CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-320

Pharmacology Review(s)

MEMORANDUM

Date: 17 November 2003
From: Suzanne R. Thornton, Ph.D.
Acting Supervisory Pharmacologist, HFD-580
To: File for NDA #21-320, N000 AZ
Re: Approvability for Pharmacology and Toxicology
Plenaxis (abarelix)

Abarelix is a GnRH antagonist and the current submission is a re-submission following a non-approvable letter issued on 11 June 2002. The re-submission includes a slight change in the indication from the original NDA submission where the indication was for

[redacted] The new indication is for the 'treatment of patients with impending neurological compromise from spinal, spinal cord or epidural metastases, urinary tract obstruction from retroperitoneal adenopathy or from enlarged prostate gland or pelvis mass, and bone pain from prostate cancer skeletal metastases requiring narcotic analgesia.'

Dr. Raheja provided a comprehensive review of all non-clinical studies including integrated report summaries containing exposure multiples (AUC basis) between animal and humans during the original review cycle. In the current submission Dr. Raheja provides appropriate summaries of new information provided. Dr. Raheja recommended the original NDA for approval and continues to recommend approval. Appropriate labeling revisions have been identified and forwarded to the Sponsor.

Recommendations: The pharmacology and toxicology data supports approval of this NDA. There are no outstanding issues.

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

/s/

Suzanne Thornton
11/17/03 08:33:31 AM
PHARMACOLOGIST

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA number: 21-320
Review number: 2
Serial number/date/type of submission: 042/2-25-03/amendment
Information to sponsor: Yes ( ) No (*)
Sponsor and/or agent: Praecis Pharmaceuticals, Inc. Waltham, MA
Manufacturer for drug substance :

Reviewer name: Krishan L. Raheja, D.V.M.,Ph.D.
Division name: Reproductive and Urological Drug Products
HFD #: 580
Review completion date: 3-11-03

Drug:

Trade name: Plenaxis
Generic name (list alphabetically): Abarelix for injectable suspension
Code name: PPI-149
Chemical name:
CAS registry number:
Mole file number:
Molecular formula/molecular weight:
Structure:

Relevant INDs/NDAs/DMFs:

Drug class: GnRH antagonist

Indication:

Clinical formulation: injectable suspension

Route of administration: intramuscular

Proposed use: for treatment of prostate cancer

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

OVERALL SUMMARY AND EVALUATION:

Introduction: This is a resubmission of NDA 21-320. The initial submission of NDA 21-320 was made on 12-11-2000 and was issued a not approvable letter on 6-11-02 because of clinical, chemistry, microbiology and facilities deficiencies. The indication for the initial NDA submission was for

Sponsor now has changed the indication slightly and proposed that Plenaxis is indicated in the treatment of patients with 1) impending neurological compromise from spinal, spinal cord or epidural metastases, 2) urinary tract obstruction from retroperitoneal adenopathy or from enlarged prostate gland or pelvis mass, and 3) bone pain from prostate cancer skeletal metastases requiring narcotic analgesia.

Under the present submission, sponsor has included minor additions to the P/T data. These include the following studies reports:

1. —— Affinity and effect of acute and chronic abarelix (PPI-149) treatment of LHRH receptors in rat pituitary, testis and adrenal gland. Report dated June 200.

The significant findings of this study were:

a) apparent affinity of abarelix in the 3 tissues follows rank order of pituitary>testes>>adrenals,
b) treatment with abarelix depot (10 mg/kg i.m. on days 1 and 15) decreased receptor density by 90% in the pituitary and 40% in the testis, compared to 60% and 40% decrease, respectively after acute treatment with abarelix injectable solution (100 ug/kg sc for 30 days) and no apparent decrease of specific binding in adrenals,
c) basal testosterone concentration in the plasma was reduced below the detection limit in rats treated with depot and unchanged or increased after acute treatment,
d) a 60% decrease in pituitary receptor density after acute abarelix treatment was not enough to significantly lower testosterone secretion on an i.v. challenge injection of LHRH. However, with depot, LHRH challenge did not increase testosterone confirming down regulation and failure to trigger testosterone secretion.

2. —— Consequences of LHRH antagonist abarelix therapy on the natural history of autochthonous prostate cancer in the Transgenic adenocarcinoma mouse prostate (TRAMP) model.

In this study the effect of 4 and 8 week abarelix depot therapy on nature and incidence of prostate cancer in the TRAMP model to surgical castration was compared. Significant findings were:

a) at 4 week course of therapy, both abarelix and castration decreased serum testosterone levels and caused profound cellular atrophy in the seminal vesicles, testicles and prostate glands of the TRAMP mice. A total of 3/6 (50%) of TRAMP mice in the castrated cohort and 3/11 (27%) in the abarelix cohort were essentially cured and showed no evidence of cancer and no evidence of T-antigen expression, suggesting these mice had testosterone dependent disease and remaining developed androgen-independent disease. All mice displayed a consistent and uniform pattern of poorly differentiated tumors with evidence of metastases, invasion, high cellular proliferation activities, high mean vessel density and loss of E-Cadhesion expression.

In comparison with control mice, abarelix therapy reduced the incidence of invasive and metastatic disease.

b) The data suggested that 8-week of abarelix therapy was significantly better than castration in reducing tumor burden (including bladder, seminal vesicles, prostatic urethra and prostate weight) and was comparable to surgical castration in reducing tumor incidence.

3. Six-month subcutaneous toxicity and toxicokinetics. Study of SL 18.0185-00 in rats.

The findings of the rat repeat 6-month SC toxicity (Praecis study # TXC1092) submitted under this submission were similar to those of the previously reported 6-month study rat toxicity study (— N0020591) submitted in the original NDA.

4. Addendum to 2-year SC carcinogenicity study of PPI-149 in mice and rats. —— —) study numbers 081-001 and 081-002.

The exposure margin calculated for the carcinogenicity studies demonstrated that both Cmax and AUC in rats and mice were substantially higher than the comparable parameters in humans (50x in rats, and 200 x in mice for Cmax post last dose, and 50x and 150 x AUC0-9 days at the highest dose levels).

Safety evaluation:-

Safety issues relevant to clinical use: none

Other clinically relevant issues: none

Conclusions: The toxicity studies data confirm the safety of abarelix for clinical use.

Communication review:
Labeling review: -
RECOMMENDATIONS: NAI

Internal comments: none

External recommendations (to sponsor): none

Draft letter content for sponsor (if not same as above):

NDA issues:-

Reviewer signature:

Team leader signature [concurrence/non-concurrence]:

Memorandum of non-concurrence (if appropriate, attached):
Addendum to review (if necessary):

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

/s/

Krishan L. Raheja
3/12/03 02:47:51 PM
PHARMACOLOGIST

Alexander W. Jordan
3/17/03 09:00:23 AM
PHARMACOLOGIST

Electronic Mail Message

Date: 6/1/01 9:15:57 AM
From: Jacobs, Abigail C (JACOBSA@A1)
Subject: MY comments on abarelix action package

N21-230 Plenaxis (abarelix)

Comments on pharm tox review 6/1/01 A. Jacobs

Since the anticipated action will not be an approval, final labeling has not been reviewed. Should this product ever reach the stage that labeling is being reviewed, the pharm/tox reviewer could consider condensing and shortening his proposed Animal Toxicology section (p.79) and deleting the paragraph describing malformations not considered to be drug related (p.85, last paragraph)

Draft

Pharmacology/Toxicology Review of Plenaxis

Abarelix

NDA 21-320

Table of contents

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

KEY WORDS: Abarelix (Plenaxis), prostate cancer
Reviewer Name: Krishan L. Raheja, D.V.M., Ph.D.
Division Name: DRUDP
HFD#: 580
Review Completion Date: 5-16-2001

Review number: 1
IND/NDA number: NDA 21-320
Serial number/date/type of submission: 000/12-12-2000/original NDA submission
Information to sponsor: Yes ( ) No ( )
Sponsor (or agent): Praecis Pharmaceuticals Inc.
Manufacturer for drug substance:

Drug:

Code Name:
Research names: PPI-149 (Praecis), R-3827 (Praecis), CDB 3827 (
SF176 27-4747
Generic Name: Abarelix
Trade Name: Plenaxis (Abarelix for suspension) 100 mg injectable, proposed
Chemical Name: acetyl-D-B-naphthylalanyl-D-4-chlorophenylalanyl-D-3-pyridylalanyl-L-seryl-L-N-methyl-tyrosyl-D-asparagyl-L-leucyl-L-N( )-isopropyl-lysyl-L-prolyl-D-alanyl-amide
CAS Registry Number: 183552-38-7
Molecular Formula/ Molecular Weight: $C_{72}H_{95}O_{14}Cl$ (anhydrous, free base form of abarelix)/1414.68
Structure:

patients with advanced prostate cancer in which superagonist therapy was absolutely or relatively contraindicated because of the risk of tumor flare with complications.

Other studies in prostate cancer patients:

A European study sponsored by Sanofi-Synthelabo (ABACAS 1) compared abarelix depot versus Zoladex plus Casodex in prostate cancer patients.

Additional safety information on the use of abarelix injectable solution in patients with prostate cancer in 2 uncontrolled studies. In both studies abarelix solution was administered as a subcutaneous continuous infusion. Under study 149-96-01, patients had advanced prostate cancer or rising PSA after local therapy for prostate cancer. In study 149-97-03, patients used required prostate gland volume reduction before undergoing radiation therapy for prostate cancer.

Of a total of 1055 prostate cancer patients exposed to abarelix in studies sponsored by Praecis, 810 received the registration dose (100 mg both for induction and maintenance of medical castration). Of these 810 patients, 720 patients were exposed to abarelix depot for 6 months and 188 patients were exposed for 1 year.

PK of abarelix depot and abarelix injectable solution was conducted in 16 subjects under study No. 149-99-01 and in patients under various clinical studies i.e.149-98-02, 149-99-03, 149-97-04, 149-97-03 and 149-96-01.

Disclaimer -- use of sponsor's material

Introduction and drug history: Hormonal therapy for the palliative treatment of prostate cancer has been the standard of care for decades. The goal of hormonal therapy in prostate cancer is to suppress androgen production to medical castration levels, thereby substantially reducing the supply of prostate growth factors. Suppression of testosterone production by either medical or surgical means has proved beneficial in the management of prostate cancer. While surgical castration had been the mainstay of treatment in the past, the use of pharmaceuticals has now surpassed the use of orchiectomy.

One class of agents, the leutinizing hormone-releasing (LHRH) superagonists, is considered to act by initially stimulating the pituitary LHRH receptor leading to increased production of androgens, followed by subsequent desensitization of the receptor and eventual suppression of androgen production. The LHRH superagonists leuprolide acetate (Lupron Depot by TAP Pharmaceuticals) and goserelin acetate (Zoladex by AstraZeneca Pharmaceuticals) are widely and effectively used for the palliative treatment of advanced prostate cancer. However, as a consequence of the agonist's mechanism of action, the onset of efficacy due to suppression of testosterone to castration levels may be delayed for several weeks. Furthermore, the initial stimulation of the LHRH receptor may cause hormonal surge that has been associated with an exacerbation of prostate cancer and accompanying severe adverse effects such as increased bone pain, urinary retention, spinal cord compression.

