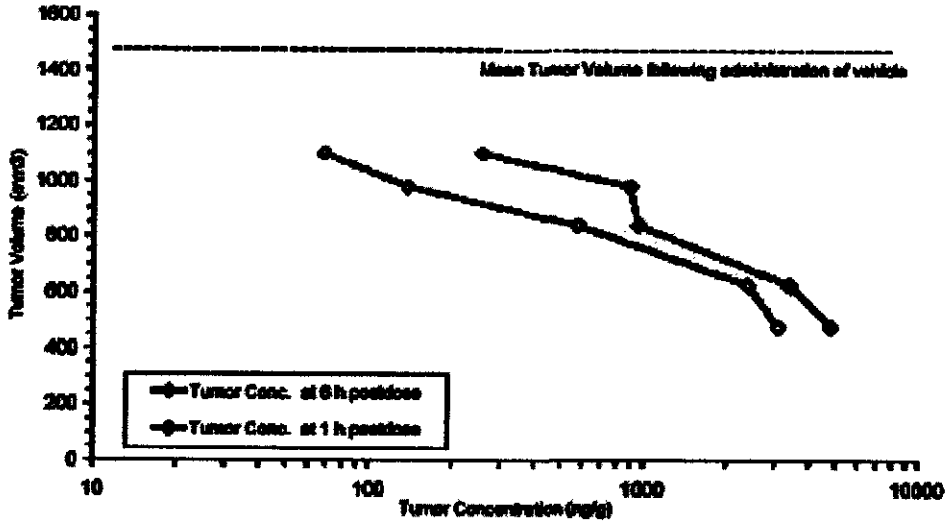
Anti-tumor efficacy and potential toxicity, along with pharmacokinetics, of erlotinib were again compared to gefitinib in a xenograft model. This study used the H460a NSCLC model. The study design included doses of gefitinib at 12.5, 100 and 150 mg/kg/day compared to erlotinib doses of 6.3, 12.5, 25, 100 and 150 mg/kg/day. Mice were administered drug for 19 days, beginning on Day 9 after tumor implantation, with 10 female mice per group. Toxicity at the higher doses of both drugs exhibited as a reddening and crusting of the muzzle, a well-documented toxicity that may be due to the high expression of EGFR in the skin. The toxicity was less prevalent in the 150-mg/kg/day erlotinib group (3/10 mice) than it was in the 150-mg/kg/day gefitinib group (10/10), and less severe. The following graph from the sponsor shows the efficacy of erlotinib at decreasing the mean tumor volume.



| | 6.25 mg/kg/day | 12.5 mg/kg/day | 25 mg/kg/day | 100 mg/kg/day | 150 mg/kg/day | 150 mg/kg/day Gefitinib |
|---|---|---|---|---|---|---|
| **Tumor Volume Change from Mean** | ↓ 28% | ↓ 36% | ↓ 46% | ↓ 61% | ↓ 71% | ↓ 34% |

A dose-response effect is seen on efficacy, as measured by decrease in tumor volume, with erlotinib tumor concentrations in this tumor model. The sponsor's graph below shows that as the concentration of erlotinib (RO0508231) in the tumor increases, with increasing doses of the drug, the tumor volume decreases, seen at both 1 and 6 hours after drug administration on the last day of the study.

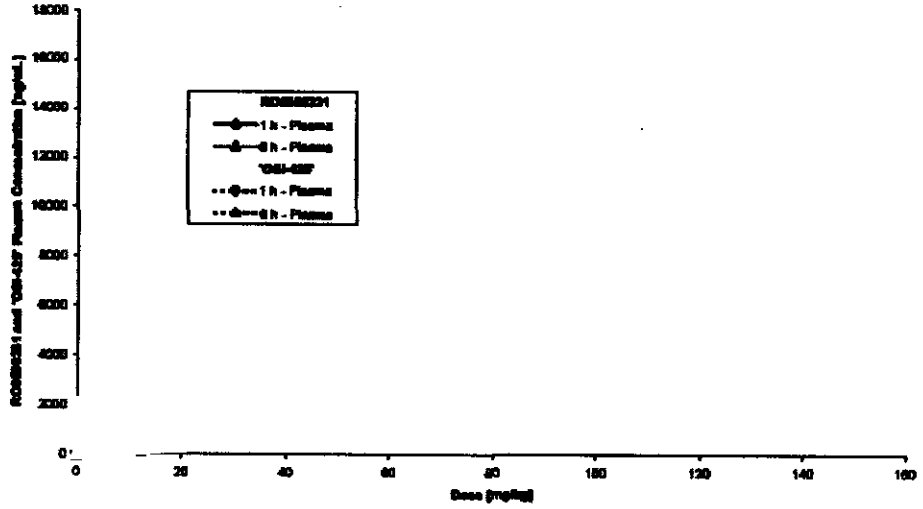
Tumor Concentration Dependence of Efficacy of Erlotinib (RO0508231) in the H460a NSCLC Xenograft Model - Grown in Female Athymic nu/nu Mice



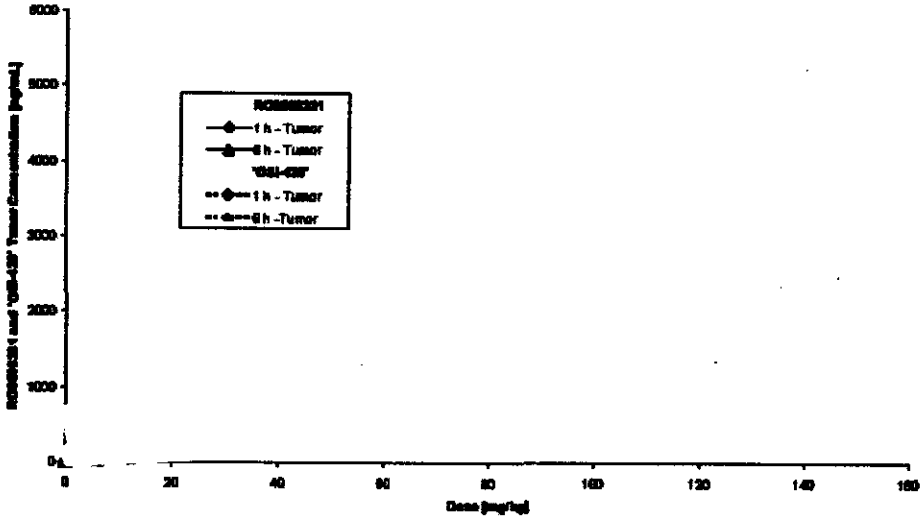

The sponsor's graphs below show the dose dependence of the drug and its purported active desmethylation metabolites in both the plasma and the tumor, as measured by drug and metabolite concentration in the plasma or the tumor cells. The figures show that the graphs of the metabolites are similar, though of smaller concentration, to that of the parent compound. Comparison of plasma and tumor concentrations again shows similar curves, with lower concentrations seen in the tumor than the plasma. Comparison of the

graphs over varying doses and over the two time points, 1 hr and 6 hrs after drug administration, indicate that induction or inhibition of metabolism is unlikely.

**Dose dependence of RO0508231 and active desmethyl metabolites'OSI-420/413' (sum of OSI-413 and OSI-420) plasma concentrations following daily oral administration of 6.3, 12.5, 25, 100, and 150 mg/kg RO0508231-001 to nu-nu athymic mice (Study Plan ONC EFF 424)**




**Dose dependence of RO0508231 and active desmethyl metabolites 'OSI-420/413' (sum of OSI-413 and OSI-420) tumor concentrations following daily oral administrations of 6.3, 12.5, 25, 100, and 150 mg/kg RO0508231-001 to nu-nu athymic mice (Study Plan ONC EFF 424)**



**OSI Study # R2002319:** RO0508231 (Tarceva/OSI-774): Pharmacokinetics in Female Fischer 344 Rats Following Single Oral Dose Administration of 20 and 100 mg/kg.

A single oral dose of RO0508231 was given to two groups of 6 female Fischer 344 rats, at doses of 20 and 100 mg/kg. Blood was drawn and plasma concentrations of both the parent compound and the purported pharmacologically active desmethyl metabolites (sum of OSI-413 and OSI-420) were measured. The sponsor's table shows the pharmacokinetic parameters. Similar results were seen with the metabolites, at levels of approximately 27% of that of the parent compound.

**Pharmacokinetic parameters of RO0508231 following single oral administration of 20 and 100 mg/kg RO0508231-001 (hydrochloride salt) to female Fischer 344 rats (Study Plan ONC PK 444)**

| | 20 mg/kg | 100 mg/kg |
|---|---|---|
| Cmax [ng/mL] ±CV% | 2150 ± 23.7% | 7800 ± 20.4% |
| Cmax/D [(ng/mL)/(mg/kg)] | 108 | 78.0 |
| Tmax [h] | 2.0 | 11 |
| AUC(0-inf.) [h·ng/mL]** | 24600 | 227000* |
| C(24h) [ng/mL] ±CV% | 9.22 ± 33.4% | 3550 ± 58.7% |
| AUC(0-24 h) [(h·ng/mL)] | 24600 | 149000 |
| AUC(0-24 h)/D [(h·ng/mL)/(mg/kg)] | 1230 | 1490 |
| CL/F [mL/min/kg]*** | 12.4 | n.c.**** |
| λz [1/h]** | 0.342 | n.c.**** |
| Vz/F [L/kg]*** | 2.2 | n.c.**** |
| T½ [h]** | 2.0 | n.c.**** |
| MRT [h] | 6.7 | n.c.**** |

* high extrapolated percentage of 34.2% compared to AUC(0-24 h).
** rough estimates (plateau)
*** calculation based on dose of free base
**** not calculated due to a plateau in the plasma concentration-time profile

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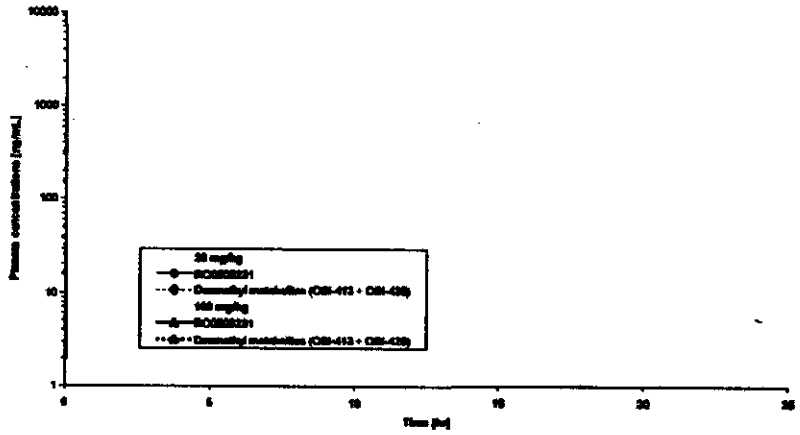
The sponsor's graph below shows rapid absorption following the PO drug administration at both doses. A plateau can be seen with both doses, between the 2 and 8 hr time points for 20 mg/kg and between 2 and 24 hrs for100 mg/kg. The pharmacokinetic profile of the metabolites is similar to that of the parent drug, most likely due to a rate limiting kinetics in the formation of the metabolites.

**Plasma concentration-time profiles of RO0508231 and main active desmethyl metabolites (sum of OSI-413 and OSI-420) from composite data following single oral administration of 20 and 100 mg/kg RO0508231-001 (hydrochloride salt) to female Fischer 344 rats (Study Plan ONC PK 444)**



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**OSI Study # D960008:** Pharmacokinetics of CP-358,774 in Dogs. Module 4.2.2.2

Data were obtained from a series of studies conducted in the male Beagle dog with both IV and PO administration of CP-358,774. A dose-response relationship between drug dose and plasma Cmax and AUC exists for both routes, each increasing with the

increasing dose. The sponsor's graph shows the dose-proportional relationship between these two parameters. The oral bioavailability varied among doses, ranging from 45-88%.

**Summary of CP-358,774 Pharmacokinetics in Dogs**

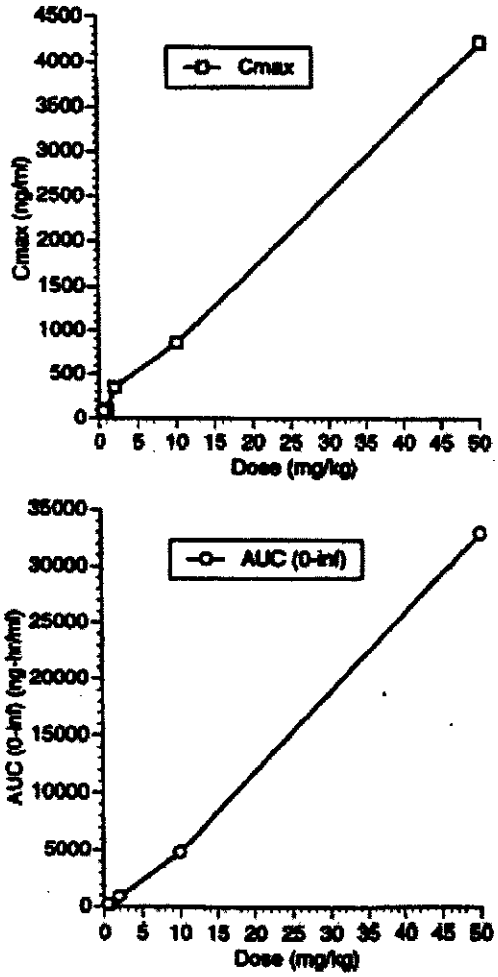
**IV Pharmacokinetics**

| Species | Dose (mg/kg) | n | AUC,0-∞ (ng-hr/ml) | Cl (ml/min-kg) | Vdss (L/kg) | T1/2 (hr) |
|---|---|---|---|---|---|---|
| Dog | 0.5 | 6 | 179 | 48 | 2.5 | 0.84 |
| | 2 | 4 | 1035 | 35 | 3.4 | 1.2 |
| | 7 | 6 | 7421 | 19 | 1.8 | 1.3 |

**PO Pharmacokinetics**

| Species | Dose (mg/kg) | n | AUC,0-inf (ng-hr/ml) | Cmax (ng/ml) | Tmax (hr) | Bioavailability (%) | T1/2 (hr) |
|---|---|---|---|---|---|---|---|
| Dog | 0.5 | 2 | 155 | 76 | 0.50 | 87 | 1.2 |
| | 1 | 4 | 181 | 88 | 0.50 | 81 | 1.2 |
| | 2 | 6 | 907 | 346 | 0.75 | 88 | 1.3 |
| | 10 | 2 | 4702 | 660 | 1.0 | 45 | 1.9 |
| | 50 | 3 | 32812 | 4206 | 2.0 | - | 2.1 |

Comparison of Mean Plasma Cmax and AUC (0-inf) Versus Dose
In Dogs Administered a Single PO Dose of CP-358,774




**2.6.4.4 Distribution**

**OSI Study # M960018:** Tissue Distribution of CP-358,774 in HN-5 Tumor-Bearing and Non Tumor-Bearing Athymic, nu/nu (nude), Female Mice. Module 4.2.2.3

Male Sprague-Dawley and Lister-Hooded rats were given a single PO dose of $^{14}$C-RO0508213-002 (5 mg free base/kg). At time points of 1, 4, 8, 24 and 72 hrs after

administration the animals were euthanized and whole body autoradiography was conducted, with blood sampling occurring immediately before sacrifice.

The peak concentrations of radioactivity were measured at the 4-hr time point. In the albino and pigmented rats, the tissue distribution of the radioactivity was fairly similar. The primary tissues for the distribution of the labeled RO0508213-002 were the liver, the kidney, the adrenal glands and the GI tract mucosa. One difference between the two strains of rats was the level of radioactivity measured in tissues containing melanin. A marked difference was seen in the radioactivity distribution in tissues such as the uveal tract, where high levels of melanin are present. Here the pigmented rat showed a much greater distribution of radioactivity than did the albino rat, showing radioactivity binding of melanin. Radioactivity was also noted in both the bile duct and the urinary tract, indicative of elimination via both biliary excretion and the urinary route.

| | Levels in the Sprague-Dawley Rat (µg equiv/g) | Levels in Lister-Hooded Rat (µg equiv/g) |
|---|---|---|
| **Liver** | 6.60 | 5.79 |
| **Kidney cortex** | 5.18 | 4.58 |
| **Kidney medulla** | 4.00 | 3.00 |
| **Adrenal cortex** | 5.11 | 3.39 |
| **Adrenal medulla** | 3.86 | Tissue not sectioned |
| **GI tract mucosa** | 3.27-6.00 | 2.29-6.95 |
| **Plasma** | 0.806 | 0.808 |
| **Uveal tract** | 0.727 | 36.2 |

By the 24-hr time point, many of the tissues showed radioactivity levels below the limit of quantification. Very few tissues had measurable radioactivity at the final time point, 72 hr.

**OSI Study # R2002318:** RO0508231: A Study of Distribution in the Rat by Quantitative Whole-Body Autoradiography. Module 4.2.2.3

Athymic female mice were administered an oral dose of [$^{14}$C]CP-358,774, 10 mg/kg, and whole-body autoradioluminography was conducted at 1, 2, 4, 8, 24, 48, 96, and 168 hr after drug administration. Two groups of mice were included, one that was tumor bearing with HN5 xenografts and one that was non-tumor bearing. Tissue distribution of radioactivity was similar between the two groups and uninfluenced by the presence of the tumors. The study does show distribution to the tumor as well. The comparison of the pituitary radioactivity levels with the levels measured in the cerebellum and cerebrum

show that there is minimal distribution of [$^{14}$C]CP-358,774 across the blood-brain barrier. The maximum concentration of radioactivity in the mice was measured at the 1 hr time point. By 24 hrs, most tissue levels were below the quantitative limits, except the levels in the alimentary canal and the liver of both groups of mice and the kidneys of the non-tumor bearing mice. The lower limit of quantification was approximately [redacted] ng equiv/g. Tissues that show means below the lower limit of quantification do so with a zero entered as the level for any single sample with a measurement below the LOQ for determination of the overall mean.

| | Tumor Bearing Mice (ng equiv/g) | Non-Tumor Bearing Mice (ng equiv/g) |
|---|---|---|
| **Liver** | 277 | 221 |
| **Kidney cortex** | 162 | 126 |
| **Pancreas** | 102 | 80 |
| **Adrenal gland** | 126 | 90 |
| **GI tract mucosa** | 808-19,234 | 788-5542 |
| **Pituitary** | 65 | 52 |
| **Cerebellum** | 12 | 11 |
| **Cerebrum** | 12 | 10 |
| **Tumor** | 59 | NA |

***Protein Binding***

**OSI Study # I2001125:** Binding of CP-358,774 in Plasma. Module 4.2.2.3

The degree of plasma binding of CP-358,774 was evaluated in plasma samples from humans, Beagle dogs, Sprague Dawley rats, and athymic Nude-*nu* mice. After 20 hrs of mixing time the samples were removed from the chambers of the [redacted] dialysis cell and stored at –20°C until analysis by HPLC and UV detection at [redacted] nm. The following table shows that plasma protein binding of CP-358,774 was significant. In the human plasma, nearly 92% of the drug was protein bound. In the mouse efficacy models, the $ED_{50}$ was shown to be 10 mg/kg, with and associated $EC_{50}$ of 8 µM. Assuming 95% protein binding in the mouse, that would mean an $EC_{50}$ of free drug to be 0.4 µM.

| Species | Mean Percent Bound (+/- SD) |
|---|---|
| Human | 91.6 (1.2) |
| Dog | 85.1 (2.9) |
| Rat | 91.5 (3.2) |
| Mouse | 94.5 (0.5) |

**OSI Study # I960015:** Protein Binding of CP-373,420 in Mouse, Rat, Dog, and Human Plasma. Module 4.2.2.3

Plasma binding of a metabolite of CP-358,774 was studied, CP-358,420. This metabolite is an O-demethylation product of CP-358,774 metabolism and an active metabolite. The binding of this metabolite is nearly identical to that of the parent compound in both the dog and human. In the rat and mouse it is slightly less protein bound than CP-358,774.

| Species | Mean Percent Bound (+/- SD) |
|---|---|
| Human | 90.9 (1.4) |
| Dog | 83.3 (1.2) |
| Rat | 77.5 (5.1) |
| Mouse | 73.0 (1.9) |

**2.6.4.5 Metabolism**

***In Vitro Metabolism***

**OSI Study # V950015:** Species Comparison of the Metabolic Half-life of EGFr Kinase Inhibitors in Rat, Dog and Human Liver Microsomes. Module 4.2.2.4.1

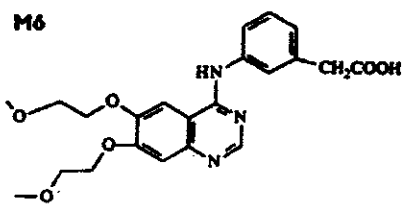
Several investigational compounds were studied for their metabolic half-life in rat, dog and human liver microsomes. For CP-358,774, the results are shown below.

| | Rat | Dog | Human |
|---|---|---|---|
| **CP-358,774** | >60 | 58 | 24 |

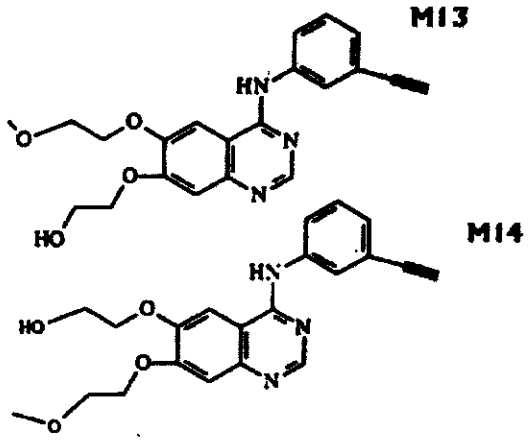
**OSI Study # V960014:** Identification of Metabolites of CP-358,774 in Rat and Human Liver Microsomes. Module 4.2.2.4.1

Identification of the metabolites of CP-358,774 was determined using [$^{14}$C]-CP-358,774-01 incubated with rat and human liver microsomes. HPLC analysis detected 5 radioactive peaks in both rat and human microsomes, with approximately 70% of the parent compound still present. When the quantity of substrate was reduced, an additional two metabolites were detected and the entire parent compound was consumed, for a total of 6 metabolites of [$^{14}$C]CP-358,774.-01. The higher concentration of substrate most likely saturated the enzyme binding sites. The major metabolite identified was the phenyl acetic acid derivative, M6.