While androgens acting on the prostatic tissue are predominantly produced through pituitary stimulation of the testes, to block the local synthesis of dihydrotestosterone (DHT) from androgen precursor synthesized in the adrenal gland, antiandrogen in combination with an LHRH

superagonist has been used to achieve maximum androgen blockade. Nonsteroidal anti-androgens however, have been reported to cause hepatotoxicity as well as the putative induction of mutations in the androgen receptor, leading to the development of androgen-independent prostate cancer.

Abarelix is a GnRH antagonist that achieves the goal of androgen suppression by preventing the binding of GnRH to gonadotrophic receptors, thereby immediately inhibiting the secretion of LH and FSH.

Studies reviewed within this submission: Only pre-clinical studies which have not been reviewed under sponsor's IND 51,710 are reviewed under this NDA.

Studies <u>not</u> reviewed within this submission: Rat and mouse carcinogenicity studies.

PHARMACOLOGY:

Mechanism of Action: In contrast to GnRH superagonists, Abarelix, a GnRH antagonist works by rapidly suppressing LH, FSH, testosterone and dihydrotestosterone without the initial surge that results in the clinical worsening of symptoms referred to as "flare". Increases in testosterone may be implicated in the pathogenesis of prostate cancer and may have the potential to stimulate cancer growth.

<u>Drug Activity Related to Proposed Indication:</u> Immediate suppression of testosterone without initial surge prevents exacerbation of prostate cancer and possible associated complications.

Ancillary Pharmacology Studies: The following studies with respect to therapeutic indication have been submitted:

1. <u>Title</u>: **Pharmacological properties of PPI-149 binding to the rat pituitary LHRH receptor**. <u>Report No. 00-171. Vol. 9 p. 144</u>

[^{125}I] PPI-149 was tested for its ability to bind to pituitary membranes over a range of tissue concentrations and various times of incubation. Specific binding was linear between — and — mg wet weight of tissues/100 ul and equilibrium binding was achieved after approximately 120 minutes.
Using saturation analysis K_D values was between — pM which was in close agreement to K_I value of — nM determined in the competition binding study to compare relative binding potencies of PPI-149 and D-Trp^6 –LHRH to inhibit [^{125}I] PPI-149 binding to rat pituitary membranes. The K_D for [^{125}I]des-Gly^{10},D-Ala^6 ethylamide was — nM and K_I was — nM. Based on these results it was concluded that binding of both PPI-149 and des-Gly^{10},D-Ala^6 is saturable and high affinity —

2. **Antagonism of LHRH-induced growth inhibition by PPI-149 in CHO cells expressing human recombinant LHRH receptor.** Praecis Report No, PR-PPI-149-02. Vol. 9 p.158

The LHRH receptor is a member of the G-protein coupled receptor family, which include neurotransmitter, chemokine, opiod, and cardiovascular peptide and gonadotropin receptors. To identify a high affinity LHRH receptor antagonist, cell line expressing recombinant human LHRH receptor (CHO — cells) was established. It was used to establish functional cellular assay that was used to screen for LHRH receptor ligands.
CHO ~ cells were treated with various LHRH agonists concentration ranging from 0 to 10^{-8} and 5 days later viable, metabolically active cells were quantified by measuring the absorbance of reduced MTT. A dose-dependent decrease in reduction of MTT reflects the decrease in number of — cells after various agonists (d-his-6-LHRH, leuprolide, gosarelin, d-trp-6-LHRH and deslorelin) exposure and permits a quantitative measure of the effect of LHRH receptor activation on the cell line.
The dose-dependent decrease in metabolism of MTT that was induced by agonists was blocked in a competitive fashion by increasing concentration of known antagonist Antide and by Abarelix.
From these findings it was suggested that PPI-149 is a pure LHRH receptor antagonist since it reverses the effect of agonist (D-His^6-LHRH) in a competitive fashion and has no activity on receptor signaling in the absence of agonist.

Note: No approved GnRH agonist was used for comparison.

3. Title: **Inhibition of serum testosterone in rats by PPI-149.** Final Report No. 00-180. Vol. 9 p.177

Male rats (10/g) were administered doses of 260, 877 or 4390 ug/kg as single SC injection. An additional group was administered 4390 ug/kg as a single IM injection. Serum testosterone was decreased in all treated groups compared to saline controls. As shown in table below, it remained suppressed for up to 24 hours in the high dose groups and up to 12 hours in the low and mid dose groups. Testosterone concentrations are expressed as ng/ml (mean of 10 rats/g). PK part of the study is described under ADME.
Table 1

Hour	Saline SC	260 SC	877 SC	4390 SC	4390 IM
-24	1.94	1.67	1.93	1.18	1.22
12	2.46	0.38	0.13	0.68	0.14
24	2.11	**3.37**	**3.16**	0.01	0.00
48	2.83	2.07	1.86	**9.57**	1.10
72	2.29	2.02	1.89	2.38	**3.13**

Note: Although not stated, it seems that there was an overshoot (rebound) after the testosterone was maximally suppressed.

The PK data shown in table below suggested that PPI-149 is highly distributed and is rapidly eliminated following SC administration and seems to be in accord with rapid onset and brief duration of testosterone suppression.

Table 2

Parameter	260 SC	877 SC	4390 SC	4390 IM
Tmax (hr)	3.00	3.30	3.30	3.00
Cmax (ng/ml)	523	1536	2304	1712
T1/2 (hr)	1.40	1.26	2.98	6.73
AUC (ng.hr/ml)	1793	8802	30729	28670
V_z/F (ml/kg)	303	188	620	1534
CL/F (ml/kg/hr)	150	103	144	159
$MRT_{SC,IM}$	3.65	4.74	11.49	15.68

Note: In sponsor's preliminary study with 2 rats/g given single SC bolus of 300 or 1000 ug/kg PPI-149 also suggested a rebound with high dose at 72 hours.

4. **Dose response study of abarelix depot on serum testosterone concentrations in male Sprague-Dawley rats.** Final report. Study No. 00-81. Vol. 9 p.190

Sponsor has stated that continuous and prolonged delivery of abarelix administered either by repeated SC injections or by minipumps effectively induces and maintains suppression of testosterone, LH and FSH in rats. In this study the relationship between the dose of abarelix depot (which provides sustained delivery) and duration of testosterone suppression was evaluated.
A single IM dose of abarelix depot (6, 9, 12, and 18 mg/kg) was used. There were 8 male rats/g. Control group was given saline.

All doses of abarelix depot caused a rapid suppression of serum testosterone during the first 24 hours following IM administration (presented as graph, no values given). Suppression was dose-proportional for the 6, 9, and 12 mg/kg doses and suppression with the 18 mg/kg depot was similar to that with 12 mg/kg depot. The duration of testosterone suppression for groups that received 6, 9, 12, and 18 mg/kg was 32, 43, 76 and 81 days. The testosterone concentrations in all groups eventually returned to normal levels. The results of the PK analysis is shown in table below:
Table 3

Parameters	Units	Group 1	Group 2	Group 3	Group 4
Tmax	Day	0.34	0.32	0.90	0.89
Cmax	ng/ml	034	1131	1453	1238
Dose normalized Cmax	ng/ml per mg/kg	156	126	121	69
T1/2	Day	4.91	7.30	8.86	6.81
AUC_{0-29}	ng.day/ml	1754	2824	4308	4448
$AUC_{0-infinity}$	ng.day/ml	1769	2878	4476	4613
Dose normalized $AUC_{0-infinity}$	ng.day/ml per mg/kg	295	320	373	256
CL/F	L/day/kg	3.6	3.2	2.8	4.1
Vz/F	L/kg	26	35	37	39
$AUC_{0-29}/AUC_{0-infini}$		0.991	0.979	0.967	0.965

The data demonstrated that there was dose proportionality in AUC and seems to compliment dose dependent suppression of testosterone for doses of 6, 9, and 12 mg/kg. Data also reveals that majority of drug exposure occurred during the first 29 days after dosing.

Summary of pharmacology: In contrast to GnRH superagonists, Abarelix, a GnRH antagonist works by rapidly suppressing testosterone without the initial surge that results in the clinical worsening of symptoms referred to as "flare".

SAFETY PHARMACOLOGY: The following studies to evaluate potential AEs were conducted:

Neurological effects:

Abarelix-depot: Assessment of effects on barbital sleeping time following single subcutaneous administration in mice. Study No. DEV-99P115. Vol. 10 p. 157.

This study was conducted by Sanofi-Synthelabo, France in compliance with GLP regulations with as study director

Six groups of Swiss OF1 male mice (10/g) were used as described below:

Group 1 (control): Diazepam vehicle -0.5% w/v methylcellulose and 0.5% w/v tween 80 SC
Group 2: (reference substance) diazepam 16 mg/kg SC
Group 3 (control): abarelix depot vehicle SC
Group 4: abarelix depot at a dose of 30 mg/kg, SC
Group 5: abarelix depot at a dose of 100 mg/kg, SC
Group 6: abarelix depot at a dose of 300 mg/kg, SC
Six hours after abarelix and one hour after diazepam, mice were administered 200 mg/kg barbital via i.p injection. Time of righting reflex loss and righting reflex recovery was recorded.

Results: are shown in table below:
Table 4

Treatment	Time to fall asleep (min)	% change	Sleeping time (min)	% change
Diazepam vehicle	29+/- 2	-	101 +/-12	-
Diazepam 16 mg/kg	23+/- 1	-21*	170 +/- 10	+68**
Abarelix vehicle	34+/- 4	-	72+/- 15	-
Abarelix 30 mg/kg	39+/- 4	+15	75 +/- 16	+4
Abarelix 100 mg/kg	33+/- 2	-3	104 +/- 10	+44
Abarelix 300 mg/kg	30+/- 2	-12	83 +/- 17	+15

Values are mean +/- s.e.m. * $p < 0.05$ ** $p < 0.01$

Note: Because of no dose-response relationship, sponsor concluded that abarelix had no effect. Data however, suggests that treatment did affect sleeping time.

Abarelix depot: assessment of effects in the Irwin observation test and on body temperature following single subcutaneous administration in rats. Study No. DEV-99P118. Vol. 10 p. 223

This study was conducted by the same laboratory as the above study in compliance with GLP regulations with as the study director.

Six group of SD, OFA male rats (5/g) were used as described below:

Group 1 (control): vehicle of haloperidol (2% w/v ascorbic acid) SC
Group 2 (ref substance): haloperidol 10 mg/kg SC
Groups 3, 4 and 5: abarelix depot at a dose of 30, 100 and 300 mg/kg SC
Group 6: (control): vehicle for abarelix depot (0.9% sod. chloride) SC

After drug, reference or vehicle administration rats were observed for the first 30 minutes and then 1, 2, 4, 6, 8 and 24 hours, 2, 5 and 7 days after treatment. Irwin observation screen consists of spontaneous activity, ptosis, spontaneous body tone, irregular breathing, slow breathing, body tone in response to pulling and catalepsy.

Results: Except for increased spontaneous activity 2 hours after treatment in all abarelix dose groups and soft feces in 2 animals from the 30 mg/kg dose group, no neurotoxic, pschotropic or neurobehavioral or body temperature changes were reported. The reference compound induced typical depressant CNS effects (decrease in spontaneous activity and in body tone, ptosis, slow and irregular breathing, and decrease in body tone in response to pulling and catalepsy) with a decrease in body temperature one hour after treatment. No change in body weight was observed.

Cardiovascular effects:

Abarelix depot: Evaluation of cardiovascular effects in the conscious normotensive rat following single subcutaneous administration. Study No. DEV-99R-106. Vol. 10 p.340

Sanofi-Synthelabo, France conducted this study as the above study in compliance with GLP regulations with . — as study director.

Six treatment groups as shown below were used:

Group A: abarelix depot at a dose of 30 mg/kg (n=5)
Group B: abarelix depot at a dose of 60 mg/kg (n=5)
Group C: abarelix depot at a dose of 100 mg/kg (n=4)
Group D: abarelix depot at a dose of 300 mg/k (n=4)
Group D: vehicle 0.9% sodium chloride, 6 ml/kg (n=5)
Group E: Verapamil at a dose of 30 mg/kg (n=5)

Telemetry transmitters capable of monitoring both PB and ECG were implanted intraperitoneally. The catheter for PB measurement was put into the aorta. Electrodes were positioned to record the lead II of the ECG. Fourteen day later, after a stabilization period of 30

minutes, conscious rats were treated SC and systolic, diastolic and mean arterial pressure, heart rate and intervals of the ECG (PR, QRS and QT) were measured before treatment and then every 30 minutes up to 480 minutes and 24 hour post-dose, using IOX, Datanalyst and ECG-auto softwares.

Results: It was reported that abarelix depot given SC at doses up to 300 mg/kg had no effect on BP, heart rate or ECG parameters tested. Reference article, Verapamil induced as expected long lasting and highly significant decrease in blood pressure. It had no effect on heart rate or ECG.

Abarelix (SL 18.0185-00): Evaluation of hemodynamic effects of the open-chest anesthetized dog following single intravenous administration. Study No. DEV-99R105. Vol, 11 p. 122

This study was conducted by the Sanofi-Synthelabo in accordance with GLP regulations.

— was the study director.

Four groups of male Beagle dogs (5/g) were used. Dogs in groups A, B and C were administered a dose of 0.1 mg/kg, 1 mg/kg and 10 mg/kg respectively. Animals in-group D were administered vehicle (5% w/v mannitol). Animals were kept under sodium pentobarbital anesthesia and all hemodynamic parameters and ECG were recorded continuously for a 30 minutes stabilization period and for 180 minutes after dosing. For each parameter abarelix-treated groups were compared to the control group by two-way analysis of variance.

Results: Effects of abarelix on main CV parameters, which were significantly modified (5 minutes after injection) are shown in table below. Values are mean +/- SEM.

Table 5

Treatment	Placebo	Abarelix 0.1 mg/kg	Abarelix 1 mg/kg	Abarelix 10 mg/kg
Systolic left ventricular pressure (mmHg)	137 +/- 9	138 +/- 7	125 +/- 13	77 +/- 2
% change	+0.4	-2.1	-9.1	-40.1***
Negative dLVP/dt max (mmHg/sec)	2510 +/- 326	2353 +/- 158	2194 +/- 230	999 +/- 92
% change	+0.3	-1.9	-10.0	-57.0***
Mean arterial blood pressure (mmHg)	124 +/- 9	122 +/- 6	109 +/- 10.2	56 +/- 4
% change	+0.2	- 2.4	- 10.2	-51.0***
Mean pulmonary artery pressure (mmHg)	14.6 +/- 1.0	13.8 +/- 2.1	15.4 +/- 1.3	24.8 +/- 4.1
% change	+1.5	+0.3	+2.4	+84.8**
Heart rate (beats/min)	169 +/- 12	150 +/- 7	156 +/- 9	147 +/- 5
% change	-0.5	+0.7	+1.6	-8.4
Peripheral resistance (mmHg/ml/min)	0.107 +/- 0.015	0.099 +/- 0.011	0.098 +/- 0.020	0.052 +/- 0.008
% change	-1,3	- 7.0	-21.3	-48.5*
Renal blood flow (ml.min)	115 +/- 18	109 +/- 16	90 +/- 10	107 +/- 31
% change	-1.9	-0.3	-5.5	-6.7
Coronary blood flow (ml/min)	12.5 +/- 3.1	10.1 +/- 2.6	10.4 +/- 2.3	6.7 +/- 1.8
% change	-4.7	+14.2	+0.9	-26.4
PR interval (msec)	95 +/- 3	105 +/- 8	91 +/- 4	98 +/- 1
% change	-0.7	-2.3	-3.4	+5.9
QTc interval (msec)	348 +/- 6	359 +/- 7	355 +/- 5	351 +/- 7
% change	+0.2	+ 0.3	-0.6	-1.2

* $p< 0.5$ ** $p< 0.01$ *** $p< 0.001$

From the results of this study sponsor concluded that in the anesthetized dog, abarelix at a dose of 10 mg/kg I.V., induced a decrease in cardiac contractility, in arterial blood pressure and in

vascular peripheral resistance and an increase in mean pulmonary artery pressure. Changes in these parameters were also observed with 1 mg/kg dose but were not statistically significant. These effects were marked and led in 1/5 dogs to a cardiac failure. They started after injection, were maximum at 3-10 minutes and lasted up to 60-90 minutes. Cardiac output, regional blood flow, stroke volume and heart rate were not significantly affected. Sponsor suggested the adverse effect due to impairment of gas exchange.

Although not mentioned in the text, data in table 28 (vol11, page 166) showed that plasma K^+ (mmol/L) was significantly greater in the 10 mg/kg dose group at 120 and 180 minutes post dose. The values at 0, 15, 30, 60, 120 and 180 minutes were 3.13, 3.45, 3.55, 3.73, 4.40, and 4.68 mmol/L, respectively.

At a dose of 1 mg/kg I.V., abarelix displayed only a slight tendency to lower cardiac contractility, arterial blood pressure and vascular peripheral resistance.
At 0.1 mg/kg I.V., abarelix had no hemodynamic effects in the anesthetized dog (NOAEL).

The electrocardiogram was not affected by abarelix up to a dose of 10 mg/kg I.V.

An Assessment of the potential cardiovascular effects of abarelix depot when administered subcutaneously to conscious, unrestrained cynomolgus monkeys. — study No. 0986-136. Final report. Vol. 12, p. 1

This study was conducted in compliance with GLP regulations by ——

The purpose of this study was to examine the effects of a single SC administration of abarelix depot on hemodynamic parameters and electrocardiographic activity in conscious unrestrained monkeys.

A total of 9 male monkeys were used in 3 groups (3/g) as follows:
Table 6

Group	Dose level mg/kg	Dose volume Ml/kg	Dose solution Conc (mg/ml)
1	0 (control)	0.5	0
2	5	0.1	50
3	25	0.5	50

Telemetry transmitters were surgically implanted SC 11 days prior to dosing for monitoring and recording CV parameters and body temperature. Each animal received a single SC dose of abarelix (lot No. 8767B) or control (0.9% sodium chloride for injection). CV parameters and body temperature were collected via telemetry prior to and at various time intervals following dose administration as shown in table below:

Table 7

Parameter	Recording period (Approximate)	Recording frequency and duration
Systolic and diastolic pressure, mean arterial pressure, heart rate, and body temperature	Prior to Day 1:for at least for 24 hours	Every 2 minutes for 20 seconds
	1 hour prior to dosing through 48 hours following dose	Every 2 minutes for 20 seconds
	48 through 144 hours following dosing	Every 15 minutes for 20 seconds
	Day 28 (3 hours)	Every 15 minutes for 20 seconds
ECG and arterial blood pressure wave forms	Prior to Day 1: for al least a 24 hour period	Every 120 minutes for 20 seconds
	1 hour prior to dosing through 2 hours following dosing	Every 10 minutes for 20 seconds
	2 through 48 hours following dosing	Every 120 minutes for 20 seconds
	48 through 144 hours following dosing	Every 4 hours for 20 seconds
	Day 28 (3 hours)	Every 15 minutes for 20 seconds.

For toxicokinetics blood samples were collected prior to dosing and then 6, 24, 48, and 72 hours post-dosing and on Days 7 and 28,

In addition all animals were evaluated for changes in clinical signs, food consumption, and body weight. At the end of the study, the animals were return to animal colony.

Results:

The effect of treatment on mean arterial pressure, heart rate and body temperature taken prior to abarelix implant administration and then at various time intervals following depot administration is shown in table below:

Table 8

	---------- Minutes post-dosing ----------								--------- Day 27 (post dosing) -------			
	Mean arterial pressure (mmHg)											
Group #	Pre-dose	30	60	90	120	480	720	1440	0	60	90	180
1	112	106	103	105	102	97	93	112	106	199	103	101
2	119	112	104	101	105	94	90	111	93	82	93	87
3	128	115	111	109	107	107	103	128	103	90	99	98
	Heart rate (beats/minute)											
1	193	177	158	162	154	139	133	184	142	134	147	149
2	202	187	168	155	172	144	132	194	123	113	110	131
3	231	195	176	164	165	154	140	226	141	126	148	150

	Body temperature (degrees celsius)											
1	37.5	37.7	38.0	38.0	38.0	37.8	36.9	37.4	37.4	37.3	37.6	37.4
2	38.2	38.2	38.4	38.5	38.5	37.9	37.3	38.0	37.6	37.9	38.1	37.8
3	38.6	38.7	39.0	39.0	39.0	38.0	37.2	38.4	38.7	38.9	38.8	38.7

ECG tracing were not included but it was stated by the examining veterinarian that all monkeys appeared electrocardiographically normal with the exception of one monkey at the 5 mg/kg dose, which had a U wave at various time intervals. It was stated that the U wave is usually not evident in monkeys and is usually associated with hypokalemia. However, serum K was not measured in this study. Since there was no dose-response relationship, this finding was considered not treatment -related.

Toxicokinetics: TK parameters are given in table below. Values are mean for 3 monkeys in each group and expressed as mean+/-SEM
Table 9

5 mg/kg				25 mg/kg			
AUC_{0-t} (ng.day/ml)	$AUC_{0-infinity}$ (ng.day/ml)	Cmax (ng/ml)	Tmax (day)	AUC_{0-t} (ng.day/ml)	$AUC_{0-infinity}$ (ng.day/ml)	Cmax (ng/ml)	Tmax (day)
1402 +/-467	1622 +/- 541	152 +/- 51	0.75 +/- 0.25	4605+/-1535	5495+/-1832	419+/-140	1.67+/-0.56

There were no PPI-149 concentrations detected in animals from the placebo group at any time point.