M6



The desmethyl metabolites CP-373,413 (OSI-413) and CP-373,420 (OSI-420) are shown as M13 and M14 respectively.




The full proposed metabolic pathway of CP-358,774 in the rat is shown on page 50.

**OSI Study # V970162:** In vitro Metabolic Stability of EGFr Kinase Inhibitor CP-358,774 in Rat Liver and Lung Microsomes - Correlation with CYP1A1 Activity. Module 4.2.2.4.1

While the liver metabolizes a large number of drugs, extrahepatic metabolism is also possible. The metabolic stability of CP-358,774 was examined in rat liver microsomes. The drug was incubated in β-napthaflavone induced rat liver microsomes that over-express both CYP1A1 and CYP1A2. Another treatment group included microsomes pre-incubated with furafylline, a selective CYP1A2 inhibitor. The metabolic stability of CP-358,774 was also measured in human lymphoblastoid cells that express transfected human CYP1A1 cDNA. Then the metabolic stability was measured in rat lung microsomes and compared to β-napthaflavone induced rat lung microsomes that over-express CYP1A1. CYP1A1 is primarily an extrahepatic enzyme while CYP1A2 is found only in the liver. The results of these studies showed that there was little effect on the metabolism of CP-358,774 when the CYP1A2 inhibitor furafylline was added, indicating that CP-358,774 is not a substrate for CYP1A2. There is, however, rapid metabolism of CP-358,774 when β-napthaflavone was added and similarly fast turnover of CP-358,774 was seen with β-napthaflavone and pre-incubation with the CYP1A2 inhibitor, indicating that although CP-358,774 is not a substrate for CYP1A2, it is a substrate for CYP1A1.

| | |
|---|---|
| Rat liver microsomes | >30 |
| Rat liver microsomes + pre-incubation with furafylline | > 30 |
| Rat liver microsomes with β-napthaflavone | <5 |
| Rat liver microsomes with β-napthaflavone + pre-incubation with furafylline | < 5 |
| Human lymphoblastoid cells (that express transfected human CYP1A1 cDNA) | 15 |

***In Vivo Metabolism***

**OSI Study # R960021:** Identification of Metabolites of CP-358,774 in Sprague-Dawley Rats Following Oral Administration of a Single 5 mg/kg Dose of [14C]-CP-358,774-01. Module 4.2.2.4.2

Sprague Dawley rats were given a single oral 5 mg/kg dose of [$^{14}$C]CP-358,774-01. The rats were cannulated for urine and bile collection at 0-6, 6-24, 24-48 hrs post-treatment. An additional group of rats were given the same drug treatment and blood was collected upon euthanasia at 1, 4 and 8 hrs post-treatment.

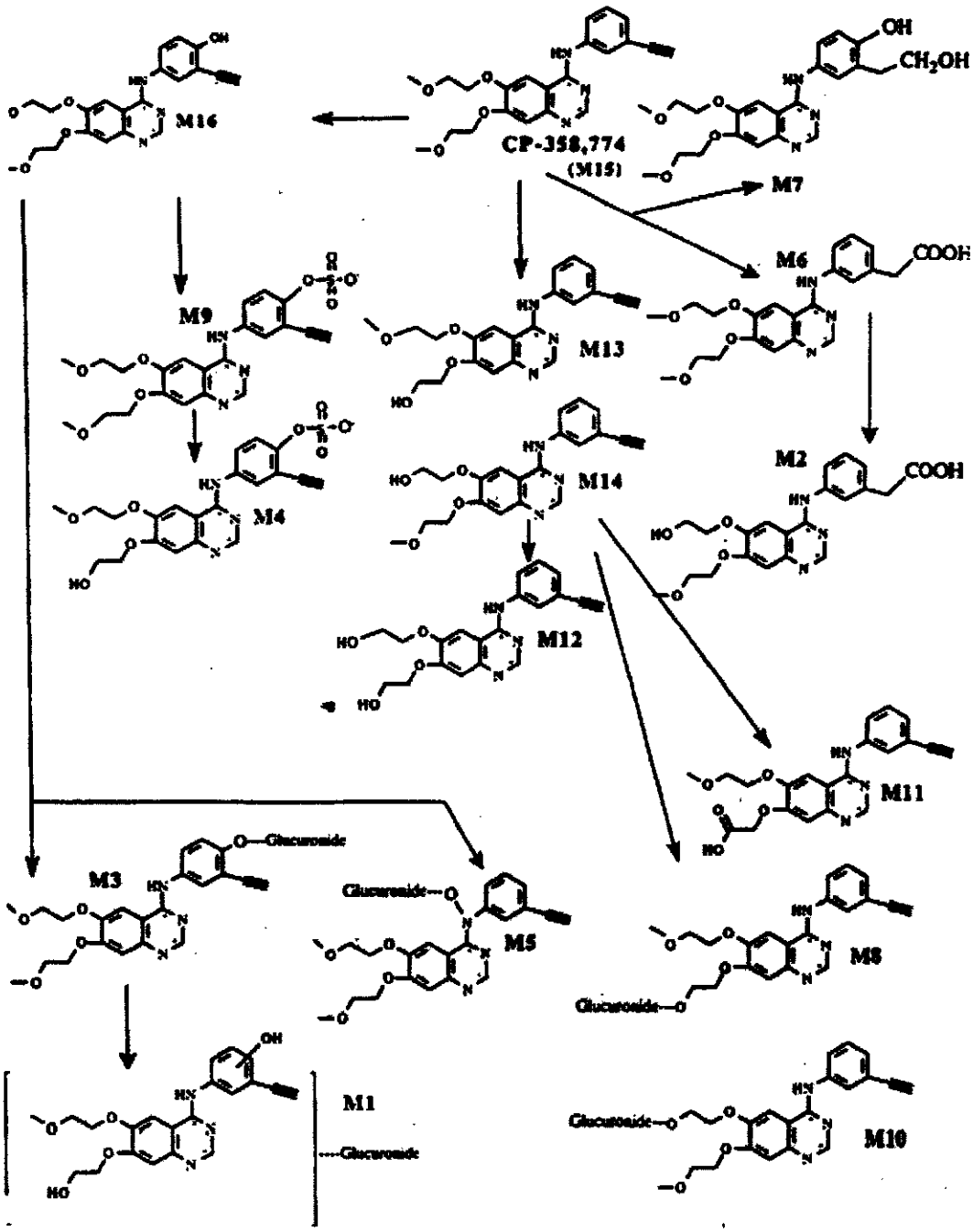
The following table shows that nearly 80% of the radioactivity is accounted for in the urine and bile by 48 hours after dosing, predominantly in the bile.

| Means | Urine 0-48 hr | Bile 0-48 hr | Total |
|---|---|---|---|
| **Males** | 27.6 | 52.7 | 80.2 |
| **Females** | 9.9 | 67.4 | 77.3 |
| **Combined** | 18.7 | 60.0 | 78.74 |

Metabolites in both the urine and bile were analyzed. Though not the major excretion route, urine did have 15 identifiable metabolites, which accounted for nearly 98% of the total radioactivity in the urine. Fifteen metabolites were also identified in the bile, with the parent compound accounting for only about 5% of the measured radioactivity and 15 metabolites accounting for about 93%. CP-358,774 is obviously extensively metabolized in the rat. The same 15 metabolites were found in both the bile and urine. When plasma was analyzed for metabolites, only 5 were found. One of these metabolites was a metabolite not found in the urine or bile, M16, while the other 4 were also present in biliary and urinary excretion. Unchanged drug accounted for 80% of the plasma sample taken form 0-8 hr. The M14 metabolite, CP-373,420 (OSI-420), accounted for 14% of the radioactivity.

Given the metabolites that were found in the bile, urine and blood, it appears there are three major routes for the metabolism of CP-358,774: (1) O-demethylation of the side

chains, (2) oxidation of the acetylene moiety followed by hydrolysis to the aryl carboxylic acid or the corresponding dihydro primary alcohol, (3) hydroxylation of the aromatic ring. The sponsor has mapped out the proposed metabolic pathway for the breakdown of CP-358,774 in the rat, as shown below.



**OSI Study # D970157:** Metabolism and Excretion of CP-358,774 in Beagle Dogs after Oral Administration of a Single 5 mg/kg Dose of [14C]-CP-358,774-01. Module 4.2.2.4.2

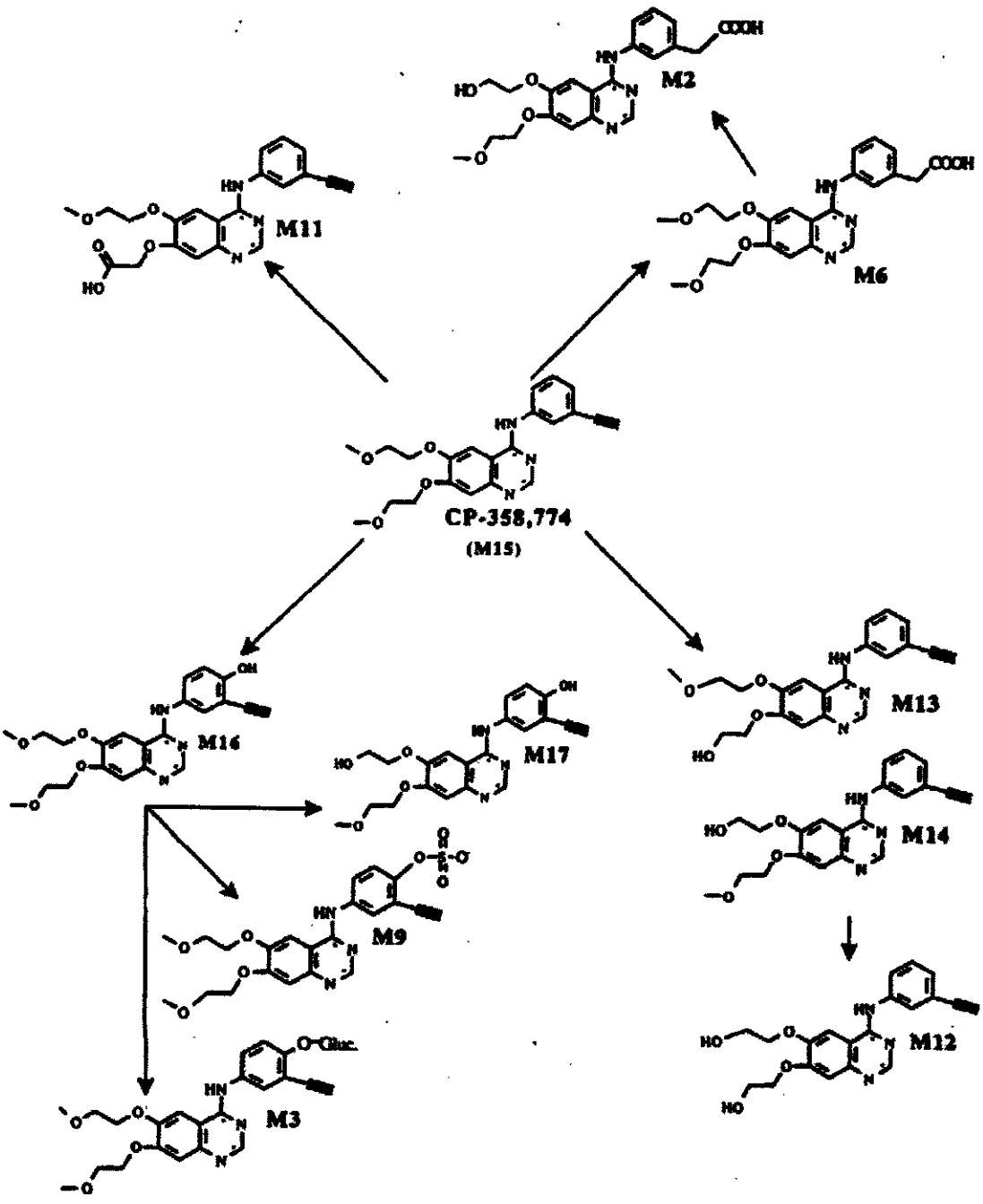
Beagle dogs were given a single oral dose of [$^{14}$C]CP-358,774-01, 5 mg/kg, and the metabolites in urine and feces were identified. Urine and feces were collected for 6 days at 0-24, 24-48, 48-72, 72-96, 96-120 and 120-144 hours post-dose. Nearly 98% of the radioactivity was recovered in the feces.

| Means | Urine 0-144 hr | Feces 0-144 hr | Total |
|---|---|---|---|
| **Males** | 2.5 | 95.3 | 97.8 |
| **Females** | 2.6 | 99.9 | 102.5 |
| **Combined** | 2.5 | 97.6 | 100.1 |

A total of 9 metabolites were identified in the urine, with the parent drug accountable for only about 1.2% of the radioactivity. In the feces, 7 metabolites were identified with approximately 9% of the radioactivity due to the parent compound. Four metabolites were identified in the plasma, along with the parent compound at about 68%. The sponsor's proposed pathway for metabolism of CP-358,774 in the Beagle dog is depicted below. Given the metabolites that were found in the feces, urine and plasma, it appears there are three major routes for the metabolism of CP-358,774 in the Beagle: (1) O-demethylation of the side chains followed by oxidation to the carboxylic acid, (2) oxidation of the acetylene moiety followed by hydrolysis to the aryl carboxylic acid or the corresponding dihydro primary alcohol, (3) O-dealkylation of the hydroxyethyl side chain, (4) hydroxylation of the aromatic ring.

APPEARS THIS WAY
ON ORIGINAL

Proposed metabolic pathway for CP-358,774 in the Beagle Dog:



**2.6.4.6 Excretion**

**OSI Study # R960020:** Mass Balance of CP-358,774 in Sprague-Dawley Rats Following Oral Administration of a Single 5 mg/kg Dose of [$^{14}$C]-CP-358,774-01. Module 4.2.2.5

Rats were administered a 5 mg/kg dose of [$^{14}$C]CP-358,774 orally and the radiolabeled mass balance of CP-358,774 determined. Urine and feces were collected for 6 days at 0-24, 24-48, 48-72, 72-96, 96-120, and 120-144 hours post-dose. Approximately 88% of the radioactivity was recovered in the feces by the end of the sampling period, with about 4% of it recovered in the urine. The metabolic profile in the intact rat is quite similar to that seen in the cannulated rat where urine and bile metabolites were analyzed, presented in Study # R960021 with the metabolism pathway presented on page 50. There were 3 metabolites not seen in that study that were seen in this study. M17 was identified in the urine of these rats and is believed to be a breakdown of the M16 metabolite. Two unidentified polar metabolites were seen in the urine of the intact rat and not the cannulated rat, but totally they accounted for less than 0.5% of the dose.

| Means | Urine 0-144 hr | Feces 0-144 hr | Total |
|---|---|---|---|
| **Males** | 3.8 | 87.6 | 91.4 |
| **Females** | 4.4 | 88.3 | 92.7 |
| **Combined** | 4.1 | 88.0 | 92.1 |

**OSI Study # R2002312:** RO0508231 (erlotinib): Excretion of Total Radioactivity in the Rat Following Oral and Intravenous Administration of [$^{14}$C]RO0508231 (Tarceva; OSI-774). Module 4.2.2.5

A study comparing the excretion of erlotinib after either oral or intravenous administration shows very similar excretion between the two routes at 96 hrs post-treatment. Nearly 97% of the radioactivity was recovered, with the majority of it (>90%) in the feces. A small amount was also recovered in the cage washing (~ 0.2%) The majority of the radioactivity was excreted within the first 24 hours, 80% in the oral group and 77% in the intravenous group.

| Means | Urine 0-96 hr | Feces 0-96 hr | Total |
|---|---|---|---|
| **Intravenous** | 5.8 | 90.4 | 96.5 |
| **Oral** | 3.7 | 92.9 | 96.7 |

**OSI Study # D970157:** Metabolism and Excretion of CP-358,774 in Beagle Dogs after Oral Administration of a Single 5 mg/kg Dose of [$^{14}$C]CP-358,774-01. Module 4.2.2.5

Previously reviewed in the metabolism section, this study showed that the excretion of [$^{14}$C]CP-358,774 in the Beagle was qualitatively very similar to the rat. The feces was the primary route of excretion, with only about 2.5% of the radioactivity recovered in the urine and over 97% of it recovered in the feces.

**2.6.4.7 Pharmacokinetic drug interactions**
None Conducted

**2.6.4.8 Other Pharmacokinetic Studies**
None Conducted

**2.6.4.9 Discussion and Conclusions**

Erlotinib is rapidly absorbed in the animal models and at most doses used in the toxicology studies a dose-proportional increase in drug exposure was seen with increasing doses of erlotinib. Erlotinib is extensively metabolized and metabolites are found in the plasma after the parent compound is no longer detected. Excretion is primarily through biliary excretion into the GI tract and >70% of the drug is accounted for in the feces. Erlotinib is generally well distributed to tissues throughout the mouse and rat, with limited distribution across the blood-brain-barrier.

The table in 2.6.4.10 shows that the toxicology information in the dog is not as complete as it could be. The dose that was too toxic during the 12-month study did yield an AUC greater than that seen in the clinic. But the highest dose from which most of the chronic toxicity data in the dog was obtained, 15 mg/kg, has an AUC nearly one-third that observed in the clinic. A dose between these two doses would help to clarify the toxicity of chronic erlotinib administration in the dog.

### 2.6.4.10 Tables and figures to include comparative TK summary

| | AUC (µg·h/mL) | | | | | |
|---|---|---|---|---|---|---|
| **Daily Dose (mg/kg)** | **Mouse** | **Rat** | | **Dog** | **Rabbit** | **Human** |
| | | ***Male*** | ***Female*** | | ***Female*** | |
| 1 | | 1.1 | 1.9 | | | |
| 10 | | 21.2 | 37<br>45.7** | | | |
| 15 | | | | 15 | | |
| 50 | 70.2 | | | 74.3 | 112 | |
| 75 | | | | | 181 | |
| 100 | | | | | 365 | |
| 150 | 119.3 | | | | | 41.3* |

* - steady state during a dosing interval
** - pregnant rats

### 2.6.5 PHARMACOKINETICS TABULATED SUMMARY

See 2.6.4.10

### 2.6.6 TOXICOLOGY

#### 2.6.6.1 Overall toxicology summary

General toxicology:
The general toxicology of erlotinib has been examined in a wide range of laboratory animals: mouse, rat, dog, and monkey using the IV and PO routes of administration. Single dose studies have previously been reviewed in the original IND for this compound. These studies were conducted in the mouse, rat and dog using the IV and PO routes. The primary toxicities seen were gastrointestinal (emesis, loose stool) and hepatobiliary (increased bilirubin), and renal (blood in urine). Oral doses as high as 6,000 mg/m$^2$/day were lethal in the rat but the highest acute oral dose tested in the dog, 5,000 mg/m$^2$/day was not lethal.