It was thus concluded that when compared with SC administration of vehicle, ECG activity, heart rate, mean arterial pressure and body temperature were unaffected by abarelix. Fluctuations in HR, MAP and BT were attributed to stress of blood sampling and dose administration activities and these fluctuations were similar in both abarelix depot and saline treated groups. Also no treatment effect was reported on clinical signs, food consumption and body weight.

Abarelix (SL 18.0185-00): Effects on the action potential of piglet purkinje fibers. Study No. DEV-99R102. Vol. 12 p.308

This study was conducted by Sanofi-Synthelabo in accordance with GLP regulations. —— was the study director.

The effects of abarelix on cardiac electrophysiological parameters were investigated in Purkinje fibers of newborn piglets by recording action potentials using the glass microelectrode technique. Purkinje fibers were dissected from the right ventricle and were pinned to the bottom of experimental organ bath. Experiments were carried on 7 fibers originating from 7 different piglets.
Fiber were superfused with oxygenated physiological control medium for 30 minutes and then with increasing concentrations of abarelix (0.01, 0.1, 1, 10 and 30 uM in DMSO) for 30 minutes. At the end of experiment, control solution was perfused for 30 minutes to assess a possible reversibility of the effects. In each experiment, action potentials were continuously recorded from the same cell.

Method of evaluation: RP (resting potential), APA (action potential amplitude), APD_0, ADP_{50} and ADP_{90} (duration of action potential at 0mV, 50% and 90% of repolarization, respectively) and dV/dt_{max} (maximum depolarization rate) were measured after 30 minutes superfusion. The effects of abarelix on action potential parameters were analyzed by comparison of the values during the treated times with those of the control time.

Results: It was stated that from 0.01 up to 10 uM, abarelix had no effects on resting potential, action potential amplitude, maximum rate of depolarization and on the duration of action potential.

However, at 30 uM, it induced a shortening of the action potential and a reduction of the maximum rate of depolarization. These effects were not completely reversed after 30 minutes washout time.
It was concluded that at 30 uM (about 42 ug/ml), abarelix inhibits ionic conductances controlling the maximum rate depolarization (sodium current) and the action potential duration, especially during the plateau phase (calcium and/or Ca-exchange currents).

Pulmonary effects:

Abarelix depot: effects on respiratory parameters in the unrestrained conscious guinea-pig following single subcutaneous administration. Study No. DEV-99R-107. Vol. 11 p.1

This study was conducted by Sanofi-Synthelabo, France in compliance with GLP regulations with —— as the study director.

Six treatment groups (7-9 animals/g) were used as shown below:

Group 1: abarelix depot vehicle (0.9% sodium chloride)
Group 2: abarelix at a dose of 30 mg/kg
Group 3: abarelix at a dose of 100 mg/kg
Group 4: abarelix at a dose of 300 mg/kg
Group 5: control (vehicle for codeine water for injection)
Group 6: reference substance: codeine at a dose of 300 mg/kg

Abarelix was administered SC and codeine or its vehicle orally.

On the study day animals were placed in bias flow ventilated whole body plethysograph for 60 minutes period of stabilization. After 30 minutes control period, test drugs were given. Respiration was recorded using —— Respiratory rate, peak inspiratory and expiratory pseudo flows, inspiration and expiration times, tidal and minute pseudo volumes were determined from the analysis of respiratory pseudo flows before treatment and then every 30 minutes during the first 8 hours and at time 24 hours after dosing.

Table 10

Parameter		Abarelix vehicle	Abarelix 30 mg/kg	Abarelix 100 mg/kg	Abarelix 300 mg/kg	Codeine vehicle	Codeine 300 mg/kg
Respiratory rate (breaths/min)	Initial value	78	79	84	88	72	71
	Emax	86	88	99	75	68	77
	Tmax (hr)	0.5	0.5	0.5	4	5	0.5
	P		NS	NS	NS		NS
Peak inspiratory flow (mL/sec)	Initial value	13.9	14.1	15.2	16.4	13.9	14.1
	Emax	15.8	16.7	18.8	19.8	**15.0**	**10.4**
	Tmax	0.5	0.5	0.5	0.5	24	3.5
	P		NS	NS	NS		*
Peak expiratory flow 9mL/sec)	Initial value	8.3	8.4	10.0	9.4	8.8	9.4
	Emax	10.0	9.8	**11.1**	**12.4**	**11.0**	**10.5**
	Tmax	2	0.5	0,5	0.5	2	24
	p		NS	*	***		**
Inspiration time (msec)	Initial value	270	275	271	245	305	329
	Emax	320	305	223	309	**347**	**444**
	Tmax	2.5	6	0.5	4	3	3.5
	P		NS	NS	NS		**
Expiration time (msec)	Initial value	505	498	459	441	537	531
	Emax	473	442	400	518	**497**	444
	Tmax	0.5	0.5	0.5	4	0.5	8
	P		NS	NS	NS		**
Tidal volume (msec)	Initial value	2.37	2.47	2.73	2.63	2.74	2.99
	Emax	2.84	2.75	2.48	3.07	**3.01**	**2.40**
	Tmax	2	0.5	7	0.5	3	3
	P		NS	NS	NS		**
Minute volume (mL/min)	Initial value	186	195	227	231	198	210
	Emax	228	**241**	**269**	**296**	**215**	**162**
	Tmax	2	0.5	0.5	0.5	0.5	3.5
	P		NS	*	***		*

From the results above it was concluded that abarelix depot at a dose of 30 mg/kg had no significant effect on respiratory parameters. At doses of 100 and 300 there was a transient increase in peak expiratory pseudo flow and minute pseudo volume for 1 hour post-dose. It was stated that they could express a respiratory stimulation, an increase in airway resistance and /or a decrease in pulmonary compliance. They were said to be present only in early time and not later when max blood levels occur.
Codeine on the other side induced a decrease in peak inspiratory flow, tidal volume and minute volume and an increase in inspiration time and these findings are stated to be consistent with respiratory depression. So abarelix treatment affected some respiratory parameters.

Renal effects:

SL18.0185-00: Effect on the hydroelectrolytic balance in rats after subcutaneous administration. Study No. ION0413. Final study report. Vol. 13 p.1

This was a non-GLP study conducted by Sanofi Recherche, Monpellier, France ——
as the study director.

Four treatment groups of OFA strain rats (10/s/g) were fasted for 4 hours and given a water load of 20 ml/kg by gavage before treatment. They were then administered a single SC injection of either saline (vehicle) or 30, 100 or 300 mg/kg of abarelix as suspension in saline.

Urinalysis was performed on overnight samples (approximately 16 hours after dosing), when blood samples were taken.
Urine pH, sp. gravity, urobilinogen, bilirubin, glucose, ketones, proteins, blood and leukocytes were determined. Electrolyte (Na^+, K^+ and Cl^-) and creatinine concentrations in blood and urine samples, blood and urine osmolality and Hct were measured.
Urine Na^+/K^+ ratio, electrolyte and creatinine excreted quantities and endogenous creatinine clearance were calculated.

Results: Urine pH was decreased and sp. gravity increased in females at 300 mg/kg dose. As shown in table below, protein was increased in a dose-dependent manner. Also incidence of ketones was also increased in male rats.
Table 11

Groups		controls		30 mg.kgSL		100 mg/kg SL		300 mg/kg SL	
Parameters		M	F	M	F	M	F	M	F
Proteins mg/dl	Neg	8/10	9/10	0/10	4/10	0/10	3/10	0/10	2/10
	Trace	2/10	1/10	6/10	4/10	3/10	4/10	2/10	3/10
	30	**0/10**	**0/10**	**4/10**	**2/10**	**7/10**	**3/10**	**8/10**	**5/10**
Ketones mg/dl	Neg	7/10	10/10	5/10	10/10	7/10	9/10	3/10	8/10
	Trace	3/10	0/10	4/10	0/10	3/10	1/10	3/10	2/10
	15	0/10	0/10	1/10	0/10	0/10	0/10	**2/10**	0/10
	40	0/10	0/10	0/10	0/10	0/10	0/10	**2/10**	0/10

The effect of treatment on urine electrolyte concentration and excreted quantities in the treated groups in relation to controls is shown in table below:
Table 12

		30 mg/kg SL		100 mg/kg SL		300 mg/kg SL	
		M	F	M	F	M	F
Sodium	Conc	/	-28%*	/	-12%	/	-36%**
	Excr Q	/	-42%*	/	-33%	/	-51%**
Potassium	Conc	+28%*	+19%	+32%**	+31%	+39%***	+44%***
	Excr Q	+28%	/	+33%*	/	+39%**	/
NA/K		-33%**	-38%**	-36%**	-33%	-42%***	-55%***
Chloride	Conc	/	/	/	/	/	/
	Excr Q	/	-43%*	/	-38%	/	-39%

Summary: The main treatment-related findings were observed in urine which included 1) decreased pH and increased sp. gravity in females only at 300 mg/dose, 2) increased amount of proteins in urine in both sexes in relation to dose, 3) decreased non dose related urine Na concentration in female and increased dose-related urine K concentration in both sexes leading to a decrease in Na/K ratio. Chloride excretion was decreased. No other significant changes were reported.

Gastrointestinal effects: none submitted
Abuse liability: none submitted

Effects of PPI-149 on the release of histamine from rat's peritoneal mast cells. Final report. Study No. 00-182. Vol. 13 p.80

This study was conducted by [redacted]. Rat peritoneal mast cells were harvested from the peritoneal fluid of SD male rats and cell concentration was adjusted to contain 1 x 10^6 cells/ml. Cell suspension was incubated for 2 hours at 37 ^{0}C and then peptide (PPI-149, Antide or PPTAA-LHRH) or buffer were added and further incubated for 1 hour. The reaction was terminated by centrifugation of the cells for 5 minutes at 120 x g and an aliquot of supernatant was assayed for histamine. Total histamine content of the cell suspension was quantified [redacted]

Results:
Results showed that only 1.3% and 1.7% of total cellular histamine were released when cells were exposed to 293 uM of either PPI-149 or Antide. PPTAA-LHRH, used as positive control caused complete release of cellular histamine at 8.2 uM with EC_{50} of 1.4 uM.
In a preliminary study, Praecis showed PPI-149 to have an EC_{50} of 42 uM compared to 0.08 uM for PPTAA-LHRH.
Based on these results it was concluded that PPI-149 is devoid of histamine release activity at pharmacologically relevant concentrations of 48 ng/ml with HTD.

Comment: The histamine releasing activity of PPI-149 however, was not compared with any approved GnRH antagonist.

Note: Histamine release in response to PPI-149 administration was also measured in the 28-day SC efficacy study in monkeys (study No. N002059A Vol.10 p.2) where monkeys were administered PPI-149 at dose levels of 0, 30, 100 and 300 ug/kg/day via osmotic pump. Only graphic presentation of histamine release data was given (p.92). Although sponsor stated that all groups demonstrated initial rise in histamine levels and there was no statistically significant increase at any time from control animals, the graphic presentation of the data showed that the histamine values for the high dose group monkeys was higher when compared to controls. In all groups histamine fell to undetectable levels by Day 15. No histamine release was observed in any group on Lupron administration on Day 29. Individual data values were not available.

Note: Positive control, PPTAA-LHRH was not used.

Study of abarelix (SL 18.0185-00, pure substance) in various binding and enzyme assays. Report No. 00-00117-EN-00. Vol. 12 p.344

This was a non-GLP study to assess the affinity of abarelix for various receptors in radioligand binding assays and to assess its effects on several enzyme activities.

It was reported that abarelix caused a concentration-dependent inhibition of radioligand specific

binding to non-selective bombesin receptors from rat cerebral cortex, human NK_1 ([redacted] cells), human NK_2 and human V_{1a} receptors (human recombinant CHO cells). Abarelix displayed little or no affinity to multitude of other receptors isolated from rat, guinea pig, chicken, human or human recombinant receptor assayed. Also abarelix did not affect enzymes activities of more than 25 enzymes tested.

Summary of safety pharmacology: Abarelix increased barbital sleeping-time in mice, although it was not dose-dependent. It had no adverse CV effects in the rat after SC administration. In dogs at an IV dose of 10 mg/kg, abarelix caused cardiac failure in 1/5 dogs. Plasma K was significantly greater compared to placebo controls. SC administration of abarelix in conscious, unrestrained monkeys had no adverse effect on CV function except that 1/3 monkeys in the mid dose had U wave at various time intervals. At a concentration of 30 uM, abarelix induced shortening of action potential and a reduction of the maximum rate of polarization of piglet purkinje fibers.

Following single SC administration of abarelix to conscious guinea pig, respiratory parameters were not affected at a dose of 30 mg/kg. However, at doses of 100 and 300 mg/kg there was an increase in expiratory flow and minute volume for 1 hour post-dose.
In rats SC administration of abarelix caused increased urinary protein in a dose-dependent manner.
The histamine releasing activity of abarelix was negligible and comparable to Antide.
In monkeys administered SC abarelix, plasma histamine concentration was increased based on limited data submitted. In another study, histamine was determined but sponsor stated that data could not be located.

Local tolerance of PPI-149-Depot was determined by IM and SC administration in rabbits. Results showed that the incidence and severity of granulomatous inflammation were slightly greater for PPI-149-depot at both IM and SC site, and the inflammation was observed for a longer time compared to Lupron depot.

12-month pharmacokinetic, pharmacodynamic and safety study of abarelix depot in dogs.
Study #PC040497. Vol. 9 p.235

This study was conducted by Praecis Pharmaceuticals Inc. Cambridge, Serum chemistry and hematology analyses were performed by [redacted] Histological evaluations were performed [redacted] No GLP statement was made.

The safety, PK and PD of abarelix depot were evaluated in 40 young adult male Beagle dogs (assigned to 8 groups of 5 each) using monthly (Q28 days) IM or SC injections of abarelix depot at doses of 1.2 mg/kg (Days 1, 57, 85, 113, 141), 0.3 or 0.6 mg/kg (Day 29) and 2.4 or 3.6 mg/kg (Days 169, 197, 225, 253, 281, 309, and 337). A total of 13 injections over 12 months with 7 drug lots were used. Three water based reconstitution vehicle (15% glycerin/5% dextrose, 4% PEG-3350/4% mannitol, 0.5% lecithin/5% mannitol) were used in the first 3 months of dosing. For the remaining 9 months of dosing, 0.9% sodium chloride solution was used.
At the end of scheduled dosing or after a period of recovery from dosing, animals were sacrificed for either complete or partial (testes and prostate for dog allowed recovery) pathological

evaluation. The pharmacodynamics of abarelix depot were evaluated by measuring plasma concentrations of testosterone, by observing gross reduction of the prostate glands and testes and grading any microscopic atrophy of prostate and testes. Reversibility from chronic testosterone suppression was evaluated by measuring testosterone levels and evaluating prostate and testes histologically after a 3 or 6 month interval of discontinuation from treatment. Gross observations of restored testes and prostatic tissue, in conjunction with microscopic confirmation, were indicative of reversibility from depot effects.

A safety evaluation of treatment with abarelix depot involved frequent monitoring of blood hematological and serum chemistry analysis and post-mortem pathological evaluation. Testosterone was measured using [redacted] radioimmunoassay kit and plasma abarelix by using [redacted] radioimmunoassay.

Results:

Safety of abarelix:

Serum chemistry and hematology: There were no significant treatment-related changes in hematology or blood chemistry during the course of the study. Beginning on Day 85, there was an increase in total cholesterol levels in treated compared with controls. However, vehicle controls also showed some increase from baseline. The increase in cholesterol was attributed to androgen ablation.

Gross and microscopic pathology: Chronic treatment with abarelix depot resulted in a marked reduction in the size of the prostate and testes in 100% of the dogs. The testes and prostate glands of all dogs allowed a 3 or 6 month recovery period had normal size.
Microscopically all animals, regardless of treatment duration and dosing regimen had marked diffuse atrophy of the testes and prostate glands. The affected prostate glands appeared microscopically no different from prostate and glands of immature animals. Testis atrophy was characterized by loss of germ cells (sperm and sperm precursors) and decreased size of seminiferous tubule lumens and sometimes streaming of sertoli cells into the lumen. Interstitial cells, which are hormone responsive and produce testosterone, were also atrophic and decreased in size and number. Although no histological changes were observed in the pituitary glands after 3 or 6 months treatment, minimal hyperplasia was reported in 2/3 and 3/3 dogs after 9 and 12 months of treatment.
The recovery dogs had minimal testes giant cells, focal prostate gland inflammation, and minimal scattered seminiferous tubule atrophy. These changes were not severe and suggested to be spontaneous or incidental findings. The testes and prostate of the recovery dogs were reported to be within normal limits with spermatozoa development and progression. It was concluded that the suppression of spermatogenesis associated with several months of treatment appeared to be completely reversible following recovery period.

PHARMACOKINETICS/TOXICOKINETICS:

Pharmacokinetics:

28-day continuous subcutaneous efficacy study of PPI-149 with a 14 day Lupron challenge in cynomolgus monkeys. — Study No. N002059A. vol. 10 p.1

This was a non-GLP study conducted to determine the efficacious dose for Lupron-induced testosterone flare and potential toxic effects of PPI-149 following continuous SC administration via implanted — osmotic pumps designed to deliver for 28 days abarelix at a dose level of 0, 30, 100 and 300 ug/kg/day (3 males/g). On Day 29, osmotic pumps were removed and each animal received a single IM administration of 0.2 mg/kg Lupron and animals observed for further 18 days.
Animals were observed for signs of toxicity twice daily. Serum chemistry and hematology determinations were done prior to initiation of dosing and on Day 7, 14, 21, 28, 35, and 42. Body weights were recorded 8 days prior to dosing and weekly thereafter. Serum for analysis of testosterone and plasma for abarelix concentration analysis were taken from each monkey prior to initiation of dosing and on at various days from day 2 through 46. Also on various time intervals from day 2 through 42 plasma samples were obtained for histamine analysis.
Under this section only PK data is reviewed and the toxicity data is reviewed under toxicology sub-section.

Summary of plasma PPI-149 concentration on select days for 28 days is given in table below:

Table 13

Study day	Mean concentration (ng/ml)			
	0 ug/kg/day	30 ug/kg/day	100 ug/kg/day	300 ug/kg/day
2	<0.8	48.2+/-11.2	89.5+/-25.3	93.6+/-52.6
7	<0.8	84.7+/-2.8	114+/-12.7	498+/-359
14	<0.8	54.9+/-11.9	83.9+/-18.0	318+/-276
21	<0.8	47.5+/-13.3	73.4+/-19.3	368+/-313
28	<0.8	57.7+/-18.1	73.6+/-18.3	271+/-168
35	<0.8	<0.8	6.2+/-6.5	27.8+/-37.9
42	<0.8	<0.8	1.5+/-1.0	1.4+/-0.9

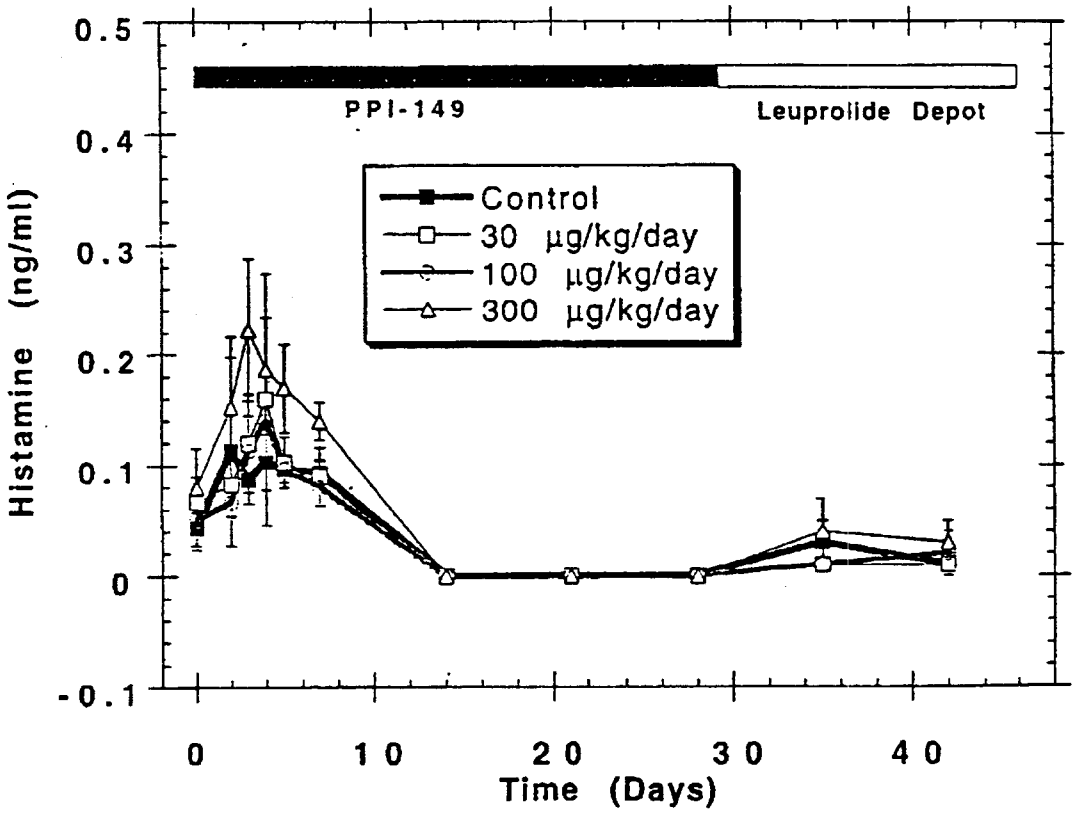
The limit of quantitation for the assay was — ng/ml. Values are means+/-standard deviations

The data showed that an increase in plasma concentration occurred with increasing dose. However, there was wide within group variation as in each dose group broad range of peak concentrations was measured: from 85.5 to 233 ng/ml in the low dose group; from 113 to 259 ng/ml for the mid dose group; and 215 to 1350 ng/ml for the high dose group. The average daily plasma concentration during the 28-day dosing period was said to be 58.6, 86.9 and 310 ng/ml for the low, mid and high dose groups, respectively. Some drug accumulation was noted in the mid and high dose animals.
A dose of 100 ug/kg/day induced a complete pharmacological castration, which was maintained throughout 28 days of dosing. Also the same dose was able to induce a complete blockade of he testosterone surge response to the depot formulation of Lupron seen in control animals as well as animals treated with a dose of 30 ug/kg/day. The proposed calculated HTD is 51 ug/kg/day and as such may be able to maintain castrate state and block testosterone surge.