Repeat-dose toxicity was examined in the mouse, rat, dog and monkey, primarily using the PO route, but several IV studies have also been conducted. The pivotal rat study was a six-month PO study. In this study the effects seen with erlotinib administration included decreases in body weights and food consumption. Histopathology showed ovarian atrophy, renal papillary necrosis with tubular dilatation and multifocal necrosis, angiectasis of the adrenal glands, and degeneration/inflammation of the skin. These effects were seen at both the 30 and 60 mg/m$^2$/day dose, with increasing frequency and severity with the higher dose and are more common and more severe in the female rats than in the males. The renal necrosis and tubular dilatation was seen despite the fact that

mass balance studies have shown the primary route of erlotinib excretion is fecal, with little renal excretion noted. Many of these effects are not surprising, considering the pharmacology of erlotinib. In particular, the skin effects are most likely a direct EGFR inhibitory effect. Studies have shown erlotinib inhibits UDP-glucuronosyltransferase. This enzyme is responsible for the conjugation of bilirubin. Inhibition by erlotinib no doubt is at least part of the reason for the increases in bilirubin seen in this and other studies. The chronic rat study also showed evidence of liver toxicity following long-term treatment with erlotinib. Liver weights were increased and histopathological changes in the liver were noted. In addition, a significant increase in liver enzymes was noted in the HD rats in this study, a finding that increased over the course of the study. "Hy's Law" would indicate the potential for liver toxicity with chronic administration of Tarceva clinically. The principal route of excretion of erlotinib is hepatobiliary, as mass balance studies have shown the majority of the drug is found in the feces. The increases in ALT, AST and total bilirubin, combined with the histopathology of the liver seen in the rat after 6 months of dosing, warrant the concern for liver toxicity.

The pivotal dog repeat-dose study was conducted for 12 months using doses up to 300 $mg/m^2$/day. The study began with a dose of 1,000 $mg/m^2$/day as the HD group. This group was suspended after 12 days of dosing due mostly to corneal ulcers, emesis and decreased body weights. Upon necropsy, these animals showed kidney papillary necrosis and GI inflammation, erosion, congestion and glandular dilation. Increased ALT and bilirubin were both seen in this group of dogs. Reddening of the skin and buccal mucus membrane were seen in all the dose groups, considered a direct EGFR inhibition effect. While the sponsor refers to the MD, 150 $mg/m^2$/day, as the NOAEL in this study, it appears that the HD had little to no adverse effects either. The only significant adverse effect was a decrease in body weight gain in 3/4 male dogs. There was no question that 1,000 $mg/m^2$/day was too high a dose for a 12-month study. The highest dose tested for the full 12 months, 300 $mg/m^2$/day, appears to be too low a dose to fully characterize the toxicity of erlotinib in the dog. An additional dose could have been added, > 300 $mg/m^2$/day but < 1000 $mg/m^2$/day, that would have shown significant toxicities but not have impacted the ability to dose for the full timeframe of the chronic study.

Most of the toxicities seen in the dog 12-month study were not surprising and while an additional dose group above 300 $mg/m^2$/day could have been tested in this study, it may not have added any additional worthwhile information. The ophthalmological effects of erlotinib seen in this study were previously unknown. While certainly an adverse event such as this might be very worrisome, this toxicity has not proven to be problematic during human exposure to erlotinib. Initial studies with Tarceva included very thorough examinations for the potential of corneal ulceration due to drug exposure. Only one incidence was noted and it was believed due to a problem with the patient's contact lenses. The HD dogs also showed a decrease in heart rate with subsequent increases in PR and QT interval. The potential for QT prolongation was further explored in safety pharmacology studies with conscious dogs and erlotinib did not cause QT prolongation, and has not been seen in the clinic.

Genetic toxicology:
Erlotinib was tested for mutagenicity and clastogenicity in the *in vitro* Ames test, human lymphocytes and the Chinese Hamster Ovary assay and in the *in vivo* mouse micronucleus assay. In the doses tested in these studies, with and without metabolic activation, erlotinib was not mutagenic or clastogenic.

Studies submitted but not reviewed in this report examined [ ] a potential impurity in the erlotinib drug product, at levels less than —, for mutagenicity and clastogenicity, as well. It too was negative under the test conditions, in the Ames assay and the chromosome aberration test with human peripheral blood lymphocytes. This impurity was present in some, but not all, general toxicology studies, but not in the genetic toxicity studies with erlotinib. Of the 14 batches of erlotinib tested throughout the nonclinical program, this impurity was present in about half, always at levels below the — limit.

Carcinogenicity:
To date, no carcinogenicity studies have been done with erlotinib. The carcinogenic potential of erlotinib is unknown.

Reproductive toxicology:
Reproductive toxicology of erlotinib was studied in both the Wistar rat and the New Zealand White rabbit. Studies included Segment I, II, and III reproductive tests.

In the rat, doses of erlotinib up to 60 mg/m$^2$/day were tested for an effect on fertility in both male and female animals. No effects were seen on the mating and fertility parameters, although toxicity was noted at the highest dose based on decreased weight gains. Erlotinib appears to have no effect on the ability of the male rats to impregnate untreated females or for treated females to become pregnant by untreated male rats.

During the fertility study, it was noted that although the treated female rats showed no effect on fertility, the number of implantation sites, corpora lutea and live fetuses were decreased slightly but significantly from control at 60 mg/m$^2$/day and the number of live fetuses decreased at 30 mg/m$^2$/day when the dosing occurred for 14 days before mating and until GD 7. In both these groups the number of early resorptions was also increased.

In a pilot study in the rat, females treated from GD 6-17 showed significant increases in early resorptions and decreases in the number of live fetuses at both 30 and 60 mg/m$^2$/day of erlotinib. When pregnant rats were dosed from GD 6-17 with erlotinib, a high dose of 120 mg/m$^2$/day had to be reduced due to the severe toxicity evident by the weight loss in the dams. This group showed a significant increase in resorptions and post-implantation loss with a decrease in the number of live fetuses, number of fetuses per litter and the fetal weights. Total litter loss was noted in 4/19 of the HD dams. Near the end of dosing, the AUC(0-24) for these rats was 16 h·µg/mL, nearly 4 times the AUC (steady state) seen in the clinic, 41.3 h·µg/mL . In the fetuses examined, a slight increase in incomplete ossification was seen in the sternal element at the HD. At both the MD and HD, small, incompletely inflated lungs were noted.

A follow-up Segment II study was conducted in the rat with the pregnant females treated from GD 6-20 with up to 60 mg/m$^2$/day. In this study, maternal toxicity was noted again by the decrease in body weights at the MD and HD, as well as increased neutrophils, bilirubin and ALT levels in the HD. This study did not replicate the findings in the pilot study. There were no increases in the resorptions of fetuses or decrease in live fetuses. The lungs of the fetuses were normal upon examination. In the HD group in this study the AUC(0-24) was comparable to that seen in the clinic, at 45.7 h·μg/mL. The effects seen in lungs of the pups from the first study were seen in both groups that received 60 mg/m$^2$/day, one having received 120 mg/m$^2$/day for the first 6 days. It is important to note that in the pilot study, only 10-20 pups per dose group were evaluated. The definitive rat Segment II study group had evaluations of over 100 pups in each dose group. This study, unlike the pilot study, was also a GLP study. Because of these differences, the results of the latter study appear to be definitive when compared to the pilot study. These results show no teratogenicity of erlotinib when it is administered to pregnant rats during organogenesis. There is also no effect on the number of live fetuses or number of resorptions with erlotinib in this study.

A Segment II study was conducted in the rat with the $F_0$ females dosed from GD 6- PND 20 with doses of erlotinib up to 72 mg/m$^2$/day. While the $F_0$ dams had decreases in body weights and food consumption, no other impact was seen of erlotinib administration in this study design. The $F_1$ and $F_2$ generations showed no effect of the $F_0$ drug treatment.

In the rabbit, doses of up to 600 mg/m$^2$/day were administered to pregnant does from GD 7-19. Maternal toxicity was seen in the decreased body weights in the HD does. A dose of 300 mg/m$^2$/day also led to slightly decreased maternal body weights. Two HD rabbits were euthanized moribund and 8/20 HD does aborted their entire litters from GD 19-22. The AUC(0-24) at Day 19 in these rabbits was approximately 3 times the AUC(steady state) seen in the clinic (112 h·μg/mL in the rabbit, 41.3000 h·μg/mL in the clinic). No teratogenicity was seen in any of the fetuses in this study. The HD does had increased resorptions and decreases in litter size, number of live fetuses and fetal body weights. These effects were not seen in the MD rabbits, with an AUC(0-24) of 37.4 h·μg/mL, approximately comparable to the steady state AUC seen in the clinic.

The results of the reproductive toxicology studies with erlotinib show that it is not a teratogen in either the rat or the rabbit. AUC levels in these studies were comparable and greater to that which has been seen in the clinic. A Segment II study in the rabbit did show erlotinib to be an abortifacient and treatment with erlotinib prior to mating in the rat, while not affecting fertility, did increase the number of resorptions and decrease the number of live fetuses in the treated dams. The Segment III study showed no significant effects of erlotinib on the rat $F_0$, $F_1$, or $F_2$ generations.

Special toxicology:
Additional studies were conducted with erlotinib to examine its effects on dermal toxicity, ocular irritation, skin sensitization, and phototoxicity. In rabbits, only minimal skin irritation was seen in 1/6 animals tested and the slight erythema was resolved within

two days. When the eyes of 3 rabbits were treated with erlotinib there was a clear discharge, slight conjunctival reddening and chemosis in all animals at 1 hr post-treatment. By 24 hrs all eyes appeared normal. Erlotinib is not considered either a corrosive material or an ocular irritant.
The Guinea Pig Maximization Test was conducted to determine the skin sensitization potential of erlotinib. Using the Kligman/Magnusson classification, erlotinib would be considered a mild skin sensitizer.

Phototoxicity of erlotinib was evaluated both *in vitro* and *in vivo*. The *in vitro* study was negative, though there were solubility problems with the erlotinib. In the hairless rat, a weak though discernable phototoxicity reaction was seen. Tissue distribution studies with erlotinib showed significant binding to melanin, which may contribute to the weak phototoxicity seen in the hairless rat. Given the degree of this reaction, it is unlikely that phototoxicity would be a significant toxicity clinically.

**2.6.6.2 Single-dose toxicity**
These studies were not reviewed for this NDA, though several were previously reviewed with the original IND. Summarized data are presented in Section 2.6.7.

**2.6.6.3 Repeat-dose toxicity**

**Study title**: 6-month oral toxicity study in Sprague-Dawley rats.

**Key study findings**:
- No significant effects were seen at the 1 mg/kg/day dose
- Female rats showed both a higher exposure to CP-358,774-01 and more toxicity than male rats
- Male rats showed decreased body weights at the HD of 10 mg/kg/day
- Histopathology and clinical chemistry data show liver toxicity, predominantly at the HD and in the female rats
- Ovarian atrophy was seen in the HD females
- Degeneration and/or inflammation of the skin were noted on histopathology. Hematology results were also indicative of an inflammatory response.

| | |
|---|---|
| **Study no.:** | **OSI Study # R980200 (Pfizer study # 98-086)** |
| **Volume #, and page #:** | **Module 4.2.3.2** |
| **Conducting laboratory and location:** | Pfizer<br>Centre de Recherche<br>37400 Amboise, France |
| **Date of study initiation:** | 6 Oct 1998 |
| **GLP compliance:** | Yes – Letter included and signed |
| **QA report:** | yes (X) no ( ) |
| **Drug, lot #, and % purity:** | CP-358,774-01; Lot # 38869-228-4F; — |

**Methods**

| | |
|---|---|
| Doses: | 0, 1, 5, and 10 mg/kg |
| Species/strain: | Rat/ CD® (SD) IGS BR – Sprague/Dawley |
| Number/sex/group or time point (main study): | 15/sex/dose |
| Route, formulation, volume, and infusion rate: | PO; in 0.5% methylcellulose, 10 ml/kg |
| Satellite groups - toxicokinetics or recovery: | 5/sex/dose |
| Age: | ~7 weeks |
| Weight (nonrodents only): | NA |
| Unique study design or methodology (if any): | None |

**Observation times and results**

Mortality: Checked daily

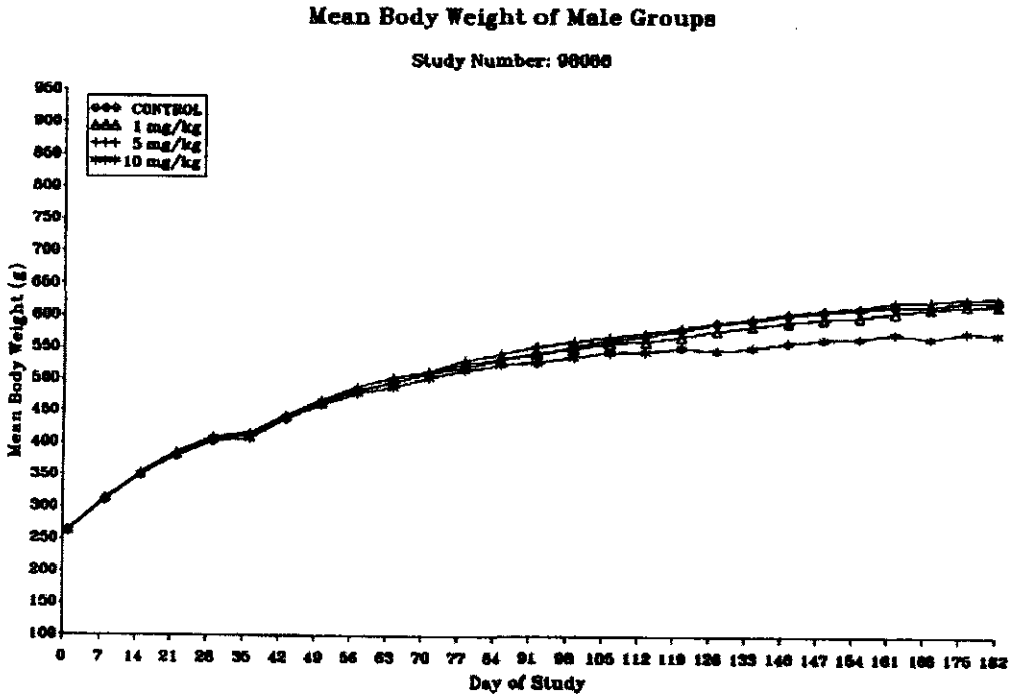
Only deaths – 1/10 LD Toxicokinetics rats on Day 113
1/10 MD Toxicokinetics rats on Day 130

No mortality in main Toxicology rats

Clinical signs: Monitored daily for the first week and then weekly thereafter.

| | Dose | | | Comments |
|---|---|---|---|---|
| | 1 mg/kg | 5 mg/kg | 10 mg/kg | |
| **Skin lesions** | | | 3-13 | From day 50. Presence of scabs on the muzzle |
| **Rough fur** | | 1-10 | 4-22 | From day 50 at the HD<br>From day 85 (♀) or 141 (♂) at the MD |
| **Thinned fur** | | | 1-11 | From day 85 (♀) or 148 (♂) |
| **Chromodacryorrhea** | 0-2 | 0-1 | 3-18 | From day 36 at HD<br>Sporadically at the LD and MD |

Body weights: Checked weekly
A significant difference was seen in the body weights of the HD males only. The sponsor's graph shows the body weights of the male rats. This effect was not seen in the female rats, as there was no difference in the body weights of any of the dose groups compared to control.




Food consumption: Measured weekly
While there was a significant increase in the food consumed by the MD and HD females during the second half of the study, there was no concurrent body weight differences. There were no differences in the food consumption of the male rats.

Ophthalmoscopy: Baseline before start of study and a follow-up conducted toward the end of the study

- No treatment-related ocular findings.

EKG:
Not conducted

Hematology: Conducted after 1 and 6 months of treatment

| | Dose | | | |
|---|---|---|---|---|
| | 5 mg/kg | | 10 mg/kg | |
| | ***Males*** | ***Females*** | ***Males*** | ***Females*** |
| Red cell distribution width | | | | |
| Day 34 | ↑ 5% | ↑ 4% | ↑ 8% | ↑ 6% |
| Day 183 | ↑ 7% | --- | ↑ 12% | ↑ 6% |
| Hemoglobin | | | | |
| Day 34 | --- | --- | --- | --- |
| Day 183 | --- | --- | ↓ 3% | ↓ 8% |
| RBC count | | | | |
| Day 34 | --- | --- | --- | --- |
| Day 183 | --- | --- | --- | ↓ 6% |
| Hematocrit | | | | |
| Day 34 | --- | --- | --- | --- |
| Day 183 | --- | --- | --- | ↓ 7% |
| Monocytes | | | | |
| Day 34 | --- | --- | ↑ 35% | ↑ 40% |
| Day 183 | ↑ 48% | ↑ 31% | ↑ 52% | ↑ 141% |
| Neutrophils | | | | |
| Day 34 | --- | --- | --- | ↑ 79% |
| Day 183 | --- | ↑ 69% | ↑ 139% | ↑ 207% |
| Eosinophils | | | | |
| Day 34 | --- | --- | --- | --- |
| Day 183 | --- | ↑ 76% | --- | ↑ 68% |
| Basophils | | | | |
| Day 34 | --- | --- | --- | --- |
| Day 183 | --- | --- | --- | ↑ 65% |
| WBC count | | | | |
| Day 34 | --- | --- | --- | --- |
| Day 183 | --- | --- | ↑ 31% | ↑ 59% |

Clinical chemistry: Conducted after 1 and 6 months of treatment

| | Dose | | | |
|---|---|---|---|---|
| | 5 mg/kg | | 10 mg/kg | |
| | *Males* | *Females* | *Males* | *Females* |
| Bilirubin | | | | |
| Day 34 | --- | ↑ 82% | ↑ 89% | ↑ 847% |
| Day 183 | ↑ 78% | ↑ 263% | ↑ 130% | ↑ 734% |
| ALT | | | | |
| Day 34 | ↑ 24% | ↑ 28% | ↑ 42% | ↑ 59% |
| Day 183 | ↑ 67% | --- | ↑ 84% | ↑ 358% |
| AST | | | | |
| Day 34 | --- | --- | ↑ 20% | ↑ 20% |
| Day 183 | ↑ 62% | --- | ↑ 107% | ↑ 295% |
| Triglycerides | | | | |
| Day 34 | ↑ 33% | --- | --- | --- |
| Day 183 | --- | --- | ↓ 49% | ↓ 26% |
| Cholesterol | | | | |
| Day 34 | --- | --- | --- | ↑ 29% |
| Day 183 | ↑ 21% | ↑ 33% | --- | ↑45% |
| Urea | | | | |
| Day 34 | --- | --- | --- | --- |
| Day 183 | --- | --- | ↑ 21% | ↑ 17% |

Urinalysis: Conducted after 1 and 6 months of treatment

| Dose | Male Rats | Female Rats |
|---|---|---|
| Control - 0 | 1/15 | 2/15 |
| 1 mg/kg/day | 0/15 | 0/15 |
| 5 mg/kg/day | 8/15 | 3/15 |
| 10 mg/kg/day | 6/15 | 10/15 |

Gross pathology:

| Finding | Control | | 10 mg/kg/day | |
|---|---|---|---|---|
| | *Males* | *Females* | *Males* | *Females* |
| Alopecia | 0/15 | 1/15 | 7/15 | 3/15 |
| Abnormal kidney surface | 0/15 | 0/15 | 1/15 | 3/15 |
| Pituitary enlargement | 0/15 | 1/15 | 0/15 | 6/15 |
| Cachexia | 0/15 | 0/15 | 0/15 | 3/15 |

Organ weights:

| Finding | 5 mg/kg/day | 10 mg/kg/day | |
|---|---|---|---|
| | *Females* | *Males* | *Females* |
| Liver | | | |
| Absolute weight | ↓ 12% | --- | ↓ 18% |
| Relative weight | | --- | ↓ 21% |
| Adrenals | | | |
| Absolute weight | --- | --- | ↓ 100% |
| Relative weight | --- | ↓ 28% | ↓ 26% |
| Kidney | | | |
| Absolute weight | --- | ↑ 11% | ↓ 18% |
| Relative weight | --- | ↓ 21% | ↓ 20% |

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

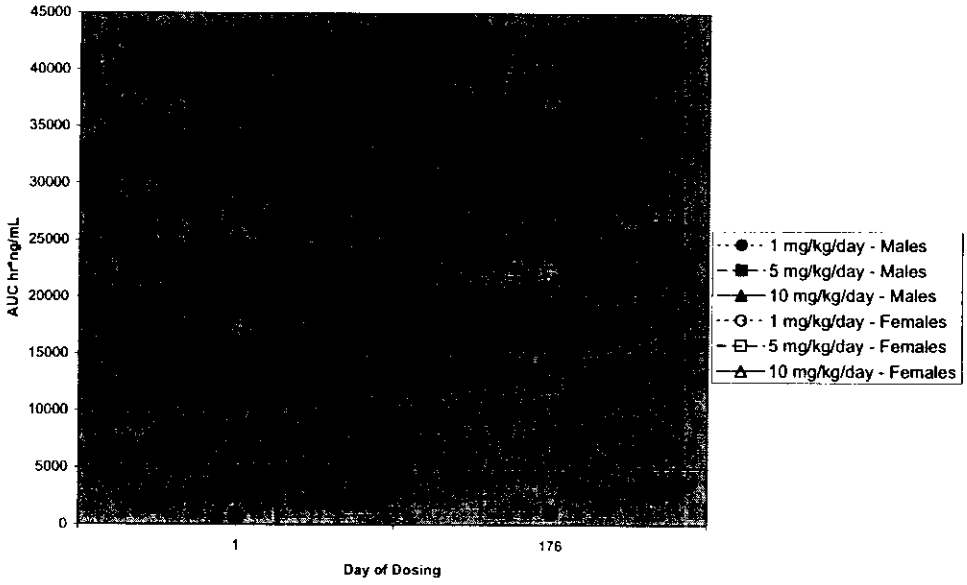
| Finding | Control | | 5 mg/kg/day | | 10 mg/kg/day | |
|---|---|---|---|---|---|---|
| | *Males* | *Females* | *Males* | *Females* | *Males* | *Females* |
| Skin | | | | | | |
| Degeneration/inflammation | 0/15 | 0/15 | 2/15 | 2/15 | 11/15 | 11/15 |
| Cervical Node | | | | | | |
| Plasmacytosis | 8/15 | 7/15 | 10/15 | 10/15 | 14/15 | 13/15 |
| Kidney | | | | | | |
| Papillary necrosis | 0/15 | 0/15 | 0/15 | 2/15 | 0/15 | 9/15 |
| Dilation, tubular | 0/15 | 0/15 | 0/15 | 1/15 | 0/15 | 9/15 |
| Chronic inflammation | 0/15 | 1/15 | 0/15 | 0/15 | 1/15 | 4/15 |
| Ovaries | | | | | | |
| Atrophy | --- | 2/15 | --- | 6/15 | --- | 14/15 |
| Adrenals | | | | | | |
| Angiectasis | 0/15 | 2/15 | 0/15 | 1/15 | 0/15 | 9/15 |
| Liver | | | | | | |
| Necrosis | 0/15 | 1/15 | 1/15 | 3/15 | 0/15 | 7/15 |
| Infiltration, mononuclear | 4/15 | 6/15 | 4/15 | 4/15 | 1/15 | 10/15 |

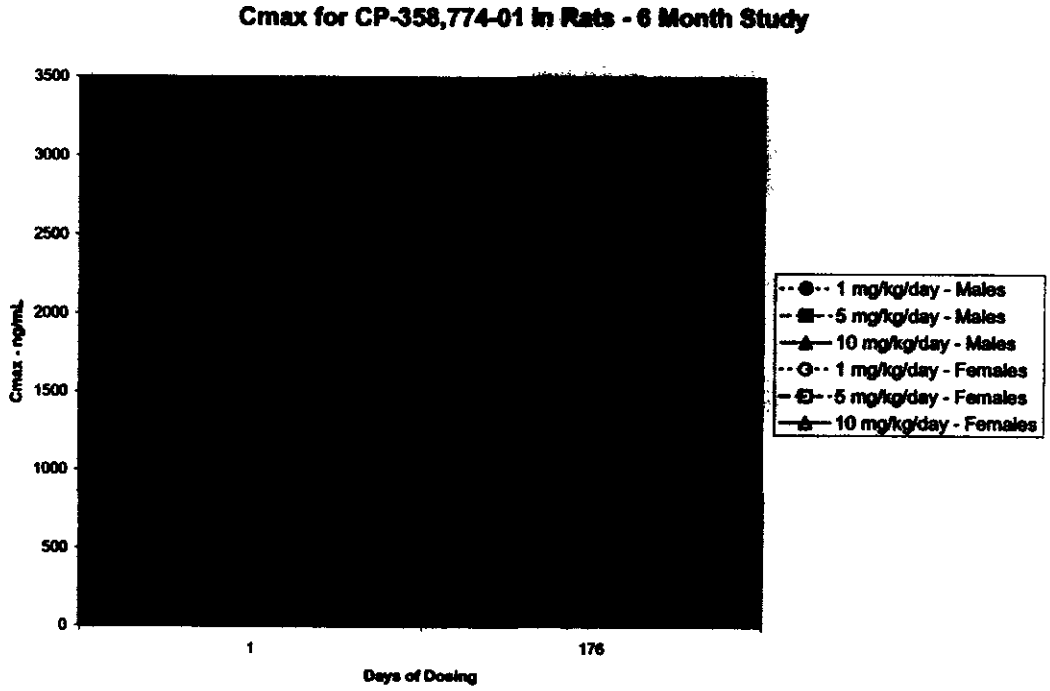
Toxicokinetics:
The table and graphs below show the toxicokinetics of CP-358,774-01 during this study. the toxicokinetic parameters increased with the higher doses, though not in a linear fashion. There was a gender difference with females exhibiting a higher AUC and Cmax in all doses and at both time points.

**Toxicokinetic Parameters CP-358,774-01 In 6 Month Rat Study**

| Dose | Sex | Day of Dosing | Cmax – ng/mL | Cmax – ng/mL Dose Normalized | $AUC_{0-\infty}$ hr*ng/mL | $AUC_{0-\infty}$ hr*ng/mL Dose Normalized |
|---|---|---|---|---|---|---|
| 1 mg/kg/day | M | 1 | 99 | 99 | 627 | 627 |
| | M | 176 | 157 | 157 | 1075 | 1075 |
| | F | 1 | 195 | 195 | 888 | 888 |
| | F | 176 | 317 | 317 | 1903 | 1903 |
| 5 mg/kg/day | M | 1 | 857 | 171 | 9124 | 1825 |
| | M | 176 | 1230 | 246 | 12375 | 2475 |
| | F | 1 | 1378 | 276 | 17066 | 3413 |
| | F | 176 | 1943 | 389 | 22042 | 4408 |
| 10 mg/kg/day | M | 1 | 2002 | 200 | 25669 | 2567 |
| | M | 176 | 1885 | 189 | 21174 | 2117 |
| | F | 1 | 3006 | 301 | 41811 | 4181 |
| | F | 176 | 2861 | 286 | 37036 | 3704 |

**AUC for CP-358,774-01 in Rats - 6 Month Study**






Other:
None

**Study title**: 12-Month Oral Toxicity Study in Beagle Dogs.

**Key study findings**:

- 50 mg/kg/day was too toxic, with dogs losing weight, exhibiting clinical signs of toxicity and having corneal ulcerations
- While 50 mg/kg/day was clearly too toxic, the modified HD of 15 mg/kg/day was most likely too low for a complete assessment of toxicity.
- All doses tested showed minor clinical signs: hair loss, redness of skin and redness of buccal mucus membrane with increased frequency and a dose-related time to onset – all likely pharmacological actions of the drug.
- Ophthalmologic exam showed foci of pigmentation in the tapetal fundus in 1/8 dogs in the MD and HD group, with apparent worsening in the HD dog from 6 – 12 months.

| | |
|---|---|
| **Study no.:** | **OSI Study # D980197** |
| **Volume #, and page #:** | **Module 4.2.3.2** |
| **Conducting laboratory and location:** | Pfizer<br>Centre de Recherche<br>37401 Amboise Cedex<br>France |
| **Date of study initiation:** | 1 October 1998 |
| **GLP compliance:** | Letter included and signed |
| **QA report:** | yes (X) no ( ) |
| **Drug, lot #, and % purity:** | CP-358,774-01, lot# 38869-228-4F, not given |

**Methods**

| | |
|---|---|
| Doses: | **Final doses:**<br>2.5, 7.5 and 15 mg/kg/day<br>**Starting doses:**<br>5, 15, 50 mg/kg/day<br>(on Day 13 50 mg group stopped dosing – 4/8 euthanized Day 14 other 4/8 euthanized on Day 96)<br>(on Day 50, 5 mg group →7.5 mg)<br>(a 2.5 mg group was added) |
| Species/strain: | Dog/Beagle |
| Number/sex/group or time point (main study): | 4/sex/dose |
| Route, formulation, volume, and infusion rate: | Oral, methylcellulose, 1 mL/kg |
| Satellite groups - toxicokinetics or recovery: | No additional groups |
| Age: | 8-9 months |
| Weight (nonrodents only): | 10.1 ± 0.7 kg ♂ and 8.0 ± 0.9 kg ♀ |
| Unique study design or methodology (if any): | None |

**Observation times and results**

Mortality: Daily before dosing and 5 hrs after

50 mg/kg/day – 4/8 dogs euthanized moribund on Day 14 – 3 ♂ and 1 ♀
4/8 stopped treatment and euthanized on Day 96 – 1 ♂ and 3 ♀

Clinical signs: Daily before dosing and 5 hrs after

50 mg/kg/day – Beginning on Day 8 - 13
No food intake 6/8 dogs
Blood in stool 3/8
↓ activity 3/8
Tremor 2/8
Emaciation 4/8
Prostration 3/8
Severe corneal ulceration 7/8

The corneal lesions were preceded by

Redness of palpabral and bulbar conjunctiva 8/8
Lacrimation 8/8
Purulent discharge 6/8
Protruding nictitating membranes 7/8

Unilateral focal opacities of the cornea in 1/4 of this group that was allowed a recovery period

| Frequency of Clinical Signs | | | |
|---|---|---|---|
| **Clinical Sign** | **Dose – mg/kg/day** | | |
| | **2.5** | **7.5** | **15** |
| **Reddening of skin and/or hair loss** | 2/8 | 3/8 | 5/8 |
| **Redness of buccal mucus membrane** | 4/8 | 6/8 | 8/8 |

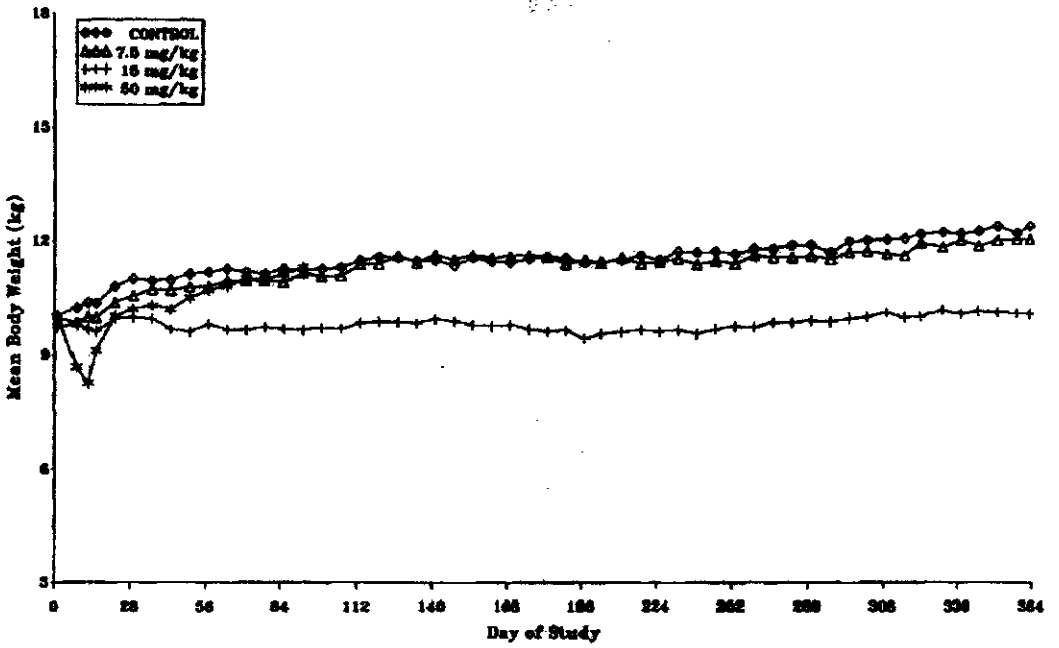
Body weights: Several weights before study began, then weekly during treatment

The discontinued group of 50 mg/kg/day had significant weight loss compared to controls. The animals that were not euthanized and remained in the laboratory, without drug treatment, for 80+ more days, had fully recovered to body weights comparable to control. The sponsor's graph below shows the body weights of the 7.5, 15 and 50 mg/kg/day groups. All dose groups showed reductions in mean body weight gain compared to control, though this was only significant in 3/4 HD males, 15 mg/kg/day. At the end of the study, the HD males had gained 0.4 kg while the control males had gained 2.4 kg.

APPEARS THIS WAY
ON ORIGINAL

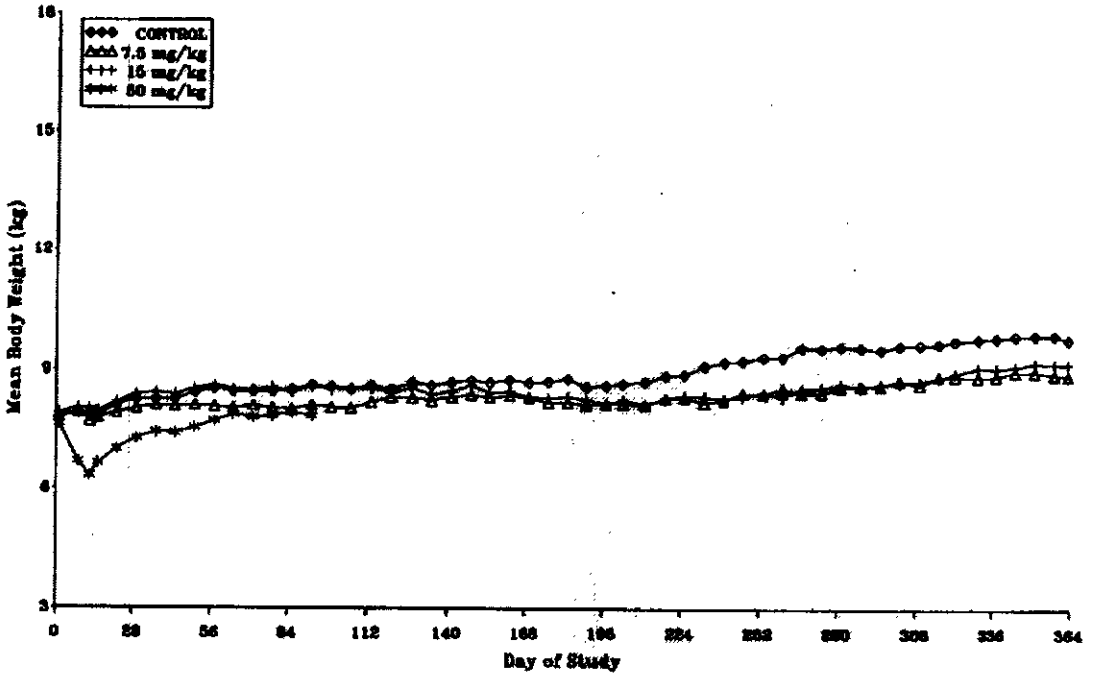
**Mean Body Weight of Male Groups**

Study Number: [illegible]




**Mean Body Weight of Female Groups**

Study Number: [illegible]



Food consumption: Daily

Results of the food consumption were not presented.

Ophthalmoscopy: Prior to study and then Days 30, 181, 286 and 358

50 mg/kg/day – ophthalmological exam on Day 14 showed:
Edema – 7/8
Ulceration – 4/8
Unilateral perforation of cornea – 2/8

15 mg/kg/day - ophthalmological exam at 6 months showed:
Foci of pigmentation - 1/8 with 5 focal spots
15 mg/kg/day - ophthalmological exam at 12 months showed:
Foci of pigmentation - 1/8 with 8 focal spots

7.5 - ophthalmological exam at 6 months showed:
Foci of pigmentation - 1/8 with 1 spot
7.5 - ophthalmological exam at 12 months showed:
Foci of pigmentation - 1/8 with 1 spot

EKG: Prior to study then after about 20, 27, 34, 44 and 51 weeks – conducted predose and then 2 hr after dosing

No effect on systolic blood pressure

HD group – heart rate ↓ 18% relative to pre-dose heart rate on Week 51
But within the historical controls of the laboratory

HD group – ↑ 4% in PR interval – relative to pre-dose value
↑ 5% in QT interval – relative to pre-dose value
Also within historical controls of the laboratory and were concomitant with the ↓ heart rate. Heart rate is known to have an inverse relationship with PR and QT interval durations.

Hematology: Twice before study then after 8, 34, 173 and 364 days

50 mg/kg/day – neutrophils and fibrinogen were significantly increased over their Day –1 pre-dose measurement

| | Fibrinogen | Neutrophils |
|---|---|---|
| Males | | |
| Day 9 | ↑ 135 | ↑ 47 |
| (% Δ from control) | (↑ 120) | (↓1.2) |
| Day 14 | ↑ 221 | ↑ 376 |
| (% Δ from control)* | --- | --- |
| Females | | |
| Day 9 | ↑ 182 | ↑ 60 |
| (% Δ from control) | (↑ 157) | (↑ 57) |
| Day 14 | ↑ 219 | ↑ 189 |
| (% Δ from control) | --- | --- |

* - No control groups measured on Day 14

There were also some minor changes in RBC, HCT, and HGB in this group, less than 10% changes from their pre-dose levels. These parameters increased in the male dogs and decreased in the female dogs by Day 14.

No treatment-related effects in any of the remaining groups

Clinical chemistry: Twice before study then after 8, 34, 173 and 364 days

| | Bilirubin | Alkaline Phosphotase | Urea | Triglyceride | Cholesterol |
|---|---|---|---|---|---|
| Males | | | | | |
| Day 9 | ↑ 156 | ↓ 2 | ↑ 20 | ↑ 37 | ↑ 72 |
| (% Δ from control) | (↑173) | (↑13) | (↑20) | (↑59) | (↑ 85) |
| Day 14 | ↑ 166 | ↑ 137 | ↑ 146 | ↑ 120 | ↑ 50 |
| (% Δ from control)* | --- | --- | --- | --- | --- |
| Females | ** | | | | |
| Day 9 | ↑ 67 | ↓ 26 | ↑ 50 | ↑ 36 | ↑ 34 |
| (% Δ from control) | (↑62) | (↓ 23) | (↓ 1) | (↑17) | (↑ 19) |
| Day 14 | ↑ 84 | ↑ 120 | ↑ 158 | ↑ 70 | ↑ 26 |
| (% Δ from control) | --- | --- | --- | --- | --- |

* - No control groups measured on Day 14
** - One female with extremely high bilirubin levels

All dogs also showed a 19-49% decrease in albumin on Days 9 and 14, with an associated decrease in calcium.

No treatment-related effects in any of the remaining groups

Urinalysis: Once before study then on Days 34 and 364

No treatment related effects were seen

Gross pathology: Upon necropsy

50 mg/kg/day – animals euthanized on Day 14
- 2/4 – cachexia and dehydration
- 4/4- marked bilateral ocular changes – red/dark discoloration on anterior chamber and/or the cornea
- 3/4 – abnormal corneal surface
- 3/4 – marked bilateral changes in kidney – white/green discoloration of papilla and/or red discoloration at corticomedullary junction
- 1/3 – small prostate

GI tract macroscopic changes
- 4/4 – stomach - dark discoloration and abnormal surface
- 3/4 – duodenum – red/dark discoloration and abnormal surface
- 2/4 - jejunum – red discoloration
- 2/4 – ileum – red discoloration
- 2/4 – rectum – abnormal surface

50 mg/kg/day – animals euthanized on Day 96
- 1/4 – minimal changes, discoloration in GI, abnormal stomach surface, enlarged cervical lymph nodes

All other doses showed no signs of treatment-related gross pathology

Organ weights : Upon necropsy

50 mg/kg/day - Organs were not weighed

No significant treatment-related effects on organ weights

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

| | Dose Group (mg/kg/day) | |
|---|---|---|
| | 50– SAC at Day 14 | 50– SAC at Day 96 |
| Corneal atrophy | | |
| Marked, bilateral, diffuse | 4/4 | --- |
| Corneal ulcers | | |
| Marked/severe | 3/4 | --- |
| Uveal inflammation | 3/4 | --- |
| Kidney | | |
| Acute papillary necrosis | 3/4 | --- |
| Mild congestion | 1/4 | --- |
| Moderate tubular dilation | 1/4 | --- |
| Esophagus | | |
| Inflammation | 3/4 | --- |
| Stomach | | |
| Hemorrhage | 1/4 | --- |
| Erosion/ulcer | 1/4 | --- |
| Duodenum | | |
| Congestion | 2/4 | --- |
| Dilation, glandular | 4/4 | --- |
| Jejunum | | |
| Congestion | 1/4 | --- |
| Dilation, glandular | 1/4 | --- |
| Ileum | | |
| Congestion | 1/4 | --- |
| Dilation, glandular | 2/4 | --- |
| Mild, focal necrosis | --- | 1-4 |
| Cecum | | |
| Dilation, glandular | 1/4 | --- |
| Rectum | | |
| Inflammation w/ glandular dilation | 1/4 | --- |
| Prostate | | |
| Moderate diffuse atrophy | 1/3 | --- |
| Skeletal muscle | | |
| Minimal, multifocal, degeneration | 2/4 | --- |
| Cervical lymph node | | |
| Mild,/moderate sinusal histiocytosis | 4/4 | 1/4 |

No other treatment-related histopathological changes were seen in the remaining dose groups.