Plasma histamine levels were presented only in graphic form as shown below. There seemed to be an increase in plasma histamine levels with high dose SC abarelix administration when compared to vehicle controls. Sponsor stated that there was no statistically significant increase at

any time compared to control animals. However, since complete data was not submitted, the extent and variability of increase can not be ascertained.

Figure 1: Histamine release in monkeys on abarelix administration via osmotic pump



Note: The toxicology part is reviewed under Toxicology section

Comments: significantly lower reticulocyte values in group 3 animals on Day 14 and in group 2, 3 and 4 animals on Day 21 as compared to respective control values may suggest possible bone marrow toxicity.

ADME

Sponsor has conducted many studies in various animal species as summarized below where PK for PPI-149 is provided.

Inhibition of serum testosterone in rats by PPI-149. Final report (00-180). Vol.28 p. 184

This study was conducted by [redacted]

Male rats (10/g) were administered doses of 260, 877 or 4390 ug/kg as a single SC injection. An additional group of 10 rats was administered 4390 ug/kg as a single IM injection. Blood was collected from each animal 24 and 48 hours prior to treatment and 3, 6, 12, 18, 24, 36, 48 and 72

hours after treatment. Plasma testosterone was assayed using radioimmunoassay and abarelix using HPLC.

Results: The PK parameters for PPI-149 are given in table below:

Table 14

Parameter	Dose (ug/kg)/Route			
	260/SC	877/SC	4390/SC	4390/IM
Tmax (hr)	3.00 +/- 0.00	3.30 +/ - 0.95	3.30 +/- 0.95	3.00 +/- 0.00
Cmax (ng/ml)	523 +/- 65	1536 +/- 247	2304 +/- 423	1712 +/- 447
$T_{1/2}$ (HR)	1.40 +/- 0.44	1.26 +/- 0.24	2.98 +/- 0.78	6.73 +/- 2.70
AUC_{0-t} (ng.hr/ml)	1793 +/- 334	8802 +/- 1721	30729 +/- 2494	28670 +/- 5867
Vz/F (ml/kg)	303 +/- 101	188 +/- 52	**620 +/- 182**	**1534 +/- 670**
CL/F (ml/kg/hr)	150 +/- 29	103 +/- 18	144 +/- 12	159 +/- 34
$MRT_{SC/IM}$ (hr)	3.65 +/- 0.10	4.74 +/- 0.40	11.49 +/- 0.91	15.68 +/- 3.87

Values are means +/- SD.

The difference in V_d was attributed to a decreased terminal elimination rate after IM administration. Both AUC and Cmax after SC administration showed dose proportionality with increasing dose levels. The R-values were 0.99 and 0.80, respectively.

In all dosed groups serum testosterone was suppressed by 3 hours. Following SC administration, with low and mid dose it remained suppressed for 12 hours and with 4390 ug/kg it remained suppressed for at least 24 hours. Testosterone in the 4390 ug/kg dose group returned to or exceeded normal levels between 36 and 48 hours. With IM administration it was suppressed for 24 to 36 hours.

Although sponsor has stated that values returned to control level or exceeded in the high dose group, data in the table below suggests that it occurred in all groups and may suggest increased sensitivity of testes to GnRH after the effect of GnRH antagonist is over.

Table 15

Hour	Dose (ug/kg)/Route				
	Saline/SC	260/SC	877/SC	4390/SC	4390/IM
-24	1.9 +/- 1.2	1.7 +/- 1.1	1.9 +/- 1.2	1.2 +/- 0.5	1.2 +/- 0.6
3	1.6 +/- 0.8	0.3 +/- 0.1	0.2 +/- 0.1	0.2 +/- 0.1	0.2 +/- 0.1
6	3.1 +/- 1.0	0.7 +/- 0.1	0.7 +/- 0.1	0.7 +/- 0.1	0.7 +/- 0.1
12	2.5 +/- 1.1	0.4 +/- 0.3	0.1 +/- 0.1	0.7 +/- 0.1	0.1 +/- 0.0
18	1.5 +/- 0.8	**3.2 +/- 1.4**	0.9 +/- 1.2	0.1 +/- 0.1	0.1 +/- 0.1
24	2.1 +/- 0.8	**3.7 +/- 1.4**	**3.2 +/- 1.0**	0.0 +/-0.0	0.0 +/-0.0
36	1.0 +/- 0.6	1.0 +/- 0.5	1.7 +/- 1.9	0.4 +/- 0.6	0.0 +/- 0.0
48	2.8 +/- 1.4	2.1 +/-1.0	1.9 +/- 0.8	**9.6 +/- 4.7**	1.1 +/- 3.1
72	2.3 +/- 0.7	2.0 +/- 0.8	1.9 +/- 0.9	2.4 +/- 1.8	**3.1 +/- 4.0**

Values are mean +/- SD for 10 rats in each group.

Dose response study of abarelix depot on serum testosterone concentrations in male sprague-Dawley rats. Final report (00-181). Vol 28 p.197

This study was conducted by —— The effect of a single IM dose of abarelix depot (6, 9, 12, and 18 mg/kg) on the duration of testosterone suppression was determined in male rats (8/g). Blood was collected for testosterone and abarelix determination prior to administration and then at various time intervals up to 119 days after administration. Serum abarelix was determined by — method.

PK parameters are given in table below. Values are mean +/-SD.

Table 16

Parameters	Group 1 (6 mg/kg)	Group 2 (9 mg/kg)	Group 3 (12 mg/kg)	Group 4 (18 mg/kg)
Tmax (day)	0.34 +/- 0.4	0.32 +/- 0.4	0.90 +/- 0.3	0.89 +/- 0.3
Cmax (ng/ml)	936 +/- 369	1131 +/- 384	1453 +/- 397	1238 +/- 315
Dose-normalized Cmax (ng/ml per mg/kg)	156 +/- 62	126 +/- 43	121 +/- 33	69 +/-18
$T_{1/2}$ (day)	4.9 +/- 2.8	7.3 +/- 4.6	8.9 +/- 3.6	6.8 +/- 1.4
AUC_{0-29} (ng.day/ml)	1755 +/- 439	2824 +/- 573	4308 +/- 1001	4448 +/- 1030
$AUC_{0-\infty}$ (ng.day/ml)	1769 +/- 433	2879 +/- 552	4476 +/- 1103	4613 +/- 1092
Dose-normalized $AUC_{0-\infty}$ (ng.day/ml per mg/kg)	295 +/- 72	320 +/- 61	373 +/- 92	256 +/- 61
CL/f (L/day/kg)	3.6 +/- 1.0	3.2 +/- 0.6	2.8 +/- 0.6	4.1 +/- 1.0
V_z/F (L/kg)	26.0 +/- 16.4	35.1 +/- 25.8	37.1 +/- 20.2	39.3 +/- 8.2
$AUC_{0-29}/AUC_{0-\infty}$	0.991 +/- 0.0	0.979 +/- 0.0	0.967 +/- 0.0	0.965 +/- 0.0

PK analysis of the data showed that majority of the drug exposure occurred during the first 29 days after dosing. There was a dose-proportional increase in Cmax and AUC for 6, 9 and 12 mg/kg doses, but no it was not true for the 18 mg/kg dose.

All doses of abarelix caused a suppression of serum testosterone during the first 24 hours following IM administration. The duration of suppression for groups that received 6, 9, 12 and 16 mg/kg was 32+/-15, 43+/-8, 76+/-16 and 81+/-11 days (mean+/-SD). Testosterone concentrations in all groups eventually returned to normal levels. The PK data thus complimented the PD data.

Absolute bioavailability, metabolism and excretion of ^{14}C-abarelix following intravenous, subcutaneous and intramuscular administration in cynomolgus monkeys. Final report [redacted] study No. PK# 100769. Vol. 29 p.1

This non-GLP study was designed to examine the bioavailability, metabolism and excretion after a single dose of 1 mg/kg of labeled abarelix in 5% mannitol. The study was conducted in 3 stages using the same 3 monkeys. In the first stage, all 3 monkeys were administered abarelix via IV route. After which, blood, urine and feces were collected. After washout periods, SC and IM dosing were done sequentially and the samples were collected. Voided urine was collected at various periods up to 48 hours for the IV and SC animals. For the IM animals, urine was collected up to 240 hours post-dose. Feces collection interval periods were same as for the urine intervals. Clinical chemistry parameters were evaluated on the 6th day following IV dosing. Serum abarelix was analyzed using [redacted] analysis was used for metabolite identification after enzymatic hydrolysis.

Results:

PK parameters of abarelix are shown in table below:

Table 17

	Tmax Day	Cmax ng/ml	$T_{1/2}$ day	AUC_{0-t} ng.d/ml	$AUC_{0-\infty}$ ng.d/ml	CL(/F) ml/d/kg	V_z(/F) ml/kg	V_{ss} ml/kg	$MRT_{0-\infty}$ Day	F %
IV	NA	17695	0.17	865	867	1160	289	143	0.12	NA
IM	0.03	3118	0.19	724	724	1436	429	NA	0.26	87.1

SC	0.03	3083	0.15	654	659	1518	325	NA	0.22	77.5

Serum abarelix was analyzed up to 48 hours post-dosing.

Mass balance and the composition of radioactivity in excreta is given in the following table:
Table 18

Route	sample	% of dose excreted	Mean percent of dose M-1	M-2	M-3	M-4	Abarelix
IV	Urine	20.0	3.0	ND	0.6	ND	15.0
	Feces	81.3	32.2	6.2	ND	20.9	8.5
	Total[a]	102.2	35.5	6.2	0.6	21.1	23.5
SC[aa]	Urine	17.3	1.0	ND	1.0	ND	15.2
	Feces	71.3	27.7	9.9	ND	8.5	12.4
	Total[a]	89.3	28.9	10.0	1.0	8.6	27.7
IM	Urine	17.8	1.1	ND	0.9	ND	15.3
	Feces	69.5	27.0	4.1	ND	15.9	7.6
	Total[a]	89.3	28.9	4.2	0.9	16.4	23.1

[a] includes cage wipes and its composition was assumed to be same as that of feces
[aa] Data from animal # 203 was not included because its serum radioactivity profile and PK profile was similar to IV dosed animals and it was thought that this animal had been dosed at least partially via the IV route. ND= not detected.

Data showed that after IV dosing, abarelix was cleared rapidly with terminal $T_{1/2}$ of 0.17 days. Only trace amount of radioactivity was present after 48 hours (15632 ng.equivalents/ml at 0.03 hours vs 27 ng.equivalents/ml at 48 hours). The bioavailability after IM and SC dosing was 87 and 78 %, respectively. Serum radioactivity was predominantly composed of abarelix and with minor amount of metabolite M-1. Majority of the radioactivity was excreted in feces. The major routes of abarelix clearance were the urinary excretion of unchanged abarelix and hepato-biliary elimination of abarelix and its hydrolytic metabolites. The metabolites observed were M-1 (hepta-peptide A-G), M-2 (penta-peptide A-E), M-3 (nona-peptide A-1) and M-4 (hexa-peptide A-F).

Clinical observations: It was stated that following IV dosing animals 101 and 103 exhibited a transient lethargy characterized by a limp demeanor and limp tail. Both animals recovered within a couple of minutes and were normal thereafter. Within 2 minutes following dosing animals **102** began to appear lethargic. His eyes began to close, tail went limp and the animal stopped moving. His gums and extremities were pale and capillary refill was slow but animal did respond to stimuli. Within 15 minutes animal began to recover and appeared normal. After the 40 minute blood collection the animal once again went in to lethargic condition and became limp and slow. Heart rate and breathing were rapid. The extremities were pale and capillary refill was slow but animal was responsive to stimuli. The animal appeared exhausted and tired and for all appearances was asleep. Animal was treated with Lactated Ringer's Solution and within minutes animal's condition improved, became alert and very responsive.
This animal exhibited similar transient lethargy after IM dosing but of much less severity and recovered within a minute.
No noteworthy clinical signs were reported for animals following SC dosing.
All clinical chemistry parameters appeared normal.

Comments: Sponsor stated that no clear explanation for this observation can be presented and suggested that it could be that animal was more sensitive to test product which was acid in nature (tri-fluoroacetate salt) and formulated in 5% mannitol.
Sponsor however, did not try only dosing with tri-fluoroacetate salt to see if it caused toxicity. From the results it would seem that adverse effect was dependent on the extent and speed of drug exposure since the effect was most severe after IV injection, much less severe after IM administration and essentially with no adverse effects after SC administration of the same dose.

A pharmacodynamic/pharmacokinetic study of PPI-149 administered by subcutaneous infusion from surgically implanted osmotic pumps in Rhesus monkeys. — study number 0436-41. Vol. 29 p.138

This study was conducted by [redacted]

This study was designed to test the hypothesis that testosterone secretion could be effectively suppressed by a regimen of initial constant exposure to abarelix at high levels followed by constant exposure at levels several fold lower.

Fourteen monkeys, 26 to 64 months of age and weighing 3.9 to 5.5 kg, were used in 5 treatment groups as shown below. First implant was removed on Day 29 and a second implant was implanted.
Table 19

Group #	# of animals	Day 1 implant Predicted dose of abarelix (ug/kg/day)	Day 29 implant Predicted dose of abarelix (ug/kg/day)
1	3	50	5
2	3	50	15
3	3	100	5
4	3	100	15
5	3	No implant (control group)	No implant (control group)

The dose was calculated on body weight of 4 kg per animal. However, it was stated that mean body weight was about 5 kg and thus doses were about 20% lower. Doses were selected to be within the expected range of anticipated human exposure.

Results:

No drug-related clinical observations were noted. Some animals had bruising and incision site bleeding caused by implant procedure. Other observations were occurrence of liquid stools and nasal discharge, which was seen in both control and treated groups. Body weight was reduced in all drug-treated groups during the first 4 weeks and then increased so that there were no differences in weight by the end of the study.

Approximate group mean plasma abarelix concentrations ranged from 20 to 52, 10 to 25, 1.8 to 8.9 and 0.8 to 3.5 ng/ml for implants with intended rates of release of 100, 50, 15, and 5 ug/kg/day, respectively.

Testosterone was suppressed in groups, 3 and 4 which received implants with release rate of 100 ug/kg/day and in groups 1 and 2 which received implants with release rate of 50 ug/kg/day. Implants with release rate of 5 ug/kg/day were not effective and those with 15 ug/kg/day gave very variable results. In group 2 (50 ug/kg/day), two of three animals escaped suppression and had levels greater than 3 ng/ml on Days 32 and 53. Also in group 4 (100 ug/kg/day) two of three animals escaped but not until Day 53. In both cases there was great variability.

Note: It was stated that prior to first implant, serum testosterone in most animals tended to be in the prepubertal range. This was ascribed to seasonal effect because the study was started in the summer (late July); a period when reproductive function is depressed and testosterone levels are low. However, it was assumed that by the end of the study in September, most animals would have been expected to return to normal reproductive function, with associated increase in testosterone levels.

With this type of starting condition, it is not possible to know if the suppression observed during the first 29 days was due to treatment or the animals had seasonal low testosterone levels. One of the control monkeys during this period had testosterone level below 0.1 ng/ml but increased after Day 44.

Sponsor however, concluded that testosterone levels were effectively suppressed up to 29 days with constant infusion rate of 50 or 100 ug/kg/day. With pumps releasing 5 or 15 ug/kg/day, testosterone was not suppressed. Plasma abarelix concentrations were proportional to rate of infusion. No adverse drug related clinical effects were observed.

Comments: Since 50 ug/kg/day was barely enough to maintain testosterone levels suppressed for one month in monkeys, one would expect that in humans a dose of 100 mg depot every 28 day (51 ug/kg/day) would barely be enough to definitely and consistently suppress testosterone to castrate levels. This also suggests that the abarelix is cleared by this time and effect lasts as long as receptors are occupied. GnRH agonists testosterone suppressive effect however, last long after drug is cleared from circulation.

In fact this happened in clinical study 149-97-04, where patients were dosed with 100 mg on days 1 and 15 and then with 50 mg on days 29 and every 28 days thereafter. It was reported that the 50 mg dose was increased to 100 mg for patients whose testosterone level exceeded 50 ng/dl on or after day 29.

Biodistribution of ^{14}C-labelled PPI-149 following a single intravenous and subcutaneous administration to sprauge-dawley rats. Final report. ——— Study No. N002059D vol. 30 p.1

This study was conducted in compliance with GLP regulations.

The study design consisted of seven groups of 3 male rats/g administered 0.50 mg/kg of ^{14}C labeled PPI-149 once IV and six groups of 3 rats/g administered similar dose once SC. Each rat was bled 1-3 times during the 96 hour study period. From one group of rats each administered abarelix IV and SC, urine, feces and expired air as well as cage rinse was collected for

radioactivity counting. Fraction of dose recovered by these paths along with that recovered in the tissues was used for mass balance determination.

Results: Administration of ^{14}C-PPI-149-Depot resulted in highest recoveries of radioactivity at early time points in the tissues on the path of fecal excretion (liver and GI tract) and urinary excretion (kidney and urinary bladder). Tissue distribution in the remaining tissues was less than 1% of the administered dose. Urinary and fecal (biliary) excretion were the major pathways for elimination and most of the administered dose was recovered equally in the urine and feces within 24 hours after dosing. Data is expressed as % recoveries (mean +/- SE) are given below.

Table 20

	IV	SC
Urine 0-96 hours	50.0 +/-11.9	55.4 +/- 12.9
Feces 0-96 hours	50.4 +/- 2.2	48.5 +/- 6.4
Tissues (includes plasma 0-96 hours)	1.04 +/- 0.34	1.95 +/- 1.13
Expired air 0-96 hours	0	0
Total (mass balance) %	101.5 +/- 14.4	105.8 +/- 20.5

Similar findings were reported under Praecis study #PC070897 where SC dose of 10.5 mg/kg ^{14}C-PPI-149 depot was used.

Determination of protein binding of ^{14}C-abarelix in rat, monkey and human plasma.
—— study No. N003993B. Final report. Vol.29 p.248

Plasma protein binding of ^{14}C-abarelix was determined by equilibrium dialysis. Rat and monkey plasma was obtained from a series of male and female animals. Human plasma was obtained from 2 healthy male and female donors. The binding was studied at drug concentrations of 0.2 and 1.0 ug/ml. The equilibration time was 6 hours. Protein binding was tested using 5 plasma samples/sex in rats, 3/sex in monkeys and 5/s for each human donor for each abarelix concentration.

Results: It was demonstrated that the fraction bound to protein was independent of abarelix concentration in the range from —— ug/ml across all species. Fraction bound was also independent of the sex of the animals. The average fraction bound across all species was about 98%.
It was concluded that since the protein binding was independent of the concentration of ^{14}C-abarelix in the —— ug/ml range, it is likely that the protein binding of abarelix will be similar in the therapeutic plasma concentration range (48.6+/-13.7 ng/ml; —— Protocol No. 149-97-03) observed in humans.

In volume 30 page 135 Under . —— study # PK # 100831 entitled **" metabolism and excretion of 14C-abarelix in bile-duct cannulated SD rats"** it was demonstrated that biliary excretion was the predominant route of elimination of abarelix-related radioactivity. Mass balance and the composition of urinary/biliary radioactivity using 3 rat/s administered abarelix IM (1 mg/kg) is shown in table below.
Table 21

0-48 h samples	Mean percent of the dose									
	Male rat					Female rats				
	total	M-1	M-3	abarelix	other	total	M-1	M-3	abarelix	other
Urine	25.11	ND	ND	22.2	3.0	19.95	ND	ND	18.1	1.9
Bile	59.02	23.1	9.1	17.7	9.1	64.92	24.5	9.4	24.7	6.3
Total	84.13	23.1	9.1	39.9	12.1	84.87	24.5	9.4	42.7	8.2
% urine		ND	ND	88.2	11.8		ND	ND	90.5	9.5
% bile		39.2	15.5	30.0	15.3		37.7	14.5	38.0	9.7

In addition to abarelix, biliary radioactivity was composed of M-1 and M-3. In contrast, urinary radioactivity was primarily composed of unchanged abarelix. There were no sex differences.

In a similar study conducted by [redacted] (study No. N003993A) total recovery over a 48 hour period (bile, urine and feces) amounted to 93.0 +/- 9.9 % in male rats and 91.1 +/- 6.9 % in female rats (mean +/- SE).

Evaluation of ^{14}C-abrelix (PPI-149) metabolism after in vitro exposure in freshly isolated rat, monkey and human hepatocyte suspensions. [redacted] study number PK # 100759. Vol. 30 p.252

In this study 1 x 10^6 cells in 0.25 ml medium were used with drug concentration of 2 uM for 3, 6 and 24 hour incubation period. The results after 6-hour incubation are shown in the following table 22.

	Abarelix	M-1	M-3	Other
Male rat	93.5	ND	ND	6.5
Male monkey	67.4	25.9	ND[a]	6.7
Male human #1	39.6	45.7	13.2	1.5
Male human #2	31.3	61.6	8.6	2.8
Female human #1	37.8	45.3	16.9	ND

[a] metabolite M-3 was observed at earlier time points ND= not detected

Results showed that human and monkey hepatocytes are capable of metabolizing abarelix. It was stated that even though rat hepatocytes did not form metabolites M-1 and M-3, these two metabolites were the major components of rat and monkey excreta. This however, left unanswered the lack of ability of rat hepatocytes to metabolize abarelix.