Toxicokinetics: Blood taken on Days 1, 29, 50, 183 and 351. Dogs bled at 1, 2, 4, 6 ,8, and 24 hrs after dosing.

The sponsor's table below shows that the AUC of CP-358,774 increased with increasing doses, but actually decreased with repeated exposure as seen on Day 351. There was a large degree of variability in these parameters between dogs. While the decrease in exposure over repeated administration is indicative of a possible induction of the metabolism of CP-358,774, the data are too variable and the results inconclusive.

**Mean AUC of CP-358,774 over the 0-24 hour period (ug.h/ml)**

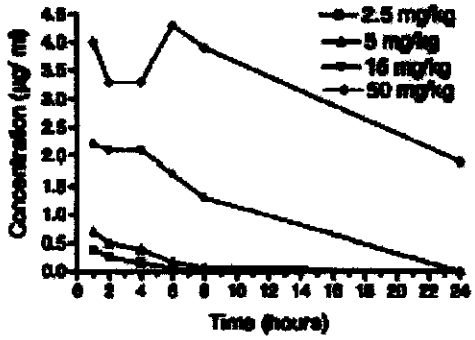
| Dose (mg/kg) | Day 1 | Day 29 | Day 50 | Day 183 | Day 351 |
|---|---|---|---|---|---|
| 2.5 | 1.4 | | | 1.4 | 0.9 |
| 5->7.5* | 3.5 | 3.1 | 6.9 | 6.5 | 2.4 |
| 15 | 24.9 | 22.2 | | 14.8 | 15.0 |
| 50 | 74.3 | | | | |

*: dose increased from 5 to 7.5 mg/kg on day 50

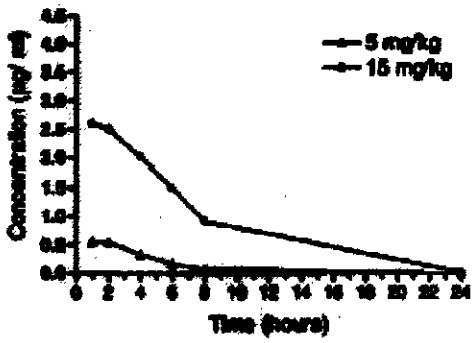
The sponsor's graphs below show that the mean concentrations of CP-358,774 peak at around 8 hours and with repeated administration the length of time that the drug is detectable in the blood is shorter. Graphs of the desmethylation metabolite CP-358,420 show a similar pattern though at $1/10^{th}$ the levels of the parent compound and the metabolite is still measurable after the 15 mg/kg dose on day 351 up to 24 hrs after administration. These data are not shown here. Similar findings have been seen in other studies reported in the Pharmacokinetics section as well as in the Reproductive Toxicology section.

Variations of mean concentrations of CP-358,774 with time

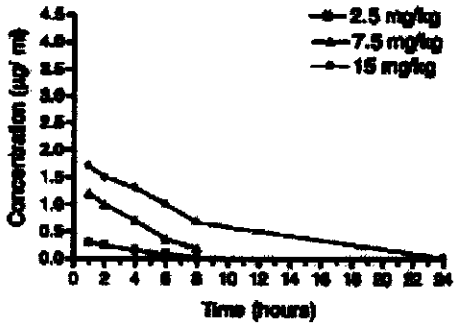
Day 1



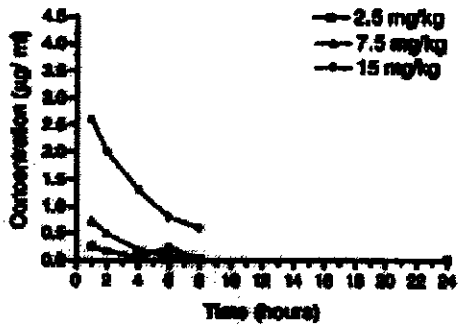

Day 29




Day 183




Day 351




Other:
None

**Histopathology inventory**

| Study | #R980200 | #D980197 |
|---|---|---|
| Species | Rat | Dog |
| Adrenals | X* | X* |
| Aorta | X | X |
| Bone Marrow smear | X | |
| Bone (sternum) | X | |
| Brain | X* | X* |
| Cecum | X | X |
| Cervix | | X |
| Colon | X | X |
| Duodenum | X | X |

| | | |
|---|---|---|
| Epididymis | X | X |
| Esophagus | X | X |
| Eye | X | X |
| Fallopian tube | | |
| Gall bladder | | X |
| Gross lesions | | X |
| Harderian gland | X | |
| Heart | X* | X* |
| Ileum | X | X |
| Injection site | | |
| Jejunum | X | X |
| Kidneys | X* | X* |
| Lachrymal gland | | |
| Larynx | | |
| Liver | X* | X* |
| Lungs | X | X |
| Lymph nodes, cervical | X | X |
| Lymph nodes mandibular | | |
| Lymph nodes, mesenteric | X | X |
| Mammary Gland | | X |
| Nasal cavity | | |
| Optic nerves | | X |
| Ovaries | X | X* |
| Pancreas | X | X |
| Parathyroid | X | X |
| Peripheral nerve | X | |
| Pharynx | | |
| Pituitary | X | X* |
| Prostate | X | X |
| Rectum | | |
| Salivary gland | X | X |
| Sciatic nerve | | X |
| Seminal vesicles | X | |
| Skeletal muscle | X | |
| Skin | X | X |
| Spinal cord | X | X |
| Spleen | X* | X* |
| Sternum | | X |
| Stomach | X | X |
| Testes | X* | X* |
| Thymus | X | X |
| Thyroid | X | X |
| Tongue | | |
| Trachea | X | X |
| Urinary bladder | X | X |
| Uterus | X | X |
| Vagina | X | |
| Zymbal gland | | |

X, histopathology performed
*, organ weight obtained

**2.6.6.4 Genetic toxicology**

**Study title: Microbial reverse mutation assays.**

**Key findings:** CP-358,774-01 was not mutagenic in the microbial reverse mutation assay at the doses tested.

| | |
|---|---|
| **Study no:** | **OSI # V950006** (95-1241-02 ) |
| **Volume #, and page #:** | Module 4.2.3.3.1 |
| **Conducting laboratory and location:** | Drug Safety Evaluation Department<br>Central Research Division<br>Pfizer, Inc.<br>Groton, CT-2106 06340 |
| **Date of study initiation:** | 28 March 1995 |
| **GLP compliance:** | Compliance included and signed |
| **QA reports:** | yes ( X ) no ( ) |
| **Drug, lot #, and % purity:** | CP-358,774-01, lot # JLD-32498-52-1A, purity not given |

**Methods:**

Strains/species/cell line: *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537
*Escherichia coli* strain $WP_2uvrA$

Doses used in definitive study: 2, 10, 50, 200, 1000 µg/plate

Basis of dose selection: A range finding study was conducted to determine the solubility of the test article in the overlay agar and cytotoxicity of the test article. Doses of 10, 50, 200, 1000, and 5000 µg/plate were used with strains TA98, TA100, TA1535, TA1537 and $WP_2uvrA$ with and without S9 activation. The doses of 200 and 1000 µg/plate had insoluble compound in both the range finding assay and the definitive assay. No cytotoxicity was seen at these doses.

Negative controls: DMSO

Positive controls:

| Strain | Without S9 | With S9 Activation |
|---|---|---|
| TA1535 | sodium nitrite | |
| TA1537 | 9-aminoacridine | All strains – |
| TA98 | 2-nitrofluorene | 2-aminoanthracene |
| TA100 | nitrofurantoin | |
| $WP_2uvrA$ | ENNG | |

Incubation and sampling times: Incubated for 48-72 hrs then revertant colonies counted

**Results:**

Study validity:

- Three replicate plates
- Methods state that counts were done "using a [redacted] colony counter" but no specifics are given.
- Criterion for a positive result is the same as the criterion used for a positive control: a dose-related, reproducible, three-fold increase in revertant colonies per plate when compared to negative controls.
- The negative and positive control values were within the historical control data ranges.
- Study design is valid. Though no evidence suggests a specific requirement of either a two- or three-fold increase in revertant colonies over background for a positive result, this is the generally accepted method of evaluation. The results showed both the positive and negative controls to be well within the historical controls, and the plates treated had comparable results to the negative controls.

Study outcome:

- CP-358,774-01 was not mutagenic in the microbial reverse mutation assay with or without S9 activation at the doses up to 1000 µg/plate.

**Study title:** ***In vitro*** **cytogenetic assays.**

**Key findings:** CP-358,774-01 was not clastogenic, as determined by the concentrations tested in the *in vitro* human lymphocyte assay.

| | |
|---|---|
| **Study no:** | **OSI # V950007** (95-1241-03) |
| **Volume #, and page #:** | Module 4.2.3.3.1 |
| **Conducting laboratory and location:** | Drug Safety Evaluation Department<br>Central Research Division<br>Pfizer, Inc.<br>Groton, CT-2106 06340 |
| **Date of study initiation:** | March 1995 |
| **GLP compliance:** | Compliance included and signed |
| **QA reports:** | yes ( X ) no ( ) |
| **Drug, lot #, and % purity:** | CP-358,774-01, lot # JLD-32498-52-1A, purity not given |

**Methods:**

| | |
|---|---|
| Strains/species/cell line: | Human peripheral lymphocytes from healthy donors |
| Doses used in definitive study: | 6.85, 8.56, 10.7, 13.4, 16.8, 21.0, 26.2, **32.8, 41.0, 51.2,** 64.0, **80.0 μg/ml – without activation**<br>**26.7, 33.4, 41.8, 52.2, 65.3,** 81.6, 102, 128, 160, 200 **μg/plate – with activation**<br>**bolded concentrations** are those with sufficient mitotic suppression, therefore these were the concentrations analyzed |
| Basis of dose selection: | A range finding study was conducted to determine the solubility of the test article in the overlay agar and cytotoxicity of the test article. Concentrations ranging from 2.0 – 500 μg/ml without activation or 4.44 – 15.35 μg/ml with activation were tested. |
| Negative controls: | DMSO |
| Positive controls: | Mitomycin-C – without activation<br>Cyclophosphamide – with activation |
| Incubation and sampling times: | Incubated for 47-49 hrs then centrifuged, suspended, and counted |

**Results:**

Study validity:

- Two replicate plates/concentration, with at least three analyzable test article concentrations.
- No details are given on the methodology or apparatus used for counting abnormal metaphase cells.
- Criterion for a positive result is a statistically significant, dose-related, and reproducible increase in the number of abnormal cells compared to the concurrent test solvent controls.
- The negative and positive control values were within the historical control data ranges.
- Study design and findings are valid.

Study outcome:

- CP-358,774-01 did not induce chromosome aberrations in human lymphocyte cultures *in vitro* with or without S9 activation at the concentrations tested.

**Study title: Mammalian mutation assays.**

**Key findings:** CP-358,774-01 was not mutagenic in a mammalian cell assay, via measurements of induction of gene mutations at the HGPRT locus in the Chinese hamster ovary cells, at the doses tested.

| | |
|---|---|
| **Study no:** | **OSI # V950008** (95-1241-12) |
| **Volume #, and page #:** | Module 4.2.3.3.1 |
| **Conducting laboratory and location:** | Drug Safety Evaluation Department<br>Central Research Division<br>Pfizer, Inc.<br>Groton, CT-2106 06340 |
| **Date of study initiation:** | October 1995 |
| **GLP compliance:** | Compliance included and signed |
| **QA reports:** | yes ( X ) no ( ) |
| **Drug, lot #, and % purity:** | CP-358,774-01, lot # JLD-32498-52-1A, — purity |

**Methods:**

| | |
|---|---|
| Strains/species/cell line: | Chinese hamster CHO- — cell line |
| Doses used in definitive study: | 38, 75, 150, 350, 650, 950 µg/ml |
| Basis of dose selection: | A range finding study was conducted to determine the solubility and cytotoxicity of the test article. Concentrations ranging from 16 – 945 µg/ml were tested. |
| Negative controls: | DMSO |
| Positive controls: | Ethylmethanesulfonate – without metabolic activation<br>3-methlycholanthrene (3-MCA) – with activation |
| Incubation and sampling times: | Incubated for 7-8 days then fixed, stained, and mutations counted. |

**Results:**

Study validity:

- Two replicate plates/concentration, with at least three analyzable test article concentrations.
- Cells were counted using a Coulter Counter.
- Criterion for a positive result is at least one concentration produces a mean mutant frequency $\geq 20$ with a statistically significant dose-related trend. It also must be reproducible (concordance between replicate plates) and repeatable (a weak increase in mutant frequency may be tested by repeating the study).
- The negative and positive control values were within the historical control data ranges.
- Study design was perhaps too restrictive in its definition of a positive result. A clear dose-related increase in percentage of cells with aberrations, with at least on concentration being statistically significant would normally be considered positive. The results did not show any dose-related increase in mutant frequency at all.

Study outcome:

- In the definitive study, showed substantial cytotoxicity (relative cell survival < 20% on day 3) in doses ≥ 650 µg/ml without metabolic activation.
- With metabolic activation, the definitive study showed moderate cytotoxicity at 950 µg/ml (< 50% relative cell survival at Day 3).
- CP-358,774-01 did not induce a substantial increase in mutant colonies, chromosome aberrations in the Chinese Hamster Ovary cell assay with or without S9 activation at the concentrations tested.

**Study title: Mouse micronucleus assay.**

**Key findings:** CP-358,774-01 did not induce chromosome damage at the doses tested in the mouse micronucleus assay.

| | |
|---|---|
| **Study no:** | **OSI # M950010** (95-1241-10) |
| **Volume #, and page #:** | Module 4.2.3.3.2 |
| **Conducting laboratory and location:** | Drug Safety Evaluation Department<br>Central Research Division<br>Pfizer, Inc.<br>Groton, CT-2106 06340 |
| **Date of study initiation:** | October 1995 |
| **GLP compliance:** | Compliance included and signed |
| **QA reports:** | yes ( X ) no ( ) |
| **Drug, lot #, and % purity:** | CP-358,774-01, lot # 34,314-181-2, — % purity |

**Methods:**

| | |
|---|---|
| Strains/species/cell line: | CD-1 mouse |
| Doses used in definitive study: | 250, 500, 1000, and 2000 mg/kg PO |
| Basis of dose selection: | Doses were chosen based on an exploratory mouse toxicity study with an oral dose of CP-358,774-01 of 2000 mg/kg where 1/6 mice died. |
| Negative controls: | 0.5% methylcellulose |
| Positive controls: | Mitomycin-C (given IP) |
| Incubation and sampling times: | Animals were euthanized 24 hrs after final of 3 CP-358,774-01 administrations |

**Results:**

Study validity:

- Two slides were prepared from each mouse
- Slides were scored for micronuclei by eye.
- Criterion for a positive result is a significant, dose-related and reproducible elevation in the number of micronucleated polychromatic erythrocytes (MNPCE) in the treated animals when compared to control.
- The negative and positive control values were within the historical control data ranges.
- Study design and findings are valid.

Study outcome:

- The HD of 2000 mg/kg was discontinued after the first administration due to mortality (3/10 in the micronuclei group and 8/18 in the toxicokinetic satellite group).
- 2/5 males in the 1000 mg/kg micronuclei group died.
- 1/5 males in the 500-mg/kg group died.
- Bone marrow cytotoxicity was seen – with a significant decrease in the % polychromatic erythrocytes (PCE) in the male mice.
- No significant increase in the % MNPCE
- CP-358,774-01 did not induce a substantial increase in mutant colonies, chromosome aberrations in the Chinese Hamster Ovary cell assay with or without S9 activation at the concentrations tested.

**2.6.6.5 Carcinogenicity**

No carcinogenicity studies were included.

**2.6.6.6 Reproductive and developmental toxicology**

## Fertility and early embryonic development

**Study title**: Segment I (Tarceva): Oral study of male fertility in the rat.

**Key study findings**:

- RO0508231-001 (Tarceva) adversely affected body weights and food consumption in the highest dose (10 mg/kg/day) administered to male rats.
- RO0508231-001 did not adversely affect fertility. All mating and fertility indices, reproductive parameters measured in the maternal rat and sperm evaluations were unaffected by the drug administration to the male rats.

| | |
|---|---|
| **Study no.:** | **OSI Study # R2003114** |
| **Volume #, and page #:** | Module 4.2.3.5.1 |
| **Conducting laboratory and location:** | F. Hoffman-L Roche Ltd. |

| | |
|---|---|
| | Non-Clinical Development – Drug Safety<br>CH-4070 Basel, Switzerland |
| **Date of study initiation:** | 15 October 2002 |
| **GLP compliance:** | Letter included and signed |
| **QA reports:** | yes (X) no ( ) |
| **Drug, lot #, and % purity:** | RO0508231-001; #BS02070001; — % purity |

**Methods**

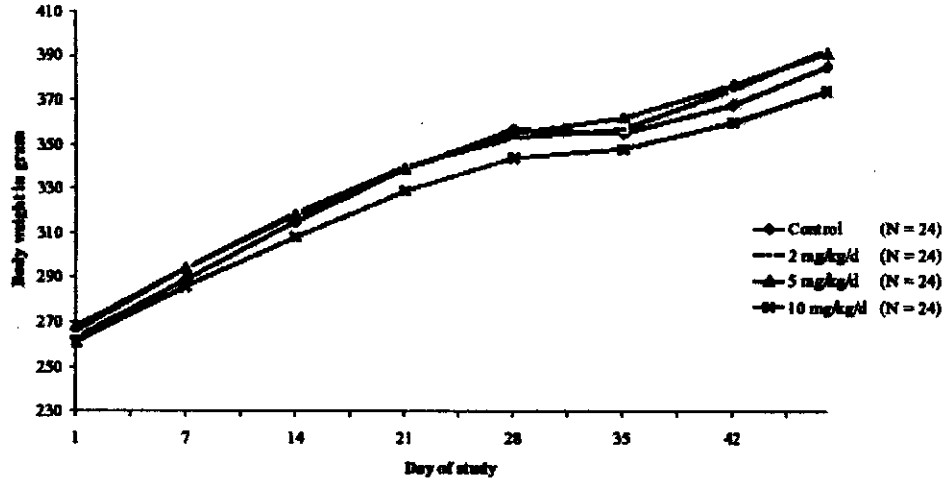
| | |
|---|---|
| Doses: | 0, 2, 5, and 10 mg/kg/day |
| Species/strain: | Rat; Wistar (HanBrl:WIST) |
| Number/sex/group: | 24/sex/dose |
| Route, formulation, volume, and infusion rate: | Oral gavage, Polysorbate 80 + Hydroxyethylcellulose; 10 mL/kg/day |
| Satellite groups used for toxicokinetics: | None |
| Study design: | Males only dosed. Dose 4 weeks prior to mating, throughout 2-week mating period and up to one day prior to sacrifice. |
| Parameters and endpoints evaluated: | Sperm motility, sperm head count, mating index, fertility index, preimplantation loss, postimplantation loss |

**Results**

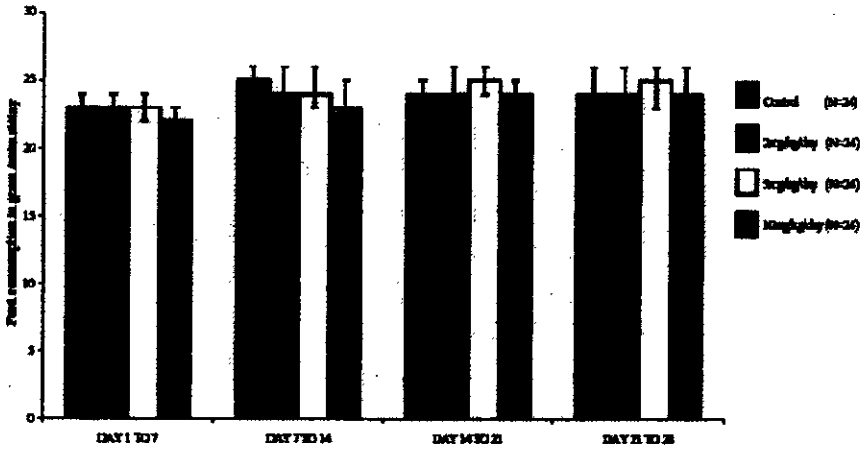
Mortality:
None

Clinical signs:
None

Body weight:
A significant decrease in body weights was seen in the HD group. During the first two weeks of drug administration the HD rats gained less weight than the controls, and the decrement was observable throughout the study. The sponsor's graph below shows the body weights of the male rats during the pre-mating, mating and post-mating time periods. No effect on body weights was seen in the LD or MD groups.




Food consumption:
Similar results to the body weight effects were seen on food consumption. The HD rats ate less food in the first two weeks of drug administration than the control group. This effect was not seen in the LD and MD groups. The sponsor's graph shows the effects of Tarceva on food consumption in male rats during the pre-mating, mating, and post-mating periods of this study.



Toxicokinetics:
Not conducted

Necropsy:
No treatment related effects were seen upon necropsy

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):
There were no treatment-related effects on any of the mating and fertility indices. The maternal reproductive parameters such as pre- and post-implantation loss, and live litter size also showed no drug-related effects. Testis weight, sperm motility and testicular sperm count were also unaffected by the drug treatment.

**Study title**: Segment I (Tarceva): Oral study of female fertility and early embryonic development in the rat.

**Key study findings**:

- Female rats treated with RO0508231-001 showed decreased food consumption and body weights at the HD (10 mg/kg/day).
- The estrous cycle and mating and fertility indices of the treated females were not affected and no effects were seen on necropsy.
- There was an adverse effect on the number of corpora lutea, implantation sites and live fetuses. These parameters were decreased in the HD group and the MD (5 mg/kg/day) group had a significant decrease in live fetuses compared to control.
- There were increases in the numbers of early resorptions seen in the MD and HD groups.

| | |
|---|---|
| **Study no.**: | **OSI Study # R2003115** |
| **Volume #, and page #**: | Module 4.2.3.5.1 |
| **Conducting laboratory and location**: | F. Hoffman-L Roche Ltd.<br>Non-Clinical Development – Drug Safety<br>CH-4070 Basel, Switzerland |
| **Date of study initiation**: | 7 October 2002 |
| **GLP compliance**: | Letter included and signed |
| **QA reports**: | yes (X) no ( ) |
| **Drug, lot #, and % purity**: | RO0508231-001; #BS02070001; — % purity |

**Methods**

| | |
|---|---|
| Doses: | 0, 2, 5 and 10 mg/kg/day |
| Species/strain: | Rat/Wistar (HanBrl:WIST) |
| Number/sex/group: | 24/sex/dose |
| Route, formulation, volume, and infusion rate: | Oral gavage, Polysorbate 80 + Hydroxyethylcellulose; 10 mL/kg/day |
| Satellite groups used for toxicokinetics: | None |
| Study design: | Females dosed daily for 14 days prior to mating, continuing throughout mating and |

| | |
|---|---|
| | up to and including Day 7 of gestation (GD7). Males not dosed. |
| Parameters and endpoints evaluated: | Estrous cyclicity, upon necropsy - live implantations, dead implantations, # of corpora lutea. Mating index, fertility index, and % pre- and post-implantation loss. |

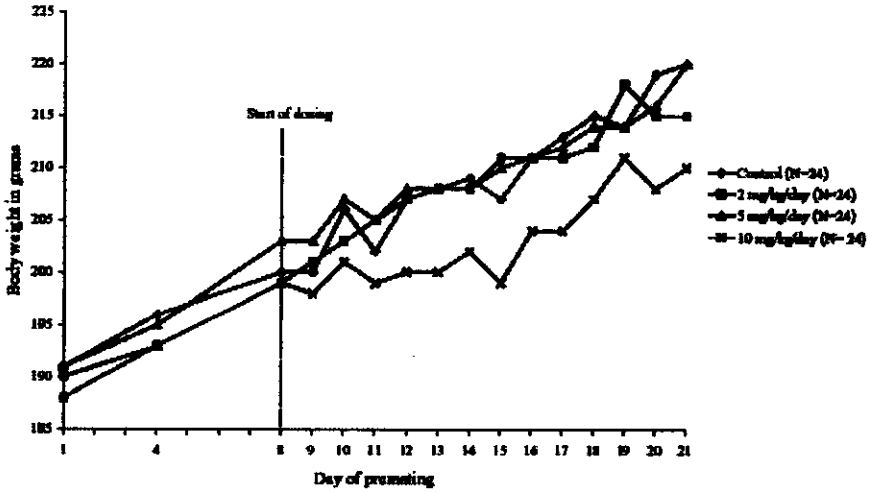
**Results**

Mortality:
No mortality was seen.

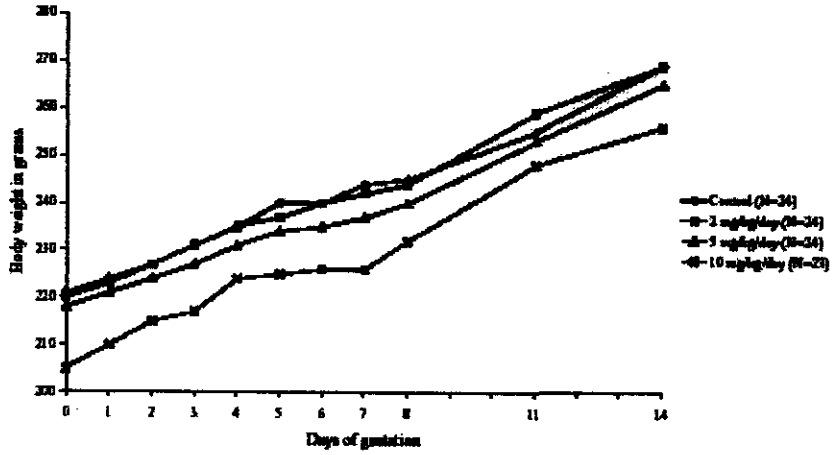
Clinical signs:
No treatment-related clinical signs were observed.

Body weight:

Gains in maternal body weights were significantly reduced once dosing began. The sponsor's graph below shows that in the HD group, the female rats, during the pre-mating phase, had significantly lower body weights.



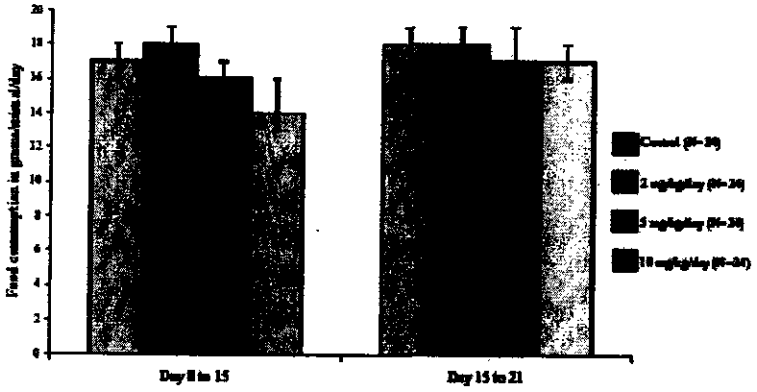

During gestation, the HD dams gained weight at comparable levels to that of the other groups. As drug administration began 14 days prior to the start of mating, and the HD rats began the gestational period at a lower starting weight of the HD rats, this caused the overall body weights to still be significantly lower than in the control group. Once dosing ceased, there was a compensatory increase in the body weights of the HD rats. The sponsor's graph below shows the body weights during gestation. Drug administration was discontinued on GD 7.






There were no significant drug effects on the body weights of the LD and MD rats.

Food consumption:
The HD females ate significantly less food than the controls. No effect of drug treatment was seen on food consumption in the LD or MD groups. The sponsor's graph shows the effects on food consumption.




Toxicokinetics:
Not conducted.

Necropsy:
No treatment-related effects on necropsy findings were noted.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):
No adverse effects were seen on the estrous cycle of the treated females.
No adverse effects were seen on the mating and fertility indices.
There were significant drug effects on the number of corpora lutea and the number of live fetuses and implantation sites.

| | Control | 2 mg/kg/day | 5 mg/kg/day | 10 mg/kg/day |
|---|---|---|---|---|
| Corpora Lutea | 14.0 | 13.0 | 13.0 | 12.0* |
| Implantation Sites | 13.0 | 13.0 | 13.0 | 12.0* |
| Live Fetuses | 13.0 | 12.0 | 12.0* | 8.0* |
| Resorbed implantations - early | 0 | 1.0 | 1.0* | 4.0* |
| % implantations - early | 0 | 7.1 | 8.0* | 33.3* |

* - Statistically significant difference from control group

## Embryofetal development

**Study title**: Oral pilot study for effects on embryo-fetal and postnatal development in the rat.

**Key study findings**:

- Significant maternal effects were seen at the MD and HD. The HD was reduced from 20 to10 mg/kg/day mid-study because of severe toxicity.
- Embryo-fetal toxicity seen at both the MD and HD as evident by the post-implantation loss and the fetal lung effects

| | |
|---|---|
| **Study no.**: | **OSI Study # R2002308** |
| **Volume #, and page #**: | Module 4.2.3.5.2 |
| **Conducting laboratory and location**: | Non-Clinical Drug Safety<br>F. Hoffmann La-Roche Ltd.<br>Basel, Switzerland. |
| **Date of study initiation**: | 6 November 2001 |
| **GLP compliance**: | Non-GLP |
| **QA reports**: | yes ( ) no (X) |
| **Drug, lot #, and % purity**: | Ro 50-8231/001, lot GPM 0442 MC 50, ~ % purity |

**Methods**

| | |
|---|---|
| Doses: | 0, 5, 10, 20* mg/kg/day (*reduced to 10 mg/kg/day from GD 12/13 on) |
| Species/strain: | Rat/HanIbm:WIST |
| Number/sex/group: | 9 females/dose reared their litters to weaning<br>10 females/dose for C-sectioning on GD 21 |

| | |
|---|---|
| Route, formulation, volume, and infusion rate: | Oral gavage, hydroxyethylcellulose (0.5%) with polysorbate 80 (0.1%), 10 mL/kg. |
| Satellite groups used for toxicokinetics: | 3 females/dose |
| Study design: | Rats dosed daily from GD 6 through to GD 17. |
| Parameters and endpoints evaluated: | Mortality and clinical signs, maternal body weights and food consumption, pre- and post-implantation loss, female fertility index, gestation index, live birth index, viability index, lactation index, resorptions, pup sex, clinical observations of pups, pup body weights, litter size, milk uptake, necropsy of dams and weanling pups, external, visceral and skeletal examinations of pups. |

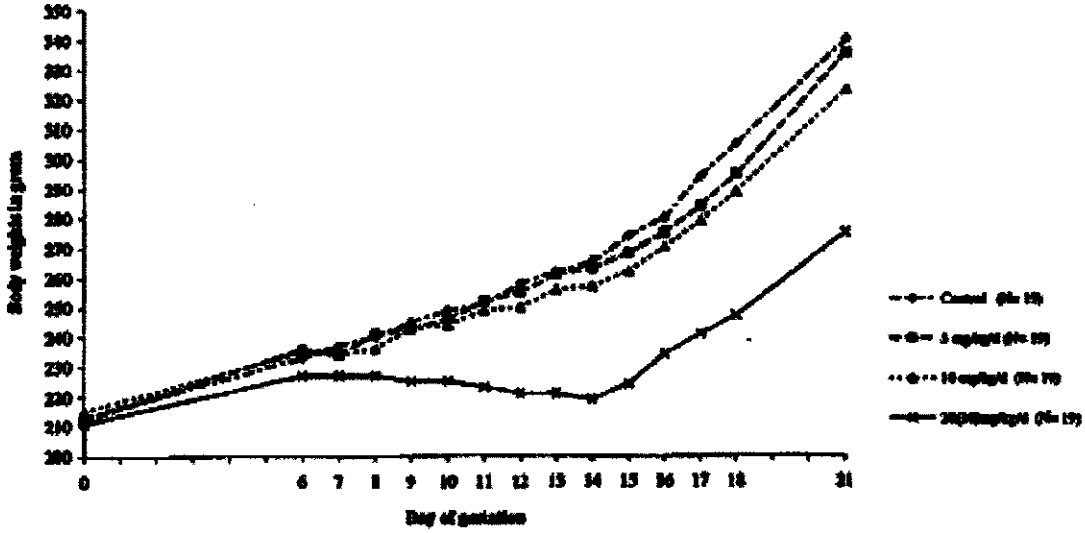
**Results**

Mortality (dams):
No maternal deaths

Clinical signs (dams):
HD dams (in 4-6 of the 19 HD dams) –
wounds/scratches, encrustation around eyes and mouth
Hemorrhagic discharge from the eyes

Body weight (dams):
Significant weight loss in the MD and HD groups, from the start of dosing on GD 6 with recovery evident at cessation of dosing (GD 17). The HD group began to show some improvement soon after the dose was reduced to 10 mg/kg/day on GD 12/13. LD group was similar to control. From the start of dosing until GD 21 the HD and MD dams exhibited a significantly reduced body weight gain compared to control. The figure below shows the maternal body weights throughout gestation.

**Median Maternal Body Weights During Gestation**

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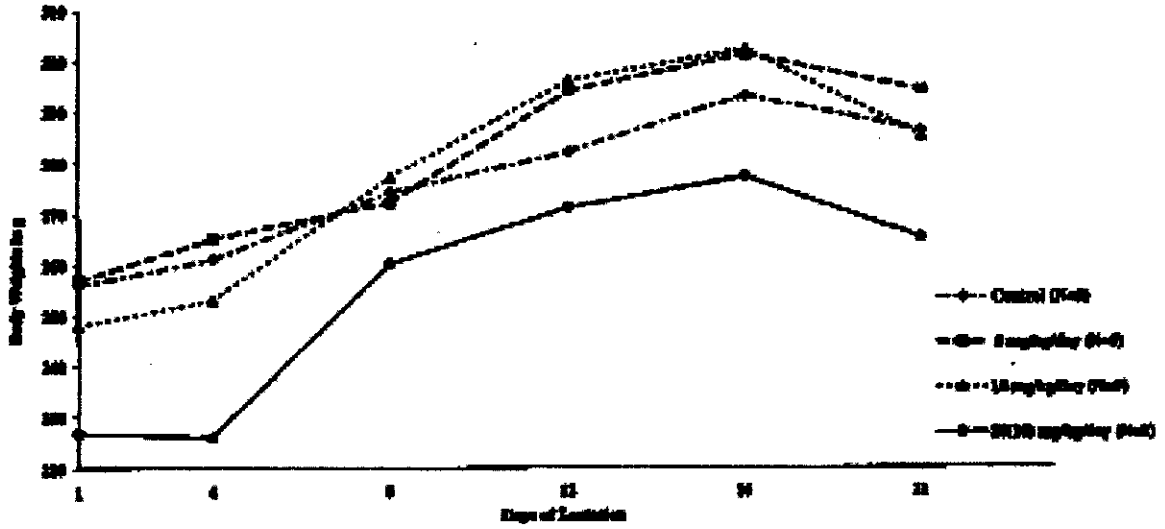
During the lactation period, the MD and HD dams had a significant increase in body weight gain, evidence of the recovery of the weight loss or decreased weight gain seen from the toxicity of Ro 50-8231/001. The figure below shows the maternal body weights during lactation. Although the HD dams weights are still below control, the recovery from Ro 50-8231/001 is evident.

**Median Maternal Body Weights During Lactation**



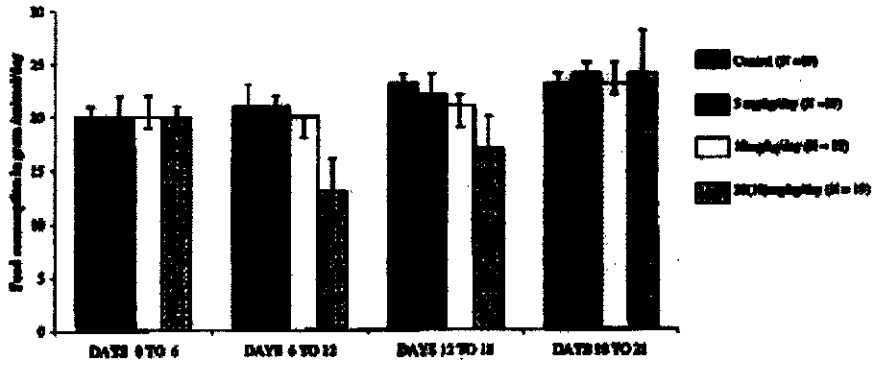


Food consumption (dams):

During drug administration, food consumption by the MD and HD dams was significantly reduced when compared to controls. After drug administration ceased on

GD 17, the food consumption across all groups was not significantly different. The chart below shows the food consumption during gestation.

**Median Maternal Food Consumption During Gestation - (1st & 3rd Quartile)**



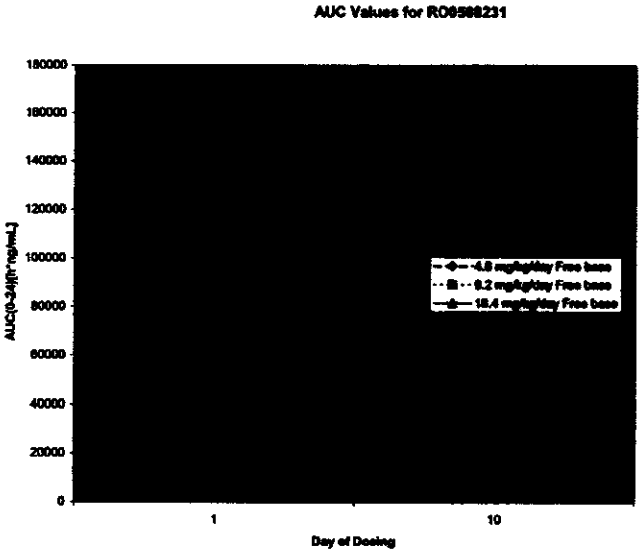
Toxicokinetics:

Three dams per dose were used for toxicokinetics. Unlike with the main study animals, the HD toxicokinetic dams received 20 mg/kg/day throughout testing, which was GD 6-15 for this satellite group. Blood was drawn on the first and tenth day of dosing at 1, 3, 7, and 24 hrs after dosing, and a pre-dose sample drawn on the tenth day.

The sponsor's table shows the toxicokinetic parameters of RO0508231-001 (Tarceva). A dose-related increase in AUC is seen, as is an increase in Cmax. Accumulation over the course of dosing only appears to be evident in the HD group. At the final day of dosing, the HD group shows a super-proportional increase in both the AUC and Cmax parameters. The main metabolites, purported to be pharmacologically active (OSI-420 and OSI-413), show similar toxicokinetic profiles, though at a smaller ratio to the parent compound. These data are not presented.

**Mean toxicokinetic parameters of [redacted] following oral administration of 4.6, 9.2, 18.4 [redacted] RO0588231-691 (calculated as free base) to [redacted] rats on Gestation Days 6 and 15 (equivalent to Dosing Days 1 and 10) – Study Plan 432r01**

| | Gestation Day 6 Dosing Day 1 | | | Gestation Day 15 Dosing Day 10 | | |
|---|---|---|---|---|---|---|
| Dose [mg/kg/day] | 4.6 | 9.2 | 18.4 | 4.6 | 9.2 | 18.4 |
| Tmax [h] | 3 | 1 | 7 | 1 | 7 | 3 |
| Cmax [ng/mL] | 1700 | 2930 | 6530 | 3150 | 2060 | 12200 |
| C(24 h) [ng/mL] | 32.7 | 71 | 848 | 61.9 | 193 | 4930 |
| ± RSD [%] | | | | ±63.1% | ±86.4% | ±45.0% |
| AUC(0-24 h) [h·ng/mL] | 20200 | 30600 | 96400 | 20800 | 31600 | 162000 |
| Cmax/Dose [(ng/mL)/(mg/kg/day)] | 370 | 318 | 355 | 685 | 224 | 663 |
| C(24 h)/Dose [(ng/mL)/(mg/kg/day)] | 7.11 | 7.72 | 46.1 | 13.5 | 21.0 | 235 |
| AUC(0-24 h)/Dose [(ng/mL)/(mg/kg/day)] | 4390 | 3330 | 5240 | 4520 | 3430 | 8800 |



Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

| Parameter | Dose | | | |
|---|---|---|---|---|
| | Control | 5 mg/kg/day | 10 mg/kg/day | 20(10) mg/kg/day |
| Total Resorptions | --- | ---- | 1/19 | 4/19 |
| Complete litter loss (between PND 1-5) | --- | ---- | ---- | 4/19 |
| # of fetuses/litter | 12.5 | 11.0 | 12.0 | 9.5* |
| # of live fetuses/litter | 12.5 | 11.0 | 12. | 9.0* |
| # resorptions/litter | 1.0 | 0.5 | 1.0 | 2.5* |
| Post-implantation loss/litter | 1.0 | 035 | 1.0 | 3.0* |
| Fetal body weight | 5.0 | 5.1 | 5.0 | 4.4* |

* - statistically significant difference from control

No significant difference in pregnancy rate, number of corpora lutea, pre-implantation loss, number of implantations and duration of gestation.

Offspring (malformations, variations, etc.):

***External examination***

No significant effects of treatment on pup external examination.

***Visceral examination***

| Finding | Dose | |
|---|---|---|
| | 10 mg/kg/day | 20(10) mg/kg/day |
| Lung<br>Small lobes, incompletely inflated | 11/55 fetuses in 5/9 litters | 17/44 fetuses in 8/9 litters |

***Skeletal examination***

| Finding | Dose | |
|---|---|---|
| | 10 mg/kg/day | 20(10) mg/kg/day |
| Abnormalities | No increased incidence | No increased incidence |
| Variations | No increased incidence | No increased incidence |
| Retardations<br>Sternal element – incomplete ossification of or unossified | No increased incidence | Significant, slight increase |

**Study title**: Segment II (Tarceva): Oral study for effect on embryo-fetal development in the rat.

**Key study findings**:

- Maternal toxicity seen at the MD and HD, evident in the decreased body weights in both dose groups during dosing and decreased food consumption in the HD group
- Hematological and clinical chemistry parameters show inflammation and hepatic toxicity
- This study did not replicate the findings of the pilot study of increased post-implantation loss and increased lung abnormalities in the offspring

| | |
|---|---|
| **Study no.**: | **OSI Study # R2003113** |
| **Volume #, and page #**: | Module 4.2.3.5.2 |
| **Conducting laboratory and location**: | Non-Clinical Drug Safety<br>F. Hoffmann La-Roche Ltd.<br>Basel, Switzerland. |
| **Date of study initiation**: | 8 October 2002 |
| **GLP compliance**: | Letter included and signed |
| **QA reports**: | yes (X) no () |
| **Drug, lot #, and % purity**: | RO0508231-001, lot BS02070001, [ ] purity |

**Methods**

| | |
|---|---|
| Doses: | 0, 2, 5 and 10 mg/kg/day |
| Species/strain: | Rat/Wistar (HanBrl:WIST) |
| Number/sex/group: | 22/sex/dose |
| Route, formulation, volume, and infusion rate: | Oral, Polysorbate 80 vehicle, 10 mL/kg/day volume |
| Satellite groups used for toxicokinetics: | 3 females per dose group |
| Study design: | Gravid rats dosed from GD 6 to GD 20 inclusive. Toxicokinetic dams dosed from GD 6 to GD 15. |
| Parameters and endpoints evaluated: | Mortality and clinical signs, maternal body weights and food consumption, maternal hematology and clinical chemistry, fetal visceral and skeletal examinations, histopathology of fetal lungs. |

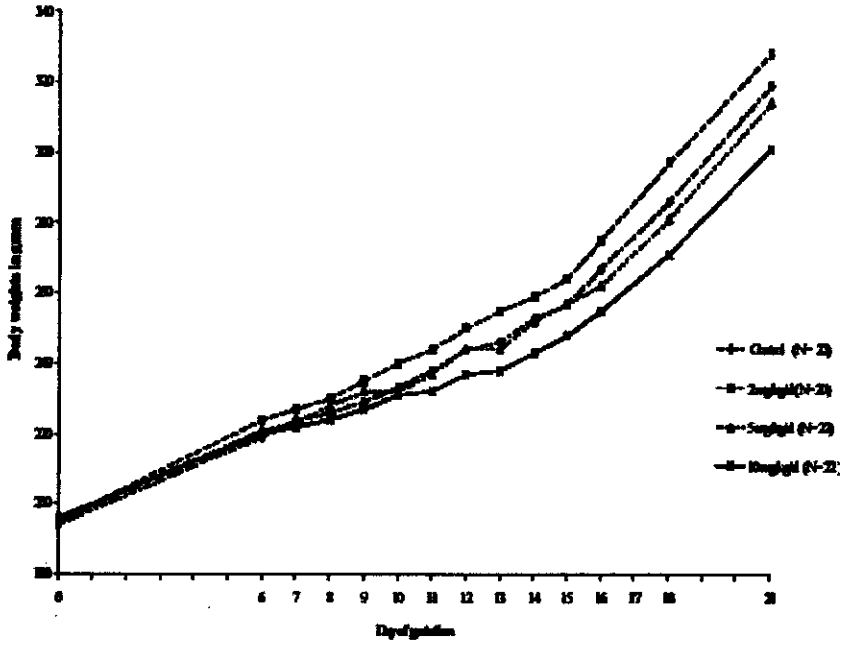
**Results**

Mortality (dams):
No maternal mortality seen in this study.

Clinical signs (dams):
No maternal clinical signs observed.

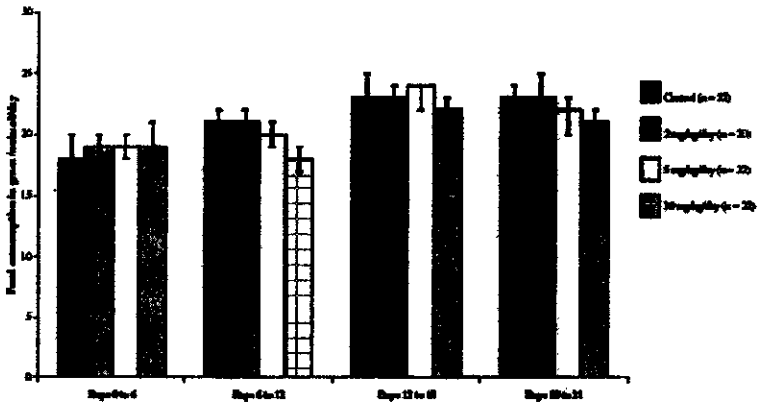
Body weight (dams):
Both the MD and HD dams gained less weight during the drug administration days of gestation than did the controls. The sponsor's graph shows the body weights throughout gestation.

**Figure 1 Median Maternal Body Weight During Gestation**



Food consumption (dams):
Food consumption was significantly decreased in the HD group for all the intervals. The MD group also consumed less food than control, significantly different only during the final interval measured (GD 18-21). The sponsor's graph shows food consumption for the four gestational intervals, with drug administration beginning during the second interval.

**Figure 2** **Median Maternal Food Consumption During Gestation (1st & 3rd Quartiles)**



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Toxicokinetics:

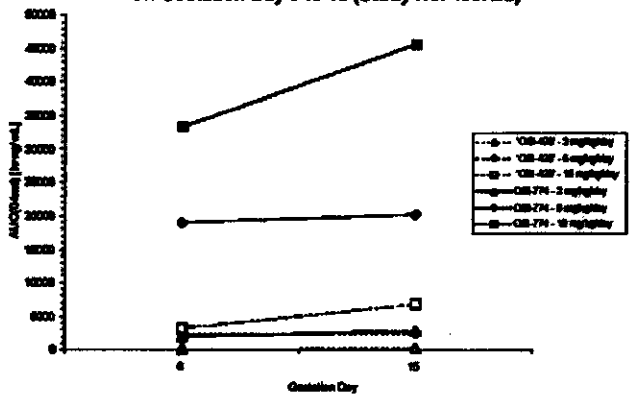
The toxicokinetics results were similar to those seen in the pilot study. High systemic exposure was achieved in the gravid female rat. The AUC and Cmax were approximately dose-proportional. Accumulation was not significant. Similar profiles were seen for the two desmethyl metabolites of RO0508231, OSI-413 and OSI-420, with a Cmax of the metabolites being approximately 15% of the parent compound.

**Table 4** **Mean toxicokinetic parameters of RO0508231 following oral administration of 2, 5, and 10 mg/kg/day RO0508231-001 to pregnant rats on Gestation Day 6 and 15 (Study No. 485R02)**

| | Gestation Day 6 Dosing Day 1 | | | Gestation Day 15 Dosing Day 10 | | |
|---|---|---|---|---|---|---|
| Dose [mg/kg/day] | 2 | 5 | 10 | 2 | 5 | 10 |
| Tmax [h] | 1.0 | 1.0 | 3.0 | 3.0 | 3.0 | 7.0 |
| Cmax [ng/mL] | 383 | 1600 | 3160 | 519 | 1490 | 3290 |
| Tlast [h] | 7.0 | 24 | 24 | 7.0 | 24 | 24 |
| C(7 h) [ng/mL] | 198 [a] | 1100 | 1720 | 320 [a] | 1320 | 3290 |
| C(24 h) [ng/mL] | BLQ | 10.6 [a] | 23.6 [a] | BLQ | 8.04 [a] | 53.2 [a] |
| ± RSD [%] | | | | | ±127% | ±84.2% |
| AUC(0-7 h) [h·ng/mL] | 2010 [b] | 9480 | 18600 | 2890 [b] | 8880 | 17300 |
| AUC(0-24 h) [h·ng/mL] | - | 18900 [b] | 33400 [b] | - | 20200 [b] | 45700 [b] |
| Cmax/Dose [(ng/mL)/(mg/kg/day)] | 192 | 320 | 316 | 260 | 298 | 329 |
| C(24 h)/Dose [(ng/mL)/(mg/kg/day)] | - | 2.12 | 2.36 | - | 1.61 | 5.32 |
| AUC(0-7 h)/Dose [(ng/mL)/(mg/kg/day)] | 1010 | 1900 | 1860 | 1450 | 1780 | 1730 |
| AUC(0-24 h)/Dose [(ng/mL)/(mg/kg/day)] | - | 3780 | 3340 | - | 4040 | 4570 |

[a] corresponds to Clast ; [b] corresponds to AUC(0-last)

**Figure 5** **Time-dependence of AUC(0-last) following oral administration of 2, 5, and 10 mg/kg/day RO0508231-001 (hydrochloride salt, calculated as free base) to pregnant rats on Gestation Day 6 to 15 (Study No. 485R02)**



Hematology and clinical chemistry:

| | 2 mg/kg/day | 5 mg/kg/day | 10 mg/kg/day |
|---|---|---|---|
| **Indicators of inflammation** | | | |
| Neutrophils | --- | --- | ↑ 47 % |
| Haptoglobin | --- | --- | ↑ 29 % |
| **Indicators of hepatic injury** | | | |
| ALT | --- | ↑ 24 % | ↑ 46 % |
| Bilirubin (direct, conjugated) | --- | --- | ↑ 184 % |

Hematology and clinical chemistry was conducted at the termination of the study, upon necropsy, to validate that the maternal toxicity seen was due to the known target organ toxicity of RO0508213-001.

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

No effects of RO0508231-001 were seen on pregnancy rate, total litter loss, number of corpora lutea, or pre- and post-implantation loss.

Fetal body weights – HD 4% ↓ compared to control fetal body weights.

Offspring (malformations, variations, etc.):

***External examination***
No significant effects of treatment on pup external examination.

***Visceral examination***
No significant treatment-related effects on fetal visceral observations.

***Skeletal examination***

| Finding | Dose | | |
|---|---|---|---|
| | 2 mg/kg/day | 5 mg/kg/day | 10 mg/kg/day |
| Abnormalities<br>Misshapen Rib | Significant increase | No increased incidence | No increased incidence |
| Variations | No increased incidence | No increased incidence | No increased incidence |
| Retardations<br>Incomplete ossification<br>Thoracic vertebrae<br>Sternal element | | Significant **decrease**<br>Significant **increase** | Significant **decrease**<br>Significant **increase** |

**Study title**: Oral (stomach tube) developmental toxicity study of OSI 774 01 in rabbits.

**Key study findings**:

- Maternal toxicity seen in the decreased body weights and food consumption of the HD does, along with the significant increase of aborted litters when compared to control
- Some toxicity seen in the MD does also, body weight and food consumption decreases
- Embryo-fetal effects seen with the HD group – increased late resorptions and decreased litter sizes, numbers of live fetuses and fetal body weights

| | |
|---|---|
| **Study no.:** | **OSI Study # B2002281** |
| **Volume #, and page #:** | Module 4.2.3.5.2 |
| **Conducting laboratory and location:** | [redacted] |
| **Date of study initiation:** | 6 Jan 2003 |
| **GLP compliance:** | Letter included and signed |
| **QA reports:** | yes (X) no ( ) |
| **Drug, lot #, and % purity:** | OSI-774-01, lot # 38869-230-3F, [redacted] purity |

**Methods**

| | |
|---|---|
| Doses: | 0, 10, 25, 50 mg/kg/day |
| Species/strain: | Rabbit/New Zealand White – Hra: (NZW)SPF |
| Number/sex/group: | 20 females/dose |
| Route, formulation, volume, and infusion rate: | Oral (stomach tube), in Captisol®, 1 mL/kg volume |
| Satellite groups used for toxicokinetics: | Toxicokinetics done in study rabbits |
| Study design: | Pregnant rabbits were administered drug once daily from GD 7 until GD 19, inclusive |
| Parameters and endpoints evaluated: | Mortality and clinical signs, maternal body weights and food consumption. Pre- and post-implantation loss, resorptions, viable pups, pup sex, clinical observations of pups, pup body weights, litter size, necropsy of dams and weanling pups, external, visceral and skeletal examinations of pups. |

**Results**

Mortality (dams):

| | Control | 50 mg/kg/day |
|---|---|---|
| Found dead | 1/20 | --- |
| Sacrificed moribund | 1/20 | 2/20 |
| Aborted litters, sacrificed | 2/20 | 8/20* |
| **Totals** | **4/20** | **10/20** |

* - Statistically significant difference from control

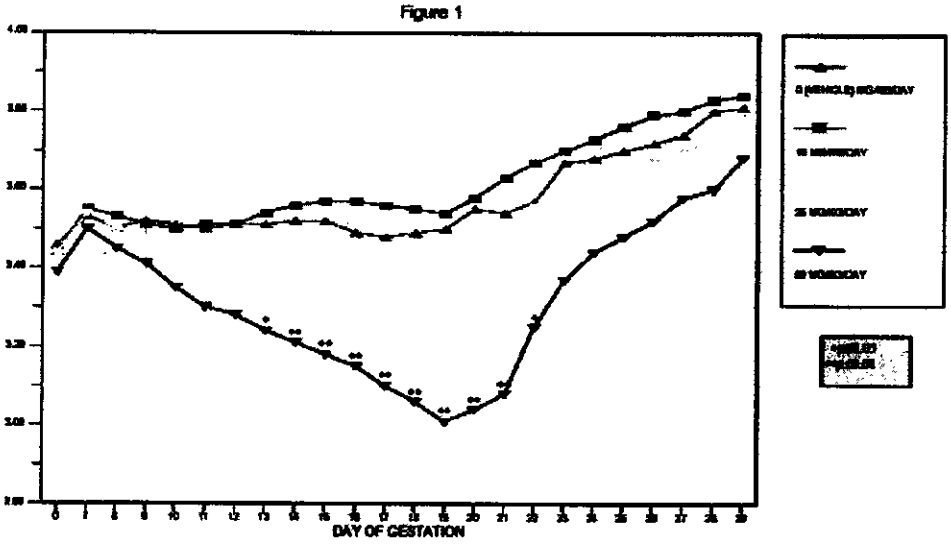
Clinical signs (dams):
Significant increase in the incidence of the following signs in the HD rabbits, when compared to controls.

- Decreased feces
- Soft/liquid feces
- Ungroomed coat
- Red substance in cage pan
- Clear/yellow perinasal discharge

Body weight (dams):
The sponsor's graph shows that body weights were significantly affected by the administration of OSI-774-01 to pregnant rabbits. The HD rabbits had significantly lower body weights from GD13-22. The MD rabbits' body weights were significantly

MATERNAL BODY WEIGHTS

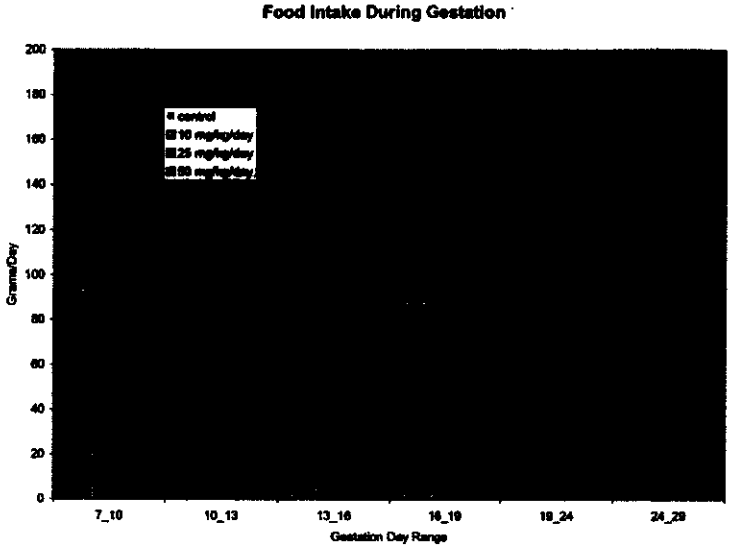
Figure 1




lower than control in the first few days of dosing, GD7-10.

Food consumption (dams):

The graph below shows that food consumption was significantly decreased from control in the MD rabbits on during the periods of GD 10-13 and GD 16-19, with a significant increase during GD 24-29, after cessation of dosing. In the HD rabbits, there was significantly less food consumed throughout dosing and during the GD 19-24 time frame, when compared to control. Significantly more food was consumed during the GD 24-29 period in the HD rabbits also.

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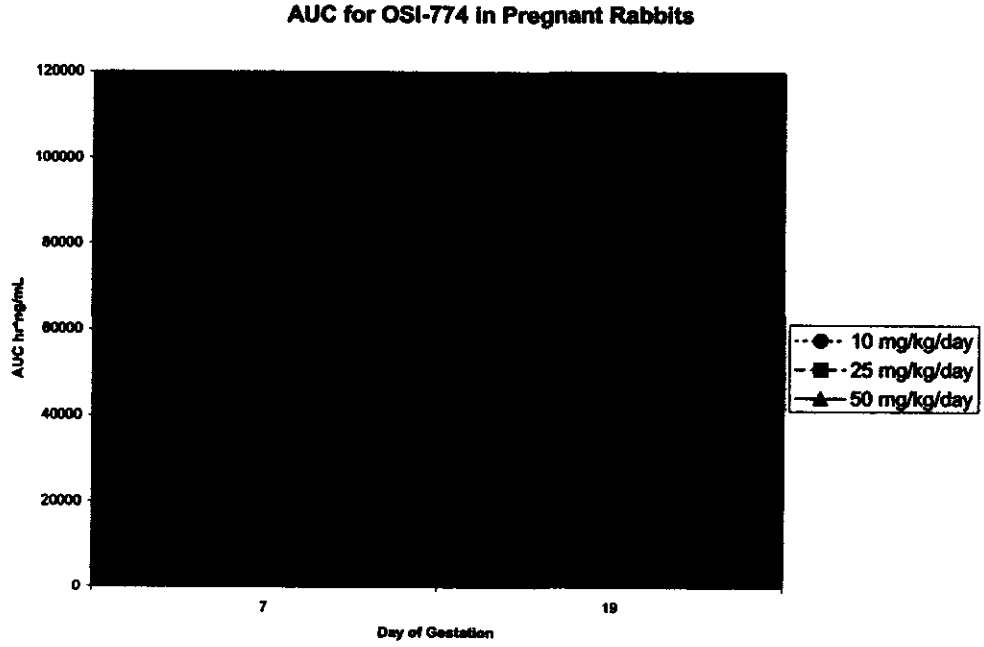
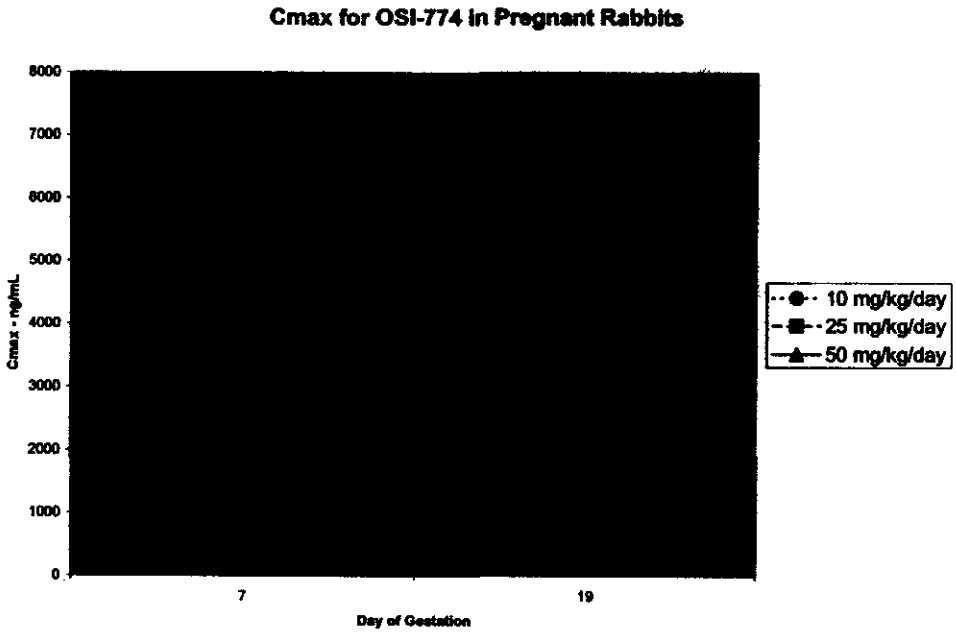
Toxicokinetics:

Toxicokinetic parameters of OSI-774 in the pregnant rabbit are presented in the following table and graphs. After the first dose of OSI-774 linear pharmacokinetics are observed. As seen in the table and graphs, this is not the case after 18 days of dosing. In the MD and HD groups nonlinear pharmacokinetics is evident. Repeat dosing in this study led to either an increase in bioavailability, accumulation of the drug substance or a decrease in clearance of the drug. The half lives in these two doses also increased with repeat dosing, while it did not in the LD group.

| | 10 mg/kg/day | 25 mg/kg/day | 50 mg/kg/day |
|---|---|---|---|
| Cmax – ng/mL | | | |
| GD 7 | 856 | 2130 | 4400 |
| GD 19 | 1260 | 2550 | 7450 |
| $AUC_{0-\infty}$ hr*ng/mL | | | |
| GD 7 | 6590 | 15500 | 40800 |
| GD 19 | 6640 | 37400 | 112000 |
| $t_{1/2}$ - hr | | | |
| GD 7 | 6.03 | 4.77 | 6.31 |
| GD 19 | 5.33 | 8.36 | 14.3 |






Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

| Parameter | Dose | | | |
|---|---|---|---|---|
| | Control N=15 | 10 mg/kg/day N=20 | 25 mg/kg/day N=20 | 50 mg/kg/day N=8 |
| Resorptions –mean | 0.1 | 0.4 | 0.3 | 1.9* |
| Late resorptions | 0.0 | 0.2 | 0.1 | 1.8* |
| Does with any resorptions - N | 1 | 6* | 5* | 5* |
| Litter size – mean | 8.3 | 8.6 | 8.0 | 4.8* |
| # of live fetuses/litter - mean | 8.3 | 8.6 | 8.0 | 4.8* |
| % dead or resorbed/litter | 0.7 | 5.2 | 3.5 | 30.6* |
| Fetal body weight | 4.375 | 43.30 | 41.78 | 35.51* |

* - statistically significant difference from control

Offspring (malformations, variations, etc.):

***External examination***
No significant effects of treatment on pup external examination.

***Visceral examination***
No significant effects of treatment on pup external examination.

***Skeletal examination***

| Finding | Dose |
|---|---|
| | **50 mg/kg/day** |
| Malformations | No significant treatment effects |
| Variations | No significant treatment effects |
| Retardations - # of ossification sites/fetus/litter | |
| Metacarpals | Significant reduction compared to control |
| Xiphoid | Reduction, not significant but below historical range |
| Phalanges | Reduction, not significant but below historical range |

## Prenatal and postnatal development

**Study title**: An oral Tarceva maternal function, and pre- and postnatal development study in rats.

**Key study findings**:
- The HD led to decreased food consumption and body weights of the $F_0$ dams
- No impact of OSI-774 administration to the $F_0$ generation females was seen on the $F_1$ or $F_2$ generations in the parameters measured in this study

| | |
|---|---|
| **Study no.:** | **OSI Study # R2002242** |
| **Volume #, and page #:** | Module 4.2.3.5.3 |
| **Conducting laboratory and location:** | OSI Pharmaceuticals, Inc.<br>2860 Wilderness Place<br>Boulder, CO 80301 |
| **Date of study initiation:** | 8 Oct 2002 |
| **GLP compliance:** | Letter included and signed |
| **QA reports:** | yes (X) no ( ) |
| **Drug, lot #, and % purity:** | OSI-774, lot # 38869-230-3F, purity [ ] |

**Methods**

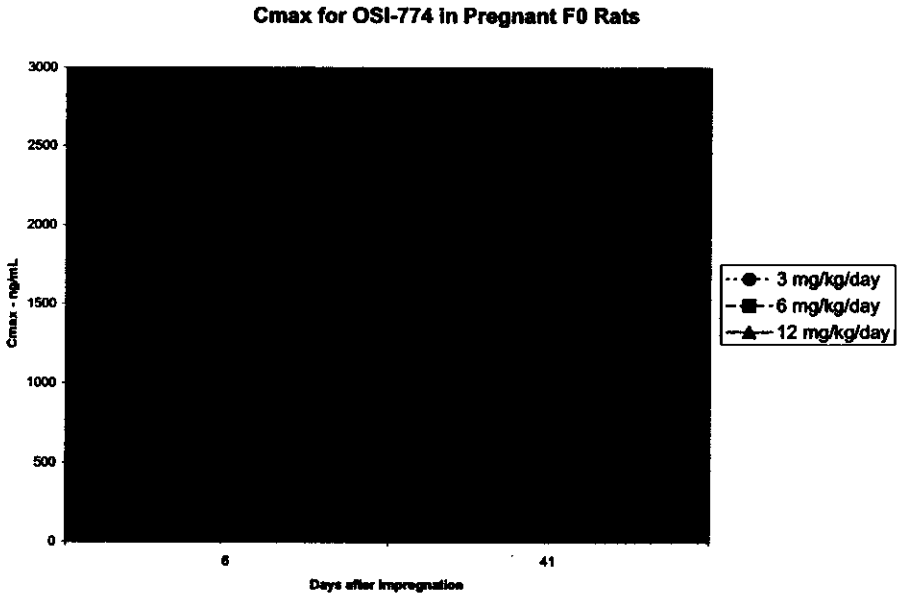
| | |
|---|---|
| Doses: | 0, 3, 6 and 12 mg/kg/day |
| Species/strain: | Rat/Wistar (HanBrl:WIST) |
| Number/sex/group: | 24 females/dose |
| Route, formulation, volume, and infusion rate: | Oral, Polysorbate 80 vehicle, 10 mL/kg/day volume |
| Satellite groups used for toxicokinetics: | 4 females per dose group |
| Study design: | Gravid $F_0$ rats dosed from GD 6 to PND 20 inclusive. Toxicokinetic dams dosed from GD 6 to GD 15.<br>$F_1$ males and females were culled at PND 4 and weaned at PND 21 then mated 1:1 within treatment groups, at sexual maturity (> 70 days).<br>$F_2$ offspring were euthanized on PND 4. |
| Parameters and endpoints evaluated: | **$F_0$** - mortality and clinical signs, maternal body weights and food consumption, nursing behavior, length of gestation, litter size (total, live, dead, cannibalized), male/female ratio, post-implantation loss<br>**$F_1$** – mortality, external examination, pinna detachment, negative geotaxis, auditory startle response, eye opening, M-maze, vaginal opening, belanopreputial separation, reproductive parameters<br>**$F_2$** – external examination, # fetuses (live, dead, cannibalized, visceral abnormalities |

**Results**

$F_0$ in-life:

- No mortality
- One LD dam euthanized on PND 20 due to adverse clinical signs – severe anal staining, difficulty using hind limbs, weight loss, soft stool
- No treatment-related effects on clinical signs
- Increase in mean post-implantation loss in the HD group compared to control (↑ 79%) – though not statistically significant
- Decreased body weight gain in the HD group from GD 6-9, 10, 11, 12 and 14.
- No difference in body weight gains from PND 0-21
- Decreased food consumption in HD group during first 2 weeks of study
- Toxicokinetic parameters in the $F_0$ dams are presented below – a non-linear increase is seen in both AUC and Cmax. With repeated administration, the levels actually went down, indicative of possibly increased clearance of the drug or lowered bioavailability

| | 3 mg/kg/day | 6 mg/kg/day | 12 mg/kg/day |
|---|---|---|---|
| Cmax – ng/mL | | | |
| GD 6 | 775 | 1580 | 2850 |
| PND 20 | 546 | 1420 | 2260 |
| $AUC_{0-\infty}$ hr*ng/mL | | | |
| GD 6 | 3690 | 14000 | 25400 |
| PND 20 | 1380 | 3730 | 9280 |
| $t_{1/2}$ – hr | | | |
| GD 6 | 2.21 | 2.62 | 2.74 |
| PND 20 | 2.62 | 4.48 | 3.38 |




$F_0$ necropsy:

- LD dam that was euthanized – distended intestines upon necropsy. Cause not determined.

$F_1$ physical development:

- No treatment-related effects on skeletal or visceral examinations of $F_1$ pups that died or were culled from the litter
- No treatment-related effects on pinna detachment or eye opening

- No treatment-related effects on either vaginal opening or preputial separation, both signs of sexual development

$F_1$ behavioral evaluation:
- No treatment-related effects on auditory startle
- The MD and HD $F_1$ pups had statistically improved scores on the negative geotaxis compared to control
- One male and one female $F_1$ pup from each litter was evaluated for vision, locomotor activity, coordination, learning and memory via the water M-maze
- Measured were the mean number of errors on each test day and mean decrease in errors over time, mean time to escape and the mean decrease in time to escape over multiple testing
- No significant treatment-related effects on any of the measures obtain in the M-maze

$F_1$ reproduction:
- No significant treatment-related effects on maternal body weights
- No biologically relevant treatment-related effects on mating performance or fertility in the $F_1$ generation
- No biologically relevant treatment-related effects on parturition parameters in the $F_1$ generation – maternal nursing behavior, gestation length, litter size, # pups born live or dead, pup cannibalization, male:female ratio, post-implantation loss.

$F_2$ findings:
- No treatment-related effects on pup survival in the $F_2$ generation
- Not treatment-related effects on $F_2$ pup group mean body weights
- No significant drug-related external malformations or variations in the $F_2$ pups
- No significant drug-related visceral malformations in the $F_2$ pups
- No treatment-related clinical observations in the $F_2$ pups

**2.6.6.7 Local tolerance**
Not Reviewed

**2.6.6.8 Special toxicology studies**
Not Reviewed

**2.6.6.9 Discussion and Conclusions**

A full battery of toxicology studies has been conducted with erlotinib in nonclinical models. The primary toxicities of erlotinib in the laboratory are the skin, the GI tract, the kidneys, and the liver. The eye was a site of toxicity with corneal ulceration seen in the longest study conducted in the dog but not in any other species or in dog studies of

shorter duration. Given that the ocular toxicity occurred in the dog at a dose tested for one month without incidence, and it occurred within 2 weeks of the start of the study, it is unclear why it was only seen in the 12-month study. This toxicity has not proven to be problematic in the clinic.

The majority of the toxicities seen with erlotinib are most likely an extension of the pharmacological action of the drug. Skin lesions and reddening, seen in the rat, dog and monkey, are known actions of drugs of this class. Other EGFR tyrosine kinase inhibitors have this same effect and skin rashes are a primary toxicity seen in man. The emesis, loose stools and GI histopathological changes are likely due to the inhibition of the EGFR located within the gastric mucosa. Again, this toxicity is one also seen in man, as diarrhea has been the DLT in clinical trials. The increased bilirubin seen in nearly all the animal studies is to be expected due to the inhibitory effect that erlotinib has on the enzyme that conjugates bilirubin.

Erlotinib was not mutagenic or clastogenic in the battery of genotoxicity studies conducted for this submission.

Erlotinib did not affect male or female fertility or the $F_1$ and $F_2$ generations in a Segment III study. Erlotinib was an abortifacient in rabbits, at AUCs nearly 3 times that seen in the clinic at the standard dose of 150 mg/day. Erlotinib also increased resorptions in rats when the treatment began prior to mating. Erlotinib was not a teratogen in either the rat or the rabbit.

### 2.6.6.10 Tables and Figures

See Text of Review

## 2.6.7 TOXICOLOGY TABULATED SUMMARY

Table adapted and modified from the original IND review with information contained in this review.

| Species | Route | N/sex/ dose | mg/kg | $mg/m^2$ | Significant findings |
|---|---|---|---|---|---|
| Mouse | Oral | 3 | 2000<br>1000<br>500 | 6000<br>3000<br>1500 | 6000 $mg/m^2$: 1/6 died, decreased activity, irregular respiration, ataxia, unkempt appearance, thin appearance, hunched walking; |
| Rat | Oral | 3 | 2000<br>1000<br>500 | 12000<br>6000<br>3000 | 12000 $mg/m^2$: 6/6 died<br>6000 $mg/m^2$: 2/6 died, mucodial stool, reddish urine, thin appearance/pale, hunched walking, weakness; |
| Dog | Oral | 3 | 200<br>100 | 4000<br>2000 | 4000 $mg/m^2$/day: emesis, dehydration, ↓ activity, salivation, pupil dilation, cold to touch, tremors, ataxia, ↑ bilirubin<br>2,000 $mg/m^2$/day: emesis, ↓ activity, salivation, pupil dilation, ↑ bilirubin in ♀ |
| Dog | Oral | 2 | 250<br>100<br>50 | 5000<br>2000<br>1000 | 5000 $mg/m^2$/day: milky-colored urine, trace blood in urine, ↓ urine pH, emesis in 1/4<br>2000 $mg/m^2$/day: milky-colored urine 1/4 |

| | | | | | |
|---|---|---|---|---|---|
| | | | 10 | 200 | |
| Mouse | IV | 3 (♂ only) | 75<br>50<br>25<br>15 | 225<br>150<br>75<br>45 | 225 mg/m$^2$: 1/2 died;<br>75-150 mg/m$^2$: convulsions, splayed hind feet or legs |
| Rat | IV | 3 (♂ only) | 50<br>35<br>25<br>15 | 300<br>210<br>150<br>90 | 300 mg/m$^2$: 2/3 died<br>150 and 210 mg/m$^2$: ↓ activity, irregular respiration, salivation, convulsions, rapid chewing, anogenital/nasal staining |
| Dog | IV | 3 | 14<br>7 | 280<br>140 | 280 mg/m$^2$/day: only 1 dog treated, convulsed, retched, urinated, salivated profusely, normal 45 minutes after dosing<br>140 mg/m$^2$/day: loose stool |

| Species | Route Duration | N/sex/ dose | mg/kg/ day | mg/m$^2$ /day | Significant findings |
|---|---|---|---|---|---|
| Mouse | Oral Daily x 14 | 5 | 300<br>150<br>50 | 900<br>750<br>150 | 900 mg/m$^2$/day: 30% mortality, ↓ activity, dehydration, slow respiration, rough coat, tremors, ↓ body weight, ↓ food consumption in ♂, ↓ in RBC indices, abnormal RBC morphology, ↓ albumin, ↓ total protein in ♀<br>750 mg/m$^2$/day: ↓ RBC indices, ↓ albumin, ↓ total protein in ♀ |
| Rat | IV Slow bolus Daily x 28 | 10 | 15<br>5<br>1.5 | 90<br>30<br>9 | 90 mg/m$^2$/day: red nasal discharge, swollen muzzle w/ encrustation, salivation, swelling/discolored tail, red penal discharge, slight ↓ in body weight ♀, slight ↑ water intake ♀, slight ↓ HCT, HGB, RBC, ↑ adrenal weights; ↓ ovary, uterus and cervix weights; reduced corpora lutea in ovary, ↑ cortical width of adrenals, ulceration and scab on muzzle skin, degranulation of striated ducts in salivary glands, medullary plasmocytosis of lymph node, extramedullary hemopoiesis of spleen in ♀, abscess at injection site..<br>30 mg/m$^2$/day: ↑ adrenal weights ♂, ↓ ovary weights, ↑ cortical width of adrenals, degranulation of striated ducts in salivary glands in ♀, abscess at injection site.<br>9 mg/m$^2$/day: degranulation of striated ducts in salivary glands in ♀ |
| Rat | Oral Daily × 28 | 10 | 10<br>5 | 60<br>30 | 60 mg/ m$^2$/d: ♀: chromorhinorrhea, ↓ body weight, ↑ total bilirubin ,↑ WBC, ↑adrenal weight |
| Rat | Oral Daily x 180 | 15 | 10<br>5<br>1 | 60<br>30<br>6 | 60 mg/m$^2$/day: skin lesions, rough fur, chromodacryorrhea, ↓ body weight in ♂, ↑ NEU, MON, BAS (♀), EOS (♀), WBC count, ↑ BIL, ALT, AST, hemoglobin in urine, pituitary enlargement ♀, alopecia, ↓ adrenal weights ♀, plasmocytosis of cervical node, skin degeneration, kidney necrosis ♀, ovary atrophy, angiectasis in adrenals ♀, liver necrosis ♀<br>30 mg/m$^2$/day: ↑ NEU, MON, WBC count, ↑ BIL, hemoglobin in urine, plasmocytosis of cervical node, skin degeneration, kidney necrosis ♀, ovary atrophy, |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | liver necrosis |
| Dog | Oral<br>Daily x 5 | 2 | 100<br>100 BID<br>50/100 | 2,000<br>2000 BID<br>1000/2000 | 2000 mg/$m^2$/day:Emesis, ↓ appetite<br>2000 mg/$m^2$/day - BID: Emesis, ↓ appetite, loose stool, salivation<br>1000/2000 mg/$m^2$/day: Emesis, ↓ appetite |
| Dog | Oral<br>Daily x 28 | | 15<br>5 | 300<br>100 | No significant toxicology |
| Dog | Oral<br>daily x 28 | 3 | 50<br>15 | 1000<br>300 | 1000 mg/ $m^2$/d: ↑ numbers of regenerating proximal tubules in the kidney, ↓RBC, HGB and HCT (90%) in ♀ |
| Dog | Oral<br>Daily x<br>356 | 4 | 50<br>15<br>7.5<br>2.5 | 1000<br>300<br>150<br>50 | 1000 mg/$m^2$/day: discontinued due to ↓ body weight, tremor, emaciation, ↓ activity, corneal lesions, ↑ FIB, NEU, BIL, ALT, UREA, TRIG, kidney necrosis, GI inflammation<br>300 mg/$m^2$/day: reddening of skin, hair loss, redness of buccal mucus membrane, ↓ body weights ♂, foci of pigmentation in eye<br>150 mg/$m^2$/day: reddening of skin, hair loss, redness of buccal mucus membrane, foci of pigmentation in eye<br>50 mg/$m^2$/day: reddening of skin, hair loss, redness of buccal mucus membrane |
| Monkey | Oral<br>Daily up<br>to 2 wk<br>(BID or<br>q3h) | 2 | 400<br>200<br>100<br>25 | 4800<br>2400<br>1200<br>300 | 4800 mg/$m^2$/day: ↑ emesis and loose stool, ↓ activity, dehydration, ↑ bilirubin, peri-oral lesions, white dots on skin, slight ↓ in lymphocytes.<br>2400 mg/$m^2$/day: ↑ emesis and loose stool, dehydration, ↓ activity, ↑ bilirubin, peri-oral lesions<br>1200 mg/$m^2$/day – ↑ emesis and loose stool, peri-oral lesions<br>300 mg/$m^2$/day – ↑ emesis and loose stool, skin redness |

## OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: The nonclinical program of erlotinib identified the target areas of toxicity to be gastrointestinal, hepatobiliary, renal, dermatological and ocular. It was neither genotoxic or teratogenic.

Unresolved toxicology issues (if any): None

Recommendations: Recommend that erlotinib (Tarceva) is approvable, with the preclinical studies adequately addressing the nonclinical safety requirements.

Suggested labeling: Suggested labeling is presented in the Executive Summary beginning on page 3.

Signatures (optional):

Reviewer Signature /S/

Supervisor Signature /S/ Concurrence Yes ___ No ___

**APPENDIX/ATTACHMENTS**

None

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

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Kimberly Benson
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John Leighton
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