Toxicokinetics

The following toxicokinetic studies were conducted:

13-week repeated-dose toxicology study of PPI-149-depot in mice: Toxicokinetic evaluation of PPI-149-depot (30, 100, 300 or 1000 mg/kg). Study No. PC073097. Vol.28 p.1

This study was conducted by Praecis Pharmaceuticals, Inc. [redacted] analyzed the Plasma PPI-149 concentrations.

The objectives of the study was to determine plasma exposure to PPI-149 following multiple SC doses of 30, 100 or 300 mg/kg PPI-149 Depot, and following a single SC injection of 300 or 1000 mg/kg PPI-149 Depot in healthy male and female mice. Depot was injected on day 1, 29, 57 and 85.

Blood samples were obtained from 5 male and 5 female animals per dose group on day 7, 14, 28, 63, 70, and 85; and from 5 males and 10 female animals on day 91 of the repeat-dose study. Also blood samples were taken from 3 male and 3 female animals on day 7 after a single SC dose of 300 or 1000 mg/kg PPI-149 Depot. Single dose animals were used for a dose-range evaluation of a mouse micronucleus pilot study. AUC values for the first (day 7-28) and last (day 63-85) dose intervals were calculated for the 100 and 300 mg dose groups. Cmax values for the 100 and 300 mg/kg doses were determined from the mean concentrations obtained on days 7 or day 63.

Results of TK portion are shown in combined table for the mouse, rat and monkey TK data. This study is also listed under toxicology as study code T 1.

6-month subcutaneous toxicity study of PPI-149 depot in Sprague–Dawley rats: multiple-dose toxicokinetics of PPI-149 (10, 30 or 100 mg/kg) following subcutaneous injection. ——— study No. N0020591.

The ——— conducted this study, and the ——— analyzed plasma. ———

Three rats/s received 10, 30, or 100 mg/kg PPI-149 Depot on days 1, 15, 29, 57, 85, 113 and 141.

Up to 6 blood samples/rat were obtained during the study at scheduled intervals at least 7 days apart. Blood was collected from 3 rats/s on day 1 (prior to dosing) and on days 2, 4, 6, 8, 10, 15, 17, 19, 21, 24, 29, 33, 57, 61, 85, 89, 113, 117, 141, 145, 152, 159, AND 169. The mean plasma PPI-149 AUC values on Day 1 – Day 15 and Day 141 – Day 169 were calculated. AUC values normalized for dose were calculated for an estimation of dose proportionality.
The dose-normalized mean Cmax PPI-149 concentrations obtained on Day 2 and Day 145 or Day 152 was also used for evaluation of dose proportionality. $T_{1/2}$ were not calculated because of he persistence of PPI-149 in plasma following administration of the depot preparation.

Results of TK portion of this study are given in combined TK table for the mouse, rat and monkey.
This study is also given under toxicology as study code T3.
12-month chronic subcutaneous toxicity study of PPI-149 depot in cynomolgus monkeys: multiple-dose toxicokinetic evaluation of PPI-149 depot (5, 15 or 40 mg/kg). ——— study No. N002059L.
This study was conducted by ——— as the above rat study. Plasma samples were analyzed by ———

Monkeys were included in 2 groups of 12 monkeys/s/g (groups 1 and 4) and 2 groups of 10 monkeys/s/g (groups 2 and 3). Group 1 monkeys received the vehicle while groups 2, 3 and 4 monkeys received PPI-149 depot at a dose level of 5, 15 and 40 mg/kg, respectively. Three monkeys/s/g were euthanized at Month 4 and Month 7. Four monkeys/s/g were euthanized following 12 months of dosing. The remaining 2 monkeys in groups 1 and 4 were maintained for a 4 month recovery period. Animals were dosed once every 4 weeks for up to 12 months or until euthanized. Blood was collected prior to first dose administration on Day 1 and on Days 2, 3, 7,

14, 21, and 28. Blood samples were also obtained prior to dosing on Day 336 and after dosing on Days 338, 339, 343, 350, 357 and 364. Blood from recovery animals was obtained on Day 427. Cmax and AUC were calculated from plasma PPI-149 concentrations obtained on Day 1 to Day 28 (first dose) and Day 336 to Day 364 (last dose). Half-life values were not calculated because of the persistence of PPI-149 in plasma following administration of the depot preparation.
Results: The TK portion of the study is given in combined table for the mouse, rat and monkey toxicokinetics and calculation of exposure multiples compared to human exposure with the proposed therapeutic dose. This study is also described under toxicology as study code T6.
Table 23

Species Study	Dosage Form	Dosage Route	Sex	Nominal dose (mg/kg)	C_{max} [a] (ng/ml)	Multiple of clinical C_{max}	AUC[b] (ng.day/ml)	Multiple of clinical AUC
human[c] 149-99-01	Depot	IM	male	100 (mg)	43	1	400	1
Mouse Toxicology 13-week[d]	Depot	SC	Male	30	–	–	–	–
				100	1049	**24**	5274	13
				300	1911	**44**	27176	**68**
			Female	30	–	–	–	–
				100	593	**14**	3090	**8**
				300	2514	**58**	19124	**48**
Rat toxicology 6-month[e]	Depot	SC	Male	10	783	**18**	1547	4
				30	1126	**26**	6215	**16**
				100	1301	**30**	20244	**51**
			Female	10	876	**20**	1048	**3**
				30	1415	**33**	5891	**15**
				100	1711	**40**	17354	**43**
Monkey Toxicology 12-month[f]	Depot	SC	Male	5	194	5	2872	7
				15	839	**20**	8053	**20**
				40	1270	**30**	18818	**47**
			Female	5	118	**3**	1030	**3**
				15	365	**8**	4334	**11**
				40	1158	**27**	8742	**22**

[a] Cmax listed for 6-month rat study is from first dose. The 13-week mouse study and 12-month monkey study are from last dose.
[b] AUC listed are from last dose
[c] Data from Praecis study 149-99-01 (— PK report 100977), the AUC value was obtained as $AUC_{0\text{-}28}$.
[d] Data from study PC073097 (— 148318)
[e] Data from study N0020591 — 148285)
[f] Data from study N002059L (— 148284)

Based on the data presented in the above table, sponsor stated that results indicate that abarelix depot has a high margin of safety in humans, based on an exposure comparison with mice, rats or monkeys.

Summary: In the 13-week SC toxicity in mice, 6-month toxicity in rats and 12-month toxicity in monkeys, multiples of clinical AUC for the high dose males and females were 68 and 48, 51 and 43 and 47 and 22, respectively.

TOXICOLOGY:

Study Title: **28-day continuous subcutaneous toxicity study of PPI-149 in Sprague-Dawley rats.** Final report
Study No: — N002059B
Amendment #, Vol #, and page #: Vo. 14. P. 1
Conducting laboratory and location: [redacted]
Date of study initiation: 5-8-1996
GLP compliance: yes
QA- Report Yes (*) No ()

Methods:
Dosing:

- species/strain: rat/sprague-dawley
- #/sex/group or time point: 15/s in groups 1 and 5; 10/s in groups 2, 3 and 4
- age: 9 weeks at study initiation
- weight: 270-324 g for males and 181-255 g for females.
- satellite groups used for toxicokinetics or recovery: 5/s of group 5 were used for 28 day recovery period.
- dosage groups in administered units: 0, 300, 1000, 3000 and 8746 (male) of 10,000 (female) ug/kg/day for 28 days via SC implanted [redacted] osmotic pumps to 5 groups of 10 rats/sex/group.
- route, form, volume, and infusion rate: SC (dorsoscapular region), solution in saline, 2.15 ul/hr. 2 pumps/animal in groups 1 and 5.

Drug, lot#, radiolabel, and % purity: Lot FPPI1499601A; expiration date 3/97
Formulation/vehicle: saline
Observations and times:

- Clinical signs: daily for signs of toxicity; twice daily for mortality and morbidity
- Body weights: Days –4, 1 (prior to and after pump implantation), and weekly thereafter, 29 (interim necropsy) and 57 (final necropsy)
- Food consumption: weekly
- Ophthalmoscopy: prior to initiation of dosing and at necropsy (days 29 or day 57)
- EKG: none
- Hematology: 10/s/g on days 29 (interim necropsy) and 5/s/g in control and high dose groups on day 57 (final necropsy)
- Clinical chemistry: same as for hematology
- Urinalysis: none
- Organ weights: at interim and final necropsy
- Gross pathology: at necropsy
- Organs weighed: adrenals, prostate, heart, lungs, liver, spleen, kidneys, brain, testes, epididymides, and ovaries with oviducts
- Histopathology: organs shown in histopathology table
- Toxicokinetics: none
- Other: statistics- analysis of variance and pairwise comparisons.

Results:

- Clinical signs: only treatment-related signs were associated with the reproductive system. Testicular atrophy was noted on day 14 of treatment and continued to termination. Abrasions/lesions around the site of pump implantation, occasional alopecia and a red nose discharge. Swelling at pump site in control and high dose groups.
- Body weights: Compared with control group, mean body weight of groups 2, 3 and 4 males was significantly lower from Day 8 to termination. For group 5 males, weight was lower on day 22 and remained lower during the recovery period.
- In treated females, body weight was increased significantly from day 15 in groups 2, 3 and 4 and from day 8 in group 5. It stayed significantly higher during the 28 day recovery period. There was no dose response relationship.
- Food consumption: there was no treatment-related effect in males but in female it was increased in group 4 and 5 females.
- Ophthalmoscopy: no treatment-related effect, some males and females in the vehicle control group and PPI-149 treatment groups showed corneal crystals, corneal opacities and occasional cataract.
- Electrocardiography: not conducted
- Hematology: Significant treatment-related findings in males on day 29 were: decreased RBC counts in groups 2, 3 and 4; increased reticulocytes (% of RBC) in group 4 and 5 (1.1, 1.2, 1.4, 1.7* and 2.0 *for groups 1, 2, 3, 4 and 5), increased methemoglobin in group 3, increased mean corpuscular volume in group 4 and mean corpuscular hemoglobin concentration in group 5. Platelet count was decreased in all treated groups but significantly in group 3 (1000 ug/kg/day).
- In female significant findings on day 29 were: decreased RBC count in groups 3 and 5, increased mean corpuscular volume in groups 2, 4 and 5 and increased mean corpuscular hemoglobin in groups 2, 4 and 5.
- Differential WBC counts: in males lymphocytes were increased in group 5 on both days 29 and 57.
- In females WBC were increased in group 2 (day 29) and group 5 (days 29 and 57), segmented neutrophils increased in group 5 on day 57, lymphocytes increased in groups 2 and 4 (days 29) and in group 5 (days 29 and 57), eosinophil increased in group 5 (day 29) and basophil in group 1 (day 29). Although only significant finding are given, increases in all female groups were observed.
- Prothrombin time and activated partial thromboplastin time were not significantly affected by treatment.
- Clinical chemistry: treatment related changes are shown in table below: