Table 24

Dose (ug/kg/day)

Males

	Day	0	300	1000	3000	8746
Alkaline phosphatase (IU/L)	29 57	144 122	106	118	144	121 91
Aspartate aminotransferase (IU/L)	29 57	83 79	83	76	**72***	**73*** 71
Alanine aminotransferase (IU/L)	29 57	38 41	36	33	37	36 37
Creatine kinase (IU/L)	29 57	397 533	444	475	267	362 208
gamma glutamyltransferase (IU/L)	29 57	0 1	0	0	0	0 1
Lactate dehydrogenase (IU/L)	29 57	367 207	335	270	**166***	**131*** **92***
Sorbitol dehydrogenase (IU/L)	29 57	22 27	18	**17***	18	**16*** **20***
Glucose (mg/dl)	29 57	101 129	105	**112***	108	109 135
Blood urea (mg/dl)	29 57	15 17	15	16	15	15 17
Creatinine (mg/dl)	29 57	0.6 0.5	**0.5***	0.5	0.5	**0.5*** **0.6***

Females

	Day	0	300	1000	3000	8746
Alkaline phosphatase (IU/L)	29 57	106 55	116	143	**141***	131 **118***
Aspartate aminotransferase (IU/L)	29 57	85 78	75	71	72	76 82
Alanine aminotransferase (IU/L)	29 57	40 39	59	43	45	42 48
Creatine kinase (IU/L)	29 57	471 150	302	229	223	349 185
gamma glutamyltransferase (IU/L)	29 57	1 0	1	1	1	2 0
Lactate dehydrogenase (IU/L)	29 57	293 213	184	87	87	167 231
Sorbitol dehydrogenase (IU/L)	29 57	17 23	16	**12***	14	15 **31***
Glucose (mg/dl)	29 57	124 112	118	121	122	115 **125***
Blood urea (mg/dl)	29 57	19 15	19	18	18	18 15
Creatinine (mg/dl)	29 57	0.6 0.6	0.6	0.6	0.6	0.6 0.6

*Statistically different from concurrent controls at $p<0.05$.

Other treatment-related changes seen in clinical chemistry were a decrease in triglycerides on day 29 and increase in chloride on day 57 in males. In treated females, triglycerides, cholesterol and phosphorus were decreased in group 5 on 57. Total protein was decreased in all treated groups on day 29.

- Urinalysis: not conducted
- Necropsy: At necropsy, treatment-related effects were confined to the organs of genital system of both male and female rats. Compared to controls, the changes
- consisted of small testes, epididymides, seminal vesicles, and prostate glands in males and small ovaries and uterine horns in females. These changes were observed in all treated and recovery rats on Days 29 and 57. In 2 rats which died after removal of the implant on day 29, showed only changes in the genital organs. The deaths were attributed to anesthesia.

Organ Weights: Treatment-related organ weight changes as organ-to-body weight ratio is shown in table below:

Interim necropsy

Table 25 Males

Treatment group	B.wt gm	Liver	Kidney	Lung	Heart	Epididymis	Prostate	Testis ovary	adrenal	thyroid	Pituitary	brain
0 ug/kg/day	411	3.156	0.748	0.512	0.378	0.271	0.319	0.795	0.017	0.006	0.004	0.497
300 ug/kg/day	376*	2.913*	0.686	0.471	0.375	0.077*	0.072*	0.180*	0.021	0.005	0.004	0.557*
1000 ug/kg/day	381*	2.978	0.742	0.571	0.395	0.067*	0.066*	0.178*	0.020	0.005	0.004	0.548*
3000 ug/kg/day	380*	2.804*	0.667*	0.500	0.355	0.070*	0.076*	0.173*	0.020	0.005	0.004	0.539
8746 ug/kg/day	382*	2.835*	0.702	0.542	0.373	0.077*	0.075*	0.180*	0.022	0.005	0.004	0.551*
Females												
0 ug/kg/day	254	2.969	0.730	0.535	0.371			0.048	0.030	0.008	0.006	0.753
300 ug/kg/day	297*	2.848	0.662*	0.631*	0.353			0.024*	0.025*	0.006	0.005*	0.658*
1000 ug/kg/day	298*	2.932	0.671	0.517	0.352			0.021*	0.026	0.007	0.004*	0.666*
3000 ug/kg/day	324*	2.816	0.633	0.557	0.347			0.021*	0.023*	0.005*	0.004*	0.609*
10000 ug/kg/day	317*	2.826	0.639*	0.559	0.373			0.022*	0.025	0.006*	0.004*	0.625*

At the final necropsy on day 57, in treated males relative weight of liver, kidney and those of genital organs was significantly lower compared to controls. Spleen weight was significantly higher (0.146 vs 0.175%). In females, relative weight of the kidney, heart, ovary, adrenal gland and brain were lower compared to respective controls. Except for treatment-related changes in genital organs, changes in organ weights could be attributed to changes in body weight with treatment.

- Gross pathology: Gross pathologic changes consisted of small testes, epididymides,
- s.vesicles, and prostate in males and small ovaries and uterine horns in females.
- Histopathology: microscopic changes considered being the direct results of drug treatment were confined to the genital organs of both male and female treated rats. These consisted of reduction of the testicular size resulting from generalized atrophy and degeneration of the germinal epithelium. Similar atrophy of s.vesicles, prostate gland and epididymides was seen. Viable spermatozoa were not observed.

  In females treatment resulted in atrophy of the uterine horns and atrophy of the endometrium and uterine glands. All these changes were of moderately severe. The squamous epithelium lining of the vaginal was significantly thinner, had large number of leukocytes resulting in supporative exudate in the lumen. Ovaries were atrophied, and follicular development appeared arrested. Corpora lutea in the ovaries of treated females were much smaller compared to those seen in the ovaries of control females. All the changes were described as consistent and similar in appearance and severity in all treated males and females regardless of dose group involved.

Microscopic changes were not reverse during the 28 days of non-treatment. Testicular degeneration and atrophy had the appearance of being more severe in the recovery males. This was suggested to be not a reflection of a continuing degenerative process, but rather morphologic result of the continued maturation of unaffected spermatocytes, their migration to the epididymis, and lack of available precursors to replace them.

All other changes were interpreted to be spontaneous in nature and not related to PPI-149 administration and not considered of toxicologic significance.
Sponsor concluded that based on the nature of the effects. NOAEL for PPI-149 may be 8746 ug/kg/day for males rats and 10,000 ug/kg/day for females rats, when administered via SC infusion for 28 days.

Toxicokinetics: none

Overall Toxicology Summary: most observations could be attributed to injection site inflammatory reaction and effect of treatment on gonadal hormones.

Addendum list: none

Title: **28-day pilot continuous subcutaneous toxicity study of PPI-149 in cynomolgus monkeys.** Study No. N002059C. Final report. Vol 18 p.1

This study was conducted in accordance with GLP regulation by ——— with

The experimental design was as follows:
Table 26

Group #	Sex	Animal numbers Core	recovery	Treatment	Dose level Ug/kg/day	Treatment regimen
1 control	Males Females	5 5	3 3	Vehicle	0	Continuous administration via SC placed catheters for 28 consecutive days
2	Males Females	5 5	- -	PPI-149	100	As for control
3	Males Females	5 5	- -	PPI-149	1000	As above
4	Males Females	5 5	3 3	PPI-149	4750	As above

In-life data collection was to include:

Twice daily observations for mortality and moribundity
Clinical observation for signs of toxicity once daily
Ophthalmic examination prior to initiation of dosing and at scheduled necropsy
Serum chemistry and hematology prior to dosing and weekly during treatment and at the end of recovery

Weekly blood drawn for serum testosterone in males and estradiol in females, **plasma histamine** and PPI-149 concentrations during treatment and at the end of recovery.
Body weight on Day 1 and weekly thereafter.
Complete necropsy with histopathological evaluation.

NOTE: It was stated that due to technical problems, which included catheter tract infections, the study was discontinued. Animals were taken off the study at different time points. Thus the results reported represent variations in the infusion duration and the amount of PPI-149 each animal received.

Results:

Clinical observations included soft feces, diarrhea, discoloration of nose, tail and ganitalia and most commonly, abrasions/lesions and/or swelling on the dorsal body. The lesions displayed hemorrhagic to purulent discharge beginning on Day 2 of the study and necessitated the termination of the study. These findings were reported for both treated and control groups. Due to the inconsistency of the duration of the infusions, no treatment effect could be deliniated. However, under the conditions of treatment, no treatment effect was reported on body weight, and food consumption. No treatment related effects were observed on hematology.
At various time points, serum chemistry differed in treated when compared to controls such as increases in gamma glutamyltransferase in low dose and high dose males on Day 7 (128 +/-6, 170 +/-21, 152+/- 8 and 168 +/- 24 for control, low, mid and high dose groups, respectively), elevated creatine kinase levels in high dose males and females on Day 29 (181 +/- 1 vs 271 +/- 19 for males and 124 +/- 1 vs 224 +/- 33 for females) and elevated sorbitol dehydrogenase values in high dose males and females on Day 29 (28 +/- vs 32 +/- 8 for males and 22 +/- 1 vs 35 +/- 1 for females). Values expressed as mean +/- SD. Serum transaminases were not increased in treated males or females and no hepatic histological damage was reported. Enzyme changes observed were not dose dependent and did not occur in both sexes.
Organ weights were not affected by treatment.
Only consistent treatment-related histological finding was inflammatory lesions in the skin at the site of administration. These were characterized as necrotizing inflammation, abscesses or ulcers with mild to minimum severity. Only one high dose animal had thymic atrophy attributed to stress secondary to inflammation at the site of administration. This animal also had an area of acute heart muscle necrosis seen at interim sacrifice, suggestive of an embolus originating from an area of necrosis in the skin.

Plasma drug concentration increased with increasing dose level but dose proportionality was not shown due to high intra-group variability. Since plasma PPI-149 concentrations were observed in recovery animals, drug accumulation was suggested.

Plasma histamine levels: Blood was collected on Days 1 (pre-dosing), 4, 7, 14/15 and 29 and plasma was analyzed by ——— for histamine. However, data was not included.

It was stated that "the sponsor has been unable to locate the original raw data for these parameters and the analytical laboratory did not retain the data". Vol 18 p.190

Title: **28-day subcutaneous toxicity of PPI-149 in cynomolgus monkeys.** ——— study No. N002059G. Final report Vol. 19. P.1

The study was conducted in accordance with GLP regulations.

This study was a repeat of the 28-day toxicity study described above, which was abandoned because of infection caused by the inserted SC cathetors. Similar dose groups were used and dose levels of 0, 100, 1000 and 5000 ug/kg/day were used instead of 0, 100, 1000 and 4750 ug/kg/day for 28 days as used in the abandoned study. Dosing regimen consisted of twice daily SC injections at a dose volume of 0.2 ml/kg/dose approximately 10 hours apart. In the abandoned study pumps were programmed to deliver at a constant (20.8 ul/kg/day) infusion rate based on animal body weight. The vehicle in the previous study was saline while in this study was 5% mannitol solution. Blood was collected on Day –21, and on Days 7, 14, 21, 29, and 57 for plasma histamine analysis.
In life data was collected as in the previous study.

Results:

Survival: With the exception of one female in the control group, which was euthenized because of an undiagnosed moribund condition, all animals survived until their scheduled termination.

Clinical observations: Only treatment-related clinical sign was inflammatory reaction at the injection site noted in high dose male and female animals. It persisted throughout the treatment period and recovery phase. Other findings unrelated to treatment were diarrhea, soft feces, abrasions and or discoloration on the extremities.

Body weight: There was no statistically significant differences in mean body weight of treated with compared with controls during the dosing phase of study.
Food consumption: Treatment did not significantly affect food consumption
Ophthalmic examination: no treatment-related effects
Clinical pathology: Sporadic significant changes were reported in some hematology parameters, which had no dose-response relationship and did not occur at all time determinations.

Some significant treatment related changes were seen in differential WBC count and in males these consisted of increased segmented neutrophils in high dose on day 29, decreased monocytes in low dose group on days 21 and 29, decreased eosinophils in the mid and high dose animals on day 29, decreased basophils in high dose on day 57, and decreased WBC in low dose on day 21. In females there was a consisted increase in segmented neutrophils and decreased eosinophils in high dose group as shown in table below:
Table 27

	Day	0 ug/kg/day	5000 ug/kg/day
Segmented Neutrophils 10^3/ul	-21	4.35	5.10
	7	8.85	8.94
	14	6.88	11.75*
	21	6.26	11.85*
	29	2.59	9.45*
	57	3.58	3.66
Eosinophils	-21	0.08	0.06

10^3/ul	7	0.16	0.07
	14	0.21	0.06*
	21	0.18	0.07*
	29	0.45	0.01*
	57	0.22	0.21

There were other sporadic changes like decreased lymphocytes in high dose on day 29, decreased monocyte in low dose on day 29 and increased WBC in low dose on day 14.

Coagulation parameters: Prothrombin time was significantly increased in low and mid dose males as shown in table below:

Table 28

	day	0 ug/kg/day	100 ug/kg/day	1000 ug/kg/day	5000 ug/kg/day
Prothrombin time (sec)	-21	11.4	11.5	11.5	11.1
	7	9.7	10.4*	10.5*	10.0
	14	9.8	10.5*	10.5*	10.1
	21	9.5	9.9*	9.9*	10.0*
	29	9.7	10.5*	10.5*	10.1
	57	10.4	--	--	10.6

In females, PT was increased on day 29 in mid dose group.

Serum chemistry: As for hematology, no consistent dose and time-related treatment effects were seen. All changes were sporadic and changes were of low magnitude compared to respective controls.

In males, there was a decrease in the lactate dehydrogenase levels in mid and high dose groups. Serum glucose and calcium was decreased, albumin, sodium and chloride increased.
In females, alanine aminotransferase was increased in low dose animals on days 7, 14 and 29 and in high dose group on Day 57. Total protein, calcium, sodium and chloride were increased.

Gross necropsy findings: On interim sacrifice, gross pathologic findings were confined to the SC tissue at the site of administration. These changes consisted of numerous firm, white nodules, and/or focal, red foci. One control monkey, which was sacrificed in moribund condition, had extensive SC hemorrhage involving right leg, chest area and site of administration, which was confirmed on histological examination. In the recovery animals, SC lesions were observed in 3 high dose females. No gross lesions were observed in 3 high dose males or in control males and females. One female had ovarian cyst.

Organ weights: There were no consistent treatment-related changes. A significant decrease in the group 3 male mean absolute spleen weight and spleen-to-brain weight was observed. Group 4 males had an increased thyroid gland to brain value and the liver-to-body weight values were significantly increased in group 3 females.
Although sponsor has stated that mean absolute testis, epididymis and prostate weights, as well as the ratio of these organs-to-brain weight and to-body weight for the testis, were decreased in all treated male groups, data presented suggested minimal change only in testis –to-body weight ratio. No changes in gonadal organ weights for treated females were observed.

Histopathology: All organs from groups 1 and 4 monkeys were examined. Also testes, ovaries, gross lesions and target organs from all groups 2 and 3 were also examined.

Based on histology of the genital organs 2, 1, 2 and 2 males in control, low, mid and high dose groups were considered adults and by the same criteria al females in each group were considered adult.
Although sponsor has stated that histomorphologically at interim sacrifice in males, the testicular changes consisted of a minimal to mild degeneration of the germinal epithelium, and in adult males, a complete cessation of spermatozoa production, only in one male of group 4 (animal # 401) had this condition. No histomorphologic differences involving the genital organs between control and treated monkeys were seen even though all females were considered adult.
Microscopic changes observed at the injection site consisted of a generalized pyogranulomatous cellulitis with numerous focal, randomly located, partially organized abscesses. The severity in control, low and mid dose was minimal to moderate but was marked in all high dose male and female monkeys. SC hemorrhage was reported in4 group 3 and 1 group 4 male monkeys, one control male and 2 control females, and one female each in groups 2 and 3.

Final sacrifice: Sponsor stated that spermatogenesis was reversible after stoppage of exposure to test article. This was based on the observation that one male (No 408) which was considered adult based on histologic examination had testes, which contained seminiferous tubules with spermatozoa, and some epididymal tubules contained viable sperm. However, review of appendix F showed that group 4 male No 401 killed at interim sacrifice had moderate testicular atrophy and was aspermic. On the other hand such condition was not described for male No. 408 recovery monkey. As with females sacrificed on Day 29, histomorphologic differences involving the genital organs between control and treated female recovery monkeys were not seen.
Histologic changes were not seen in the SC tissue at the site of administration in any of the 6 male recovery monkeys or in 3 control recovery monkeys. However, 3 high dose female monkeys revealed minimal to mild SC congestion and hemorrhage. The marked inflammatory reaction seen at day 29 was not seen in any recovery monkey of either sex.

Comments: As in the previous study which was aborted due to infection caused by the inserted cathetors, in this study also immature monkeys were used. As such no conclusion can be drawn from the data submitted if the fertility was returned after cessation of abarelix treatment or with treatment male monkeys did not attain adulthood. As for females, which were adult based on histologic examination, treatment showed no effect on genital organs weight or their histology. Plasma testosterone concentrations were not provided.

Based on the findings of this study NOAEL was established.

Toxicokinetics: Plasma PPI-149 PK parameters determined after SC administration of the first dose on Day 1 and on Day 28 is shown in table below:
Table 29

	----------Day 1 ----------				---------- Day 28 ----------			
Animal	201	211	401	411	201	211	401	411
Dose ug/kg	50	50	2500	2500	50	50	2500	2500
Cmax ng/ml	73	387	1111	498	88	457	4881	1273
Tmax h	1.5	1.5	6	4	1.5	1.5	1.5	0
AUC_{0-n} (ng/ml h)	193	1473	9770	3210	244	2645	38560	11151
T1/2 h	1.23	2.87	95.38	11.33	1.54	2.91	15.91	17.86
Cmax/dose (ug/kg)	1.45	7.74	0.44	0.20	1.76	9.14	1.95	0.51
AUC/dose								

(ug/kg)	3.85	29.47	3.91	1.28	4.87	52.90	15.42	4.46

Values for Cmax and AUC for Day 1 and Day 28 suggest that drug accumulates with time of administration.

Serum Histamine: Although individual values for histamine were not available, sponsor has provided mean values in the following table, which demonstrates that treatment with abarelix had no effect on histamine release.

Table 29 a

	Males Histamine (ng/ml)				Females Histamine (ng/ml)			
Dose	0 vehicle	100 ug/kg/day	1000 ug/kg/day	5000 ug/kg/day	0 vehicle	100 ug/kg/day	1000 ug/kg/day	5000 ug/kg/day
baseline	0.046	0.05	0.11	0.01	0.0025	0.002	0.004	0.11
Day 7	0.0025	0.006	0.002	0.01	0.00375	0.002	0.002	0.0025
Day 14	0.00125	0.004	0.008	0.0025	0	0.008	0.006	0.00875
Day 21	0.00625	0.006	0.004	0.005	0.0025	0.008	0.002	0.0025
Day 29	0.005	0.006	0.008	0.0075	0.00375	0	0.002	0.00625

Study Title: **13-week repeat dose toxicology study of PPI-149-Depot in CD-1 mice.**
Study No: PC073097

This study was designed to evaluate safety of high dose of PPI-149 Depot in preparation for a carcinogenicity study and a one week mouse micronucleus study.
Amendment #, Vol #, and page #: vol. 13 page 94
Conducting laboratory and location: Praecis Pharmaceuticals Inc. Cambridge, MA.TK conducted [redacted] Histopathology was conducted at [redacted]
Date of study initiation: 7-30-1997
GLP compliance: no statement made
QA- Report Yes () No (*)
Methods:
Dosing:

- species/strain: mice/CD-1
- #/sex/group or time point: 3/s/g dose with saline, CMC, 300 mg/kg PPI-149 and 1000 mg/kg PPI-149 for animals sacrificed on Day 8. 10/s/g for CMC, saline, PPI-149 30 mg, 100 mg or 300 mg injected on Days 1, 29, 57 and 85 and sacrificed after 13 weeks on October 30, 1997.
- age: 6 weeks
- weight: 20-25 g
- satellite groups used for toxicokinetics or recovery: TK was conducted on day 8 and week 13 samples
- dosage groups in administered units: dosing regimen is shown in table below for animals sacrificed on day 8 and after 13 weeks

Table 30
Dosing regimen for animals of the Day 8 sacrifice

Group	# of animals	Sex	Dose (mg/kg	Days dosed
F	3	M	CMC	1
G	3	M	0.9% saline	1
H	3	M	PPI-149-depot 300 mg/kg	1

J	3	M	PPI-149-depot 1000 mg/kg	1
P	3	F	CMC	1
Q	3	F	0.9% Saline	1
R	3	F	PPI-149-depot 300 mg/kg	1
S	3	F	PPI-149-depot 1000 mg/kg	1
Dosing regimen for animals of the 13-week sacrifice				
A	10	M	CMC	1,29,57,85
B	10	M	0.9% saline	As above
C	10	M	PPI-149-depot 30 mg/kg	As above
D	10	M	PPI-149-depot 100 mg/kg	As above
E	10	M	PPI-149-depot 300 mg/kg	As above
K	10	F	CMC	As above
L	10	F	0.9%saline	As above
M	10	F	PPI-149-depot 30 mg/kg	As above
N	10	F	PPI-149-depot 100 mg/kg	As above
O	10	F	PPI-149-depot 300 mg/kg	As above

Note: all doses refer to the amount of PPI-149 peptide administered

- route, form, volume, and infusion rate: SC (mid scapular region), depot every 28 days

Drug, lot#, radiolabel, and % purity: GM070797

Formulation/vehicle: Depot/CMC-saline

Observations and times:

- Clinical signs: twice daily for overt signs of toxicity, changes in behavior and appearance
- Body weights: recorded on days 1, 29, 57, 85 and 92
- Food consumption: not recorded
- Ophthalmoscopy: -none
- EKG: -none
- Hematology: hematology and CBC on day 8 and day 92
- Clinical chemistry: on days 8 and 92
- Urinalysis:-none
- Organ weights: at sacrifice
- Gross pathology: yes
- Organs weighed: yes
- Histopathology: yes
- Toxicokinetics: Blood was drawn on day 8 for CBC/chemistry/PPI-149, on days 15, 29, 64, 71, 85 for PPI-149 determination and on day 92 for CBC/chemistry/testosterone/PPI-149.

- Other: none

Results:

- Clinical signs: no clinical signs described
- Body weights: no treatment related effect in females but it was decreased by 10% in males.
- Food consumption: not recorded
- Ophthalmoscopy :-
- Electrocardiography:-
- Hematology: CBC was not affected by Day 8 but was significant changes were seen on day 92 as shown in table below:

Table 31

	CMC	Saline	30 mg/kg	100 mg/kg	300 mg/kg	CMC	Saline	30 mg/kg	100 mg/kg	300 mg/kg
	Males					Females				
Lymph x 10^3/ul	6.258	5.595	6.160	**9.635**	7.310	7.293	7.142	5.953	**10.835**	8.424
Eosin X 10^3/ul	0.545	0.433	0.528	0.984	**1.057**	0.663	0.697	0.583	0.625	**0.831**

Sponsor stated that upon scanning of stained blood smears, a low number of large, reactive lymphocytes were observed in 4 smears (3 in D group (100 mg/kg) and one in E group (300 mg/kg) animals. Sponsor further stated that there was no consistent correlation between occurrence of lymphocytosis in group D mice and these reactive lymphocytes. Significance of this finding is not explained.

Note: Review of table 10 of 11 on page 262 showed that in group N (female 100 mg/kg dose), 5/9 mice had elevated lymphocytes. It is not stated if these were also reactive lymphocytes.

- Clinical chemistry: no treatment-related effects. Serum transaminases not listed. Serum triglycerides were significantly decreased in animals treated with PPI-149 depot.
- Urinalysis:-none
- Organ Weights: no effect observed on Day 8 sacrifice but testes weight as well as liver and kidney weight were decreased when determined for 92 day sacrifice as shown in table below:

Table 32

	Male	Female	Male	female	Male	Female
Treatment	Kidney		Liver		Testes	Ovaries
CMC	0.663	0.400	2.501	1.669	0.231	0.027
Saline	0.650	0.393	2.134	1.551	0.249	0.028
30 mg/kg PPI-149	0.561	0.387	1.887	1.354	0.215	0.024
100 mg/kg PPI-149	0.436	0.345	1.675	1.269	**0.043**	**0.012**
300 mg/kg PPI-149	0.401	0.345	1.546	1.228	**0.025**	**0.009**

Values are mean of 10 animals

Gross pathology: -

- Histopathology: On Day 8 sacrifice, minimal atrophy of the prostate and seminal vesicles in males and minimal atrophy of ovaries in females was reported. Both males and females showed minimal (300 mg/kg) to moderate (1000 mg/kg) inflammation of injection site. Injection site of animals dose with CMC or saline also showed inflammation but less frequent and less severe. Based on local response doses of 30, 100 and 300 mg/kg were selected for mouse micronucleus assay.
- On 92 day sacrifice, males treated with 100 and 300 mg/kg showed moderate to severe testicular atrophy with aspermia accompanied by severe atrophy of the prostate, seminal vesicles and coagulation glands. In females with these doses, ovary size was reduced and fewer corpora lutea and graafian follicles were observed. 30

mg/kg dose was comparable to controls. Injection site inflammation was observed in male (100 and 300 mg/kg) and females with all doses levels.

-

- Toxicokinetics: TK data for the 7-28 days and 63-85 days is summarized in table below:

Table 33

	Combined males and females		Males		Females	
Day 7 – 28	AUC	AUC/dose mg/kg	AUC	AUC/dose mg/kg	AUC	AUC/dose mg/kg
100 mg/kg dose	1831	18.31	2120	21.20	1426	14.26
300 mg/kg dose	18549	61.83	22268	74.23	14830	49.43
Days 63-85						
100 mg.kg dose	4153	41.53	5274	52.74	3090	30.90
300 mg/kg dose	23150	77.17	27176	90.59	19124	63.75
	Cmax	Cmax/dose mg/kg	Cmax	Cmax/dose mg/kg	Cmax	Cmax/dose mg/kg
Day 7 – 28						
100 mg/kg dose	338	3.38	395	3.95	281	2.81
300 mg/kg dose	1851	6.17	2192	7.31	1510	5.03
Day 63-85						
100 mg/kg dose	821	8.21	1049	10.49	593	5.93
300 mg/kg dose	2212	7.37	1911	6.37	2514	8.38

With 30 mg/kg dose plasma concentration of 2.82 ng/ml was reported on Day 7, value could not be calculated or BLQ on Day 14 and 0.99 ng/ml on Day 28. Plasma concentrations on Day 7 for the 300 and 1000 mg/kg dose levels were —→and — ng/ml respectively. On Day 7 dose-normalized plasma PPI-149 concentrations (ng/ml/dose mg/kg) for the 100, 300 and 1000 mg/kg doses were respectively —— and —ng/ml.

Results thus demonstrated that dose-proportionality was not observed for plasma PPI-149 AUC or Cmax following 100 and 300 mg/kg doses of PPI-149 depot on day 7 – 28 and day 63 – 85. Day 7 PPI-149 plasma concentrations for the micronucleus study were proportional to dose following SC administration of 100 and 1000 mg/kg depot but not for the 300 mg/kg dose.

Key Study Findings: Inflammation at the injection site and atrophy of testes and ovaries were observed at dose of 100 and 300 mg/kg, expected pharmacological action of abarelix. A few large, reactive lymphocytes were observed in blood smear from 100 and 300 mg/kg groups.

Overall Toxicology Summary: No other significant toxic effects observed

Addendum list: none

Study Title: **6-month subcutaneous toxicity study of PPI-149 depot in Sprague-Dawley rats**
Study No: —— No. N0020591
Amendment #, Vol #, and page #: vol. 15 p. 1
Conducting laboratory and location: ————————
Date of study initiation: 5-5-1997
GLP compliance: yes

QA- Report Yes (*) No ()
Methods:
Dosing:

- species/strain: rat/Sprague-Dawley
- #/sex/group or time point: as shown in table below:

Table 33

Group #	# of core study animals		# TK and endocrine animals		Treatment	Dose level (mg/kg)	Dose conc mg/ml	Route of administra-Tion
	Males	Female	Male	Female				
1 control	35	35	0	0	Vehicle	0	0	SC
2	35	35	0	0	Excipient (CMC)	22	22	SC
3	35	35	0	0	Vehicle (castrate)	0	0	SC
4	25	25	12	12	PPI-149	10	10	SC
5	25	25	12	12	PPI-149	30	30	SC
6	35	35	12	12	PPI-149	100	100/50*	SC

* dose concentration was reduced to 50 mg/ml beginning Day 29 for males and Day 15 for females due to formulation difficulties.

PPI-149-Depot, excipient or vehicle (0.5% lecithin/5% mannitol) control was administered on Day 1, 15, 29, 57, 85, 113, and 141 to 6 groups of 25 rats/s/group at dose levels of 0, 10, 30 and 100 mg/kg. An additional 10 rats/s/group were administered PPI-149-Depot, CMC or vehicle (groups 1, 2, 3 and 6) for up to 6 months and following the sacrifice on day 169, were included in and 84 day non-treatment recovery period. Animals were surgically castrated on Day 1 and administered vehicle throughout the study. Surgically castrated male and female rats acted as positive controls for gonadal ablation. Five or 10 rats/sex/group from core group were euthanized on Day 29, 85 or 169. Doses were administered in the dorsoscapular region.

- age: 6 weeks
- weight: 199 to 293 for males and 162 to 226 for females
- satellite groups used for toxicokinetics or recovery: as indicated in table
- dosage groups in administered units: as shown in table
- route, form, volume, and infusion rate: as shown in table

Drug, lot#, radiolabel, and % purity: Lot 050597 and Lot 070797
Formulation/vehicle: Depot (70-85% peptide, 12-22% CMC and water less than 10%)
Observations and times:

- Clinical signs: once daily for signs of toxicity, twice daily for mortality and morbidity
- Body weights: at randomization, prior to dosing on Day1 and weekly thereafter
  Food consumption: weekly
- Ophthalmoscopy: prior to dosing and at termination
- EKG: not conducted
- Hematology and coagulation: at termination on Day 39, 85, 169 and 253
- Clinical chemistry: as for hematology
- Urinalysis: not conducted
- Organ weights: at necropsy
- Gross pathology: at necropsy

- Organs weighed: heart, lungs, liver, spleen, kidneys, adrenals, brain, testes. epididymides, prostate, and ovaries with oviduct from all rats euthanized each scheduled necropsy.
- Histopathology: All collected tissues for control and high dose groups. Target organs (testis, epididymis, prostate, seminal vesicles, ovaries, uteri, vaginas, mammary glands, injection site, bone marrow except for Day 29), and adrenals (except for Day 29) from low and mid dose groups.
- Toxicokinetics: Blood was collected from 3 rats/sex/group in groups 4, 5 and 6 at various time intervals from Day 1 (predose) to Day 169. Samples were analyzed by [redacted] Testosterone was determined on samples collected on Days 183, 197, 211, 225, 239, and 253.
- Other: Analysis of variance and t test was used

Note: Sponsor has stated that retrospective analysis of archival samples of the formulated test articles showed that for the male rats analytical recoveries ranged from 5% of target to 440% of target while recoveries for female doses ranged from 16% of target to 856% of target. It was suggested that influences for this retrospective analysis include frozen storage and possible evaporation.

This would suggested that the intended dosage of PPI-149-Depot of 0, 10, 30, and 100 mg/kg at various times may be much below in some cases and much greater than the intended dose in others. Also if such variations also occur in the serum drug levels determination, where samples are normally frozen and assayed together at the end of the study, the values will be unreliable.

Results:

Survival: Four rats died and all belong to TK groups. One mid dose male died on Day 6 with possible urinary obstruction. One low dose female died on Day 39, which had caudate liver mass and cause of death determined to be ruptured liver. Two high dose males died on Day 253 of the study and deaths attributed to anesthesia.

- Clinical signs: treatment-related clinical signs were associated with the reproductive system and the injection site. Testicular atrophy was first noted on Day 12 of PPI-149 administration. SC tissue mass/nodule was noted in 4 high dose males and 20 high dose females. The mean first day observed for nodule was Day 130 for females and Day 132 for females. Only group 6 females showed mass/nodule during the recovery period.
- Clinical findings observed in all dose groups including controls included abrasions/lesions on the dorsal body, ventral body, head, neck, and shoulder regions, occasional alopecia and reddened and swollen ears. It is not clear as to why lesions occurred at various places when doses were administered in the dorsoscapular region, suggesting possibly a systemic effect.
- Other treatment related findings were in one group 5 male that was lethargic and unresponsive on Day 6 and cause of his death was attributed to urinary obstruction. Five TK animals, 2 group 4 males, 2 group 5 males and 1 group 5 female were observed with head tilt until termination of the study, Day 253. Because there were no gross necropsy findings, sponsor considered it not related to PPI-149 Depot. Eight TK

animals had mydriasis during the recovery phase and presumed to be related to retro-orbital blood sampling. Group 3 animals, both males and females (castrated on Day 1) had unusually high incidence of corneal opacities, attributed to exposure during surgery and lack of normal lacrimation. Although not pointed out by the sponsor, head tilt was probably due to viral middle ear infection.

- Body weights: Beginning Day 8, mean body weight of PPI-149 Depot treated animals in groups 4, 5 and 6 was significantly decreased from control animals during dosing phase and continued during recovery period. It was similar for all groups regardless of the dose level. Also similar decrease was observed in castrated rats.
-
- In females on the other hand, body weight was significantly increased beginning on Day 8 in groups 4 and 5 and on Day 15 for group 6 without any dose-response relationship. Similar weight changes were seen in surgically castrated animals.
- Food consumption: Food consumption in the PPI-149 Depot treated males was significantly decreased as compared to vehicle control animals from Day 15 through the end of the study including recovery period in group 6. In treated females, it was significantly increased beginning on Day 8 in groups 4 and 5 and Day 22 in group 6 compared to the vehicle treated group 1 females up to Day 84. From Day 92, food consumption in treated females was comparable to vehicle treated females. Food consumption of castrated male and females were affected as the PPI-149 Depot treated animals.

- Ophthalmoscopy: Commonly observed finding was occurrence of corneal opacities and corneal crystals in most males and females in treated as well as in control animals. The occurrence was unusually high in the castrated rats.

- Electrocardiography: none
- Hematology: Significant treatment-related changes when compared to vehicle controls in male rats were:
- Increased mean corpuscular volume on Day 85 and 253 for high dose group (55.5 vs 57.4 and 55.3 vs 58.0 fL); mean corpuscular Hb on Day 85 for mid and high dose groups (18.7, 18.8 vs 18.0 pg for control) and on Day 169 for high dose group (18.5 vs 19.4 pg), and decreased platelet count for low dose group on Day 169 (1089 vs 830 x 10^3/ul).
- In females RBC count was increased in groups 3 and 6 on Day 253, reticulocytes increased in group 3 on Day 85, Hb and Hct increased in groups 3 and 6 on Day 253, mean corpuscular volume decreased in mid and high dose groups on Day 169 and platelet count decreased in mid dose group on Day 85.
- Differential white blood cell count: in males, lymphocytes were significantly increased in group 3 on Day 85 and eosinophils decreased in group 2 on Day 29.
-
- Significant changes reported in female rats are shown in table below:

Table 34 -

	Study day	Vehicle	22 mg/kg CMC	0 mg/kg castrate	10 mg/kg PPI-149	30 mg/kg PPI-149	100 mg/kg PPI-149
WBC (10^3/ul)	85	4.5	6.3	9.0*	11.3*	9.7*	8.7*
Segmented neutrophils (10^3/ul)	85	0.54	0.79*	1.28*	1.31*	0.98	1.28*
Lymphocytes 10^3/ul	85	3.93	5.32	7.50*	9.66*	8.49*	7.11*
Eosinophils 10^3/ul	85	0.06	0.09	0.15*	0.15*	0.12	0.14*
	253	0.12	0.13	0.18*	–	–	0.16*

Prothrombin time was increased in group 5 and 6 males compared to vehicle control (14.9 and 14.8 vs 14.1 sec) on Day 85. Prothrombin time was also increased in female groups 3, 4, 5 and 6 when compared to vehicle controls (15.1, 14.9, 15.1 and 15.2 for treated vs 13.7 sec for controls). It was also significantly higher on Day 253.

Clinical chemistry: significant treatment-related changes compared to vehicle control in males consisted of the following:
Alkaline phosphatase was decreased in group 6 (69 vs 90 IU/L) on Day 85
Aspartate aminotransferase was increased in groups 3 and 6 (91 and 103 vs 77 IU/L) on Day169
Alanine aminotransferase was increased in low dose group 4 (47 vs 40 IU/L) on Day 85
Other changes were increased glucose in high dose on Day 85, increased creatinine in low dose on Day 29. TG was decreased and cholesterol increased.

In females significant enzyme changes are shown in table below:
Table 35

	Study day	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Alkaline phosphatase	29	163	165	178	198	243*	176
	85	46	47	61	64*	78*	75*
	169	37	34	61*	73*	77*	72*
	253	42	42	82*	-	-	86*
Alanine aminotrans -ferase	29	46	50	52	55	56	60*
Creatine kinase	253	138	152	260*	-	-	155

Other changes seen at different times were increased glucose, decreased TG and increased cholesterol, decreased total protein and albumin and increased globulin with decreased A/G ratios. Calcium was decreased, sodium, potassium and chloride increased.

- Urinalysis: none
- Organ Weights: Consistent treatment-related changes in organ weights expressed as percent of body weight consisted of decrease in testis, epididymides and prostate in males and ovaries in females in all PPI-149 depot treated groups at all times sacrifice. Also liver, kidney, adrenal gland, thyroid, pituitary and brain relative weights were decreased in castrated and drug treated females on 3 and 6 month sacrifice. These decreases seem to be reflection of increased body weight. All other changes were not observed in all groups and at all times.

Gross pathology: At one month sacrifice 5/5 males in group 3, 4, 5 and 6 had small testis, epididymis, prostate and seminal vesicles. In females all five had small uterus and 2/5 in low dose and 1/5 in high dose had small ovaries. No injection site nodules were reported in groups 1,2 and 3 males or females, However, 1, 4 and 3 males and 1, 4, and 2 females in groups 4, 5 and 6 had nodules.
At 3 month sacrifice all 10 males and females in groups 4, 5 and 6 had small genital organs. Injection site nodules were in 2 and 7 males and in 4 and 10 females of groups 5 and 6.
At 6 month sacrifice all 10 males and female/g had small genital organs. Injection site nodules were reported in 5, 10 and 10 males and 5, 10 and 10 females of groups 4, 5 and 6 respectively.
At recovery sacrifice all 10 male and females had small genital organs and had injection site nodules. One male and one female had spleen cysts.

- Histopathology: PPI-149 depot treatment-related findings in rats sacrificed on Days 29, 85, 169 and 253 were found in the testis, epididymis, prostate, seminal vesicle, ovary, uterus, vaginal, mammary gland, injection site, bone marrow and adrenals at all dose levels.
-
- Changes in testis consisted of spermatogenic arrest and interstitial cell atrophy. Fewer spermatogonia and spermatocytes were observed on Day 85 than in rats sacrificed on Day 29. Epididymal atrophy was characterized by a decreased tubular diameter and aspermia. Prostate and seminal vesicle were atrophic and lacked acinar development.
-
- In females there was atrophy of the ovaries with absence of corpora lutea, increased number of atresia of preovulation follicles and an absence of mature graafian follicles.
- There was an increase in nuclear density of interstitial cells in the ovaries of all PPI-149 treated rats and it was suggested to be due to condensation of interstitial cell nuclei as the result of cytoplasmic atrophy and not hyperplasia of these cells.
- Atrophy of the uterus was characterized by a decrease in diameter of the uterine horn accompanied by atrophy of uterine glands. Vaginal mucosal atrophy associated with suppurative exudate was seen at all sacrifices including after the recovery period.
-
- Mammary glands of all males and females of PPI-149 treated rats as well as of castrated rats were atrophied.
-
- The prostate, seminal vesicles, uterus, vagina and mammary gland of castrated rats were affected to similar degree as PPI-149 Depot treated rats.
-
- Hypertrophic/hyperplasia of gonadotrophs was observed only in the pituitary glands of castrated male and female rats. Granulomatous inflammation of the administration site was observed in almost all animals treated with PPI-149 depot examined on Day 29, 85, 169 and 253. Mean severity grade based on size and number of foci, was greater in 30 and 100 mg/kg dose groups than in the 10 mg/kg group.
- Adipose tissue was increased in the bone marrow of the femur and sternum of castrated male and female rats and in all PPI-149 treated rats sacrificed at Day 85, 169 and 253. This was attributed to aging as it was not observed at Day 29.
-
- In the adrenal glands, degenerative/atrophy of the zona reticularis near the junction with zona fasiculata was observed in all PPI-149 treated and castrated rats and only in one rat from excipient group.
-
- All histological changes observed on Day 169 were also present at Day 253, suggesting that recovery period was not sufficient and was consistent with the lack of recovery of plasma testosterone and estrogen.
-

Toxicokinetics: Sponsor stated that PK analysis was initially analyzed by _____ The method was not validated and data was not technically sound. Residual samples were analyzed at _____ analysis showed that the concentration of PPI-149 in rat plasma samples ranged from below the limit of quantitation _____ ng/ml. Results are shown under TK subtitle in Table on page .

Results of testosterone determination showed that testosterone levels in male rats were suppressed for 42 days in the 10 mg/kg dose group and greater than 112 days in the 30 and 100 mg/kg dose groups following discontinuation of treatment with PPI-149 Depot. In females treated with 10, 30 and 100 mg/kg PPI-149 Depot, estradiol levels were low (<20 pg/ml) or suppressed (<10 pg/ml) up to 42 days after discontinuation of treatment and recovery appeared dose-dependent.

Key Study Findings: Increased GPT and SOT levels, which were not dose-dependent and not observed at all time points. Degenerative/atrophy of the zona reticularis in all PPI-149- treated and castrated rats.

Overall Toxicology Summary: All observations could be attributed to pharmacological action of abarelix.

Addendum list: none

Study Title: **12-month chronic subcutaneous toxicity study of PPI-149-Depot in cynomolgus monkeys.**
Study No: —— study No. N002059L
Amendment #, Vol #, and page #: vol. 20, p. 1
Conducting laboratory and location: ————————
Date of study initiation: 9-3-1997
GLP compliance: yes
QA- Report Yes (*) No ()
Methods:
Dosing:

- species/strain: monkey/cynomolgus
- #/sex/group or time point: as shown in experimental design table below:

Table 36 Experimental design

Group #	Number of animals				Treatment	Dose level mg/kg	Route and duration
	Month 4 sacrifice	Month 7 sacrifice	Month 13 sacrifice	Recovery			
1 (control)	3/sex	3/sex	4/sex	2/sex	Vehicle	0	SC; every 4 weeks
2	3/sex	3/sex	4/sex	–	PPI-149-depot	5	SC, every 4 weeks
3	3/sex	3/sex	4/sex	–	PPI-149-depot	15	SC, every 4 weeks
4	3/sex	3/sex	4/sex	2/sex	PPI-149-depot	40	SC, every 4 weeks

- age: not known
- weight: 2.2 to 3.6 kg for males and 1.9 to 2.9 kg for females
- satellite groups used for toxicokinetics or recovery: yes as shown in table
- dosage groups in administered units: as shown in table
- route, form, volume, and infusion rate: SC, depot, 1.0, 0.125, 0.375 and 1.0 ml/kg/dose. Dose for group 1 and 4 divided into 2 sites.

Drug, lot#, radiolabel, and % purity: lots 082797, 112697 and 030498. Peptide content 70-85%. 98% pure
Formulation/vehicle: depot/saline
Observations and times:

- Clinical signs:daily
- Body weights: 4 days prior to initiation and then weekly
- Food consumption: noted with clinical observations
- Ophthalmoscopy: prior to initiation and at scheduled termination
- EKG: EKG and blood pressure measurement conducted prior to initiation and then at
- scheduled sacrifice
- Hematology: 2 weeks prior to initiation and at scheduled sacrifice. Also coagulation
- parameters
- Clinical chemistry: same as for hematology
- Urinalysis: none
- Organ weights: at necropsy at 4, 7 and 12 month of test article or vehicle administration and then 4 months of recovery. Organ weighed were adrenal glands, brain, epididymides, heart, kidneys, liver, lungs, ovaries with oviducts, pituitary, thyroid, prostate, spleen, testes, and thymus.

- Histopathology: for all animals. Tissues examined listed in histopathology table.

Toxicokinetics: plasma drug levels determined prior to dosing and on Days 2, 3, 7, 14, 21 and 28 following first dose administration on Day 1 and then prior to Day 226 and on Days 338, 339, 343, 350, 357 and 364 after the last dose administration on Day 337.

- Other: plasma testosterone and estradiol determined prior to treatment and weekly during the recovery period. Dose formulation samples were analyzed retrospectively.

Results:

Survival: All monkeys survived until their scheduled sacrifice

- Clinical signs: SC swelling (nodules) at the injection site noted in groups 2, 3 and 4 males and females which appeared within 2 days following depot administration. Nodules persisted throughout the treatment period with erythema and thickening. Eventually nodules erupted in 4 mid dose and 4 high dose females and 2 low dose, one mid dose and 4 high dose males. Other clinical findings consisted of diarrhea, soft feces, salivation, abrasions, alopecia, red vaginal discharge and 2 incidences of emesis in one mid dose male. All these occurred during treatment and recovery period and were not considered treatment-related.
- Body weights: As shown in table below, mean weight changes were both increased and decreased intermittently throughout the treatment and recovery period for male and female monkeys in all dose groups compared to the vehicle control. High dose females during the recovery phase had lower body weight compared to controls for 14 of the final 21 weeks and considered not treatment-related

Table 37

	Day 85		Day 169		Day 365		Day 449	
Treatment #	Male	Female	male	Female	Male	Female	male	Female
O mg/kg	3.18	2.41	3.38	2.53	3.81	2.98	4.98	3.56
5 mg/kg	2.55	2.15	3.02	2.50	3.61	2.88	–	–
15 mg/kg	2.70	2.34	3.14	2.53	3.57	2.92	–	–

40 mg/kg	2.42*	2.44	3.27	2.46	3.68	2.73	3.90	2.79*

- Food consumption: There was not treatment effect.
- Ophthalmoscopy: there were no treatment-related effects
- Electrocardiography: It was stated that all ECGs showed sinus rhythms, some with mild tachycardias. Frequent alterations in configurations of component deflections and in orientations of QRS and ST-T vectors when compared to baseline to terminal recordings were observed but these appeared as frequently in monkeys that served as vehicle controls. Heart rates were higher than baseline post-dosing in many monkeys in all groups during the entire course of the study.
- During recovery high dose group had a significantly greater mean arterial pressure (MAP) at 4-week recovery.
- Significant alterations in ST-T appeared in high dose monkeys 407 (13month sacrifice) and 422 (4 month sacrifice) at their terminal examinations. It was suggested that these changes can be consistent with ventricular myocardial hypoxia/injury, however, the occurrence was so infrequent that may be of neither physiological nor toxicological significance. ECG tracings were not submitted. During recovery
- males in the high dose group had significantly greater mean arterial pressure and both males and females had higher heart rate.
- No statistically significant treatment-related changes were reported in mean systolic or diastolic pressures. Heart rate was higher in low dose male and female groups combined during week 52 and in high dose females alone and combined with males during the recovery period.

- Hematology: There were not consistent treatment-related effects. Statistically significant changes consisted of increased reticulocyte counts in mid dose males on Day 85, decreased platelet count in mid dose females on Day 169, basophils in low dose males and segmented neutrophils in high dose females were increased on Day 169, lymphocytes were increased in high dose males on Day 449 and hematocrits were increased in mid and high dose females on Day 169. All these changes were stated to be within historical ranges and with absence of dose-response were considered of no biological relevance. Increased segmented neutrophils may indicate inflammation due to nodules at the injection site. Treatment had no effect on prothrombin time or activated partial thromboplastin time.

- Clinical chemistry: There was no consistent PPI-149 depot- related effect on any chemistry value. Significant increase in mid and high dose males blood urea nitrogen and significant decrease in low and mid dose male globulin was observed on Day 85. Calcium values for mid and high dose males were significantly decreased relative to control animals on Day 169.
-
- In low and high dose females, glucose was increased on Day 85 and in low dose on Day 169. Creatine values for low and high dose females were increased and TG for low dose females decreased relative to controls on Day 85.
- At the end of treatment on Day 365, alkaline phosphatase was decreased in low and high dose males, Ca and Na were increased in mid and high dose males and chloride increased in high dose males.
- In mid dose females on Day 365, alkaline phosphatase was increased while globulin was decreased in low and high dose groups.

- Since none of the changes were dose-related and differences were of small magnitude, these were not considered biologically relevant.
- Urinalysis: none conducted
- Organ Weights: expressed as organ-to-body weight ratios, no changes were observed at interim sacrifice on Day 85 in males or females. At interim sacrifice on Day 169, prostate was decreased in mid dose males and lung weight was increased in mid dose females. At interim sacrifice on Day 365, heart weight was higher in all treated groups (0.35, 0.44*, 0.43* and 0.43 for the control and treated groups). Also ovary weight was decreased in low dose, and spleen and thymus increased in mid dose group. At necropsy Day 449, epididymis and prostate weights were lower and spleen higher in high dose males and thymus weight higher in high dose females.

Gross pathology: Treatment-related gross lesions consisted of SC nodules at the injection site and small ovaries, uteri, testes, epididymides, prostate and seminal vesicles. Nodules were observed at all doses levels at all time points. The number of nodules at 13-week sacrifice was not different in the low and mid dose but was higher in the high dose male and females. For males total number of nodules for group were 0, 30, 37 and 67 and for female these were 0, 27, 25 and 53 for respective controls and treated groups. The decreased size of genital organs was not associated with significant decrease in organ weights. At necropsy after recovery, there were still 34 nodules in males and 24 in females.

- Histopathology: At months 4 and 7 sacrifices, findings attributed to treatment included foci of granulomatous inflammation at the injection sites, ovarian atrophy, atrophy of the vaginal epithelium and atrophy/attenuated glandular development of mammary glands. For the male sex and accessory sex organs, treatment effect was suggested. However, sponsor stated that because of immaturity in some control animals, treatment effect could not be determined with assurance at this stage.
- At month 13 sacrifice, findings attributed to treatment in females were similar as described for 4 and 7 months sacrifice. However, in males there was arrested development of the testes, prostates and seminal vesicles and hypospermia/aspermia in the epididymides. Nodules were determined to be foci of granulomatous inflammation.
- At recovery sacrifice, findings attributed to treatment were similar as described under 13 month sacrifice in both males and females though the severity was decreased.

The incidence and severity of all treatment-related findings is shown in table below:

Table 38 **4 month sacrifice**

	Males				Females			
Dose (mg/kg)	0	5	15	40	0	5	15	40
Number examined & necropsied	3	3	3	3	3	3	3	3
Injection site								
Inflammation-granulomatous	0	3[a] (1.7)[b]	3 (3.0)	3 (3.7)	0	3 (2.0)	3 (2.7)	3 (3.7)
Hemmorhage	1 (0.3)	2 (1.0)	1 (0.3)	1 (0.3)	0	2 (1.0)	3 (2.0)	2 (0.7)
Ovary								
Atrophy					0	2 (1.0)	3 (2.0)	3 (2.0)
Atretic preovulatory follicles					3 (2.3)	3 (1.3)	3 (1.7)	3 (1.0)
Scarred attretic follicles					3 (2.3)	3 (3.0)	3 (3.0)	3 (4.0)
Corpora lutea					-	-	-	-
Mineralization					1 (0.3)	1 (0.7)	1 (0.7)	2 (2.7)
Oviduct					0	0	2 (0.7)	0
Atrophy								
Uterus								
Glandular development					3 (3.0)	3 (1.0)	3 (1.0)	3 (1.0)
Pigment					3 (1.3)	1 (0.3)	1 (0.3)	1 (0.3)

Vagina								
Atrophy					0	3 (1.0)	3 (2.0)	3 (2.0)
Mammary gland								
Atrophy/attenuated glandular development	0	0	0	0	3 (3.0)	3 (3.0)	3 (3.0)	3 (3.0)
Testes								
Immature/arrested development	2	3	3	3				
Epididymis								
Asprmia, immature/arrested development	2	3	3	3				
Prostate								
Immature/arrested development	2	3	3	3				
Seminal vesicle								
Immature/arrested development	2	3	3	3				

7 month sacrifice

	Males				Females			
Dose (mg/kg)	0	5	15	40	0	5	15	40
Number examined & necropsied	3	3	3	3	3	3	3	3
Injection site								
Inflammation-granulomatous	0	3[a] (2.3)b	3 (4.0)	3 (4.0)	0	3 (2.7)	3 (4.0)	3 (3.3)
Hemmorhage	0	0	3 (1.3)	3 (1.3)	0	3 (1.0)	2 (0.7)	3 (1.0)
Ovary								
Atrophy					(0.7)	3 (1.3)	3 (2.0)	3 (1.7)
Atretic preovulatory follicles					3 (2.0)	1 (0.7)	3 (2.0)	3 (1.7)
Scarred attretic follicles					3 (1.7)	3 (2.7)	3 (3.3)	3 (3.3)
Corpora lutea					2	0	0	0
Mineralization					3 (2.0)	0	1 (0.3)	0
Oviduct								
Atrophy					0	1 (0.3)	2 (0.7)	0
Uterus								
Glandular development					3 (3.3)	3 (1.3)	3 (2.0)	3 (1.3)
Pigment					0	1 (0.3)	2 (0.7)	3 (2.7)
Vagina								
Atrophy					0	2 (2.0)	2 (1.7)	3 (2.7)
Mammary gland								
Atrophy/attenuated glandular development	0	0	0	0	1 (1.0)	3 (3.0)	3 (3.0)	2 (1.7)
Testes								
Immature/arrested development	1	3	3	3				
Prepubescent	1	0	0	0				
Epididymis								
Asprmia, immature/arrested development	2	3	3	3				
Prostate								
Immature/arrested development	1	3	3	3				
Prepubesent	1	0	0	0				
Seminal vesicle								
Immature/arrested development	1	3	3	3				
Prepubescent	1	0	0	0				
Femur								
Adipose tissue increased, bone marrow	0	2 (0.7)	0	2 (0.7)	1 (0.3)	2 (0.7)	1 (0.3)	2 (0.7)
Sternum								
Adipose tissue increased, bone marrow	1 (0.3)	0	1 (0.3)	2 (0.7)	1 (0.3)	1 (0.30	1 (0.3)	2 (0.7)

13 month sacrifice

	Males				Females			
Dose (mg/kg)	0	5	15	40	0	5	15	40
Number examined & necropsied	4	4	4	4	4	4	4	4
Injection site								
Inflammation-granulomatous	0	4[a] (3.3)[b]	4 (4.0)	4 (4.0)	0	4 (3.3)	4 (3.0	4 (4.0)
Hemmorhage	0	4 (3.3)	1 (0.3)	1 (0.5)	0	0	2 (0.5)	4 (1.3)

Ovary								
Atrophy					0	4 (1.8)	4 (2.5)	4 (3.0)
Atretic preovulatory follicles					3 (1.3)	4 (1.5)	4 (1.5)	3 (1.3)
Scarred attretic follicles					4 (1.8)	4 (2.3)	4 (3.0)	4 (3.3)
Corpora lutea					4	0	0	0
Mineralization					0	2 (1.3)	0	2 (1.0)
Oviduct								
Atrophy					0	1 (0.3)	0	1 (0.5)
Uterus								
Glandular development					4 (4.0)	4 (1.8)	4 (2.0)	4 (1.3)
Pigment					0	1 (0.3)	1 (0.5)	2 (0.5)
Vagina								
Atrophy					0	4 (3.0)	3 (2.0)	4 (3.0)
Mammary gland								
Atrophy/attenuated glandular development	0	0	0	0	0	4 (2.8)	4 (2.3)	4 (2.5)
Testes								
Immature/arrested development	0	3	4	4				
Prepubescent	3	1	0	0				
Epididymis								
Asprmia, immature/arrested development	2	3	4	4				
Hypospermia	1 (1.0)	1 (0.8)	0	0				
Prostate								
Immature/arrested development	0	3	4	4				
Prepubescent	3	1	0	0				
Seminal vesicle								
Immature/arrested development	0	3	4	4				
Prepubescent	3	1	0	0				
Femur								
Adipose tissue increased, bone marrow	0	1 (0.3)	0	2 (0.5)	0	0	1 (0.3)	2 (0.5)
Sternum								
Adipose tissue increased, bone marrow	0	1 (0.3)	2 (0.5)	2 (0.5)	0	0	1 (0.3)	1 (0.3)

Recovery sacrifice

	Males				Females			
Dose (mg/kg)	0	5	15	40	0	5	15	40
Number examined & necropsied	2	0	0	2	2	0	0	2
Injection site								
Inflammation-granulomatous	0	-	-	2[a] (3.0)[b]	0	-	-	2 (3.0)
Hemmorhage	0	-	-	0	0	-	-	1 (0.5)
Ovary								
Atrophy					0	-	-	2 (2.5)
Atretic preovulatory follicles					2 (2.0)	-	-	2 (1.5)
Scarred attretic follicles					2 (1.5)	-	-	2 (2.5)
Corpora lutea					2	-	-	0
Mineralization					1 (.5)	-	-	2 (2.0)
Oviduct								
Atrophy					0	-	-	0
Uterus								
Glandular development					2 (4.0)	-	-	2 (3.0)
Pigment					0	-	-	0
Vagina								
Atrophy					0	-	-	0
Mammary gland								
Atrophy/attenuated glandular development	0	-	-	0				
Testes								
Immature/arrested development	0	-	-	2				
Prepubescent	1	-	-	0				
Epididymis								
Asprmia, immature/arrested development	0	-	-	2				
Hypospermia	1 (1.0)	-	-	0				
Prostate								
Immature/arrested development	0	-	-	2				
Prepubescent	1	-	-	0				
Seminal vesicle								
Immature/arrested development	0	-	-	2				
Prepubescent	1	-	-	0				

Femur Adipose tissue increase, bone marrow	0	-	-	0	0	-	-	0
Sternum Adipose tissue increased, bone marrow	0	-	-	0	0	-	-	0

[a] number of animals in the group affects

[b] Average severity score of lesions in parenthesis, 0-4 scale wit 0= normal and 4= marked lesion. Average severity sum of individual severity grades in a group divided by the number of organs examined.

Thus the decreased size of the ovary was attributed to decreases in developing and/or atretic follicles and the absence of current or previous cycle corpora lutea. Statement was made that it is not clear whether the decreased size of the ovaries from treated monkeys was due to degeneration of the follicular development or was more of a hypoplasia.

Atrophy of the oviducts in one low dose at 7 month necropsy and 2 mid dose monkeys at 4 and 7 month necropsies was considered as possible treatment effect. Lack of uterine glandular development was suggested to be indicative of hormonal stimulation. Vaginal epithelial atrophy was also treatment related. It was not certain if the state of mammary gland development failure to develop or possibly immaturity. Sponsor stated that the lack of uniform ages for all monkeys complicates the interpretation of the observed morpholgies in male monkeys. The occurrence of increased adipose tissue in bone was not consistent.
Corpora lutea were absent from all control monkeys at 4 months, present in 2/3 control monkeys at 7 months and present in all control monkeys at 13 months and recovery necropsies. None of the treated monkeys had corpora lutea. The absence of corpora lutea in the recovery monkeys indicated that cycling had not returned to normal during the recovery period.

Sponsor concluded " it appears likely that PPI-149-Depot causes an effect on the male sex organs but the precise mechanism of action for any single monkey could not be determined without knowledge of the state of sexual maturity at the beginning of dosing. The absence of mature sex organs and accessory sex organs in recovery monkeys would indicate that 3 months is insufficient time for recovery or that there is some permanent arrest of maturation of the organ. Alternatively, the presence of nodules at injection sites may indicate that PPI-149-Depot may persist in the body".

-
- Toxicokinetics: PK data is included under Pharmacokinetics/toxicokinetics section.
- Plasma testosterone and estradiol: While mean testosterone levels of the control monkeys increased over the course of the study, testosterone levels decreased in the mid and high dose groups over the course of evaluation (365 for mid dose treated monkeys and 448 days for high dose treated monkeys). Mean testosterone levels in low dose male monkeys remained consistently low throughout the evaluation period (354 days). Thus it was unclear low testosterone levels were due to monkeys being immature or PPI-149 suppressed testicular development. In females in all groups plasma estradiol levels were mostly below the limit of quantification i.e. [redacted]pg/ml throughout the study period. While in increased in the control monkeys during the course of the study through Day 364 (10/117 determinations above 50 pg/ml), it remained low in all other groups as well as in controls during the recovery period.
- Whether low plasma estradiol was due to immaturity of monkeys or PPI-149 Depot suppressed estradiol is not clear.
-

- **Note:** Where analysis of formulations in the 6 months toxicity study showed that it varied from 5 to 850% of the target concentration, in this study formulation contained approximately correct concentrations of PPI-149.

Key Study Findings: Reaction at the injection site. Significant increase in blood urea nitrogen in mid and high dose males. Other changes included atrophy of sex hormone-dependent organs.

It was stated that all ECGs showed sinus rhythms, some with mild tachycardia.
Significant alterations in ST-T appeared in 1/3 (3-month sacrifice) and 1/4 high dose (13-month sacrifice) monkeys. It was suggested that these changes could be consistent with ventricular hypoxia/injury. Sponsor however, suggested these to be of neither physiological nor toxicological significance since these were infrequent. Three months was insufficient time for recovery.

Overall Toxicology Summary: Except for the above stated ECG changes, there were no significant treatment-related adverse findings.

Addendum list: none

Title: **Single-dose intramuscular/subcutaneous local tolerance study with PPI-149-Depot in rabbits.** Study No. —— N002059H. vol. 21 p. 311

This study was conducted in accordance with GLP regulations.

Experimental design: Thirty male NZW rabbits were assigned to one of two treatment groups a shown in table below:

Table 39

Group	# of animals	Route	Treatment
1	15	IM	2 mg/kg PPI-149 depot
			0 mg/kg (vehicle 0.5% lecithin/5% mannitol)
		SC	2 mg/kg PPI-140 depot
			0 mg/kg (vehicle)
2	15	IM	0.1 mg/kg Lupron
			0 mg/kg (vehicle, 0.9% saline)
		SC	0.1 mg/kg Lupron
			0 mg/kg vehicle

Four injections were administered at 4 distinct sites. Dose delivery volume was 0.3 ml/kg. The concentration of PPI-149 was 6.66 mg/ml and that of Lupron 0.33 mg/ml.

Three rabbits/group were sacrificed on Days 3, 8, 15, 29 and 57. Daily modified Draize evaluations and clinical observations and weekly body weights were recorded. At necropsy gross examination of the injection sites was made and sites processed for histological examination.

Results: treatment had no food consumption. Body weight was similar for PPI-149 and Lupron treated groups.
Treatment-related findings at the IM and SC injection sites consisted of mild hemorrhage, inflammation and myofiber degeneration/necrosis and vacuole formation. Histopathological examination revealed no difference between 4 treatment groups in the incidence and severity of myofiber degeneration/necrosis. Granulomatous inflammation occurred in both the IM and SC

sites with PPI-149 depot and Lupron but not with their respective vehicles. 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 period with PPI-149 depot (up to final necropsy at Day 57) than Lupron (up to Day 29). With Lupron treatment granulomatous inflammation was associated with vacuolization characteristic of lipid accumulation in macrophages.

Hemorrhage and mononuclear cell inflammation were reported to be slightly higher in PPI-149 and its vehicle SC administration than with Lupron and it vehicle administration. Hemorrhage following IM administration was observed in 3/15 PPI-149 depot and 1/15 in Lupron group, but not in either control site. Group mean daily Draize score for both PPI-149 depot and Lupron was less than 1. The ratio of treated muscle weight vs vehicle control muscle weight was comparable for PPI-149 depot and Lupron treated animals. Results thus showed that granulomatous inflammation was observed longer than nyofiber degeneration/necrosis and tended to persist longer with PPI-149_Depot than Lupron.
Sponsor concluded that neither PPI-149-Depot nor Lupron produced lesions of notable significance.

Reproductive toxicology

Study Title: **Subcutaneous fertility of PPI-14-Depot in female rats. Final report**
Study No: Protocol # 1316-003
Amendment #, Vol #, and page #: NDA review/ vol.23, p 1
Conducting laboratory and location:
Date of study initiation: not mentioned. Protocol was signed on 11-20-1997
GLP compliance: yes
QA- Report Yes (*) No ()
Methods:
Dosing:

- species/strain: rat/Crl:CD VAF/Plus (Sprague-Dawley)
- #/sex/group or time point: 25 females/group as shown in table below. Male rats of the same source and strain were used only as breeders.

Table 40

Dosage Group	Dosage mg/kg	Concentration Mg/ml	Volume ml/kg
1	0	0	0.3
2	0.3	1	0.3
3	1	3.3	0.3
4	3	10	0.3
5	10	33.3	0.3

Doses levels of 0.3, 1.0, 3.0 and 10.0 mg/kg are equivalent to 1.8, 6.0, 18.0 and 60.0 mg/m^2 and are 0.034, 0.114, 0.340 and 1.135, respectively multiples of the human therapeutic dose of 100 mg/70 kg person.

Dose selection was based on results of sponsor's earlier studies, however no data was presented.

- age: females 57 day and males 97 day on arrival

- weight: females 198 – 240 g at assignment and males 597 – 1068 g at the first cohabitation.

- satellite groups used for toxicokinetics or recovery: No TK. Return to fertility was index of recovery from duct effect

- dosage groups in administered units: as shown in table above

- route, form, volume, and infusion rate: SC, Depot, 0.3 ml/kg, single administration 14 days prior to cohabitation

Drug, lot#, radiolabel, and % purity: 082797/no/not given

Formulation/vehicle: saline

Observations and times:

Mortality: checked twice daily

- Clinical signs: clinical effects observed before and approximately 60 minutes after dosage. Observations pertained to abortions, premature deliveries and death once daily during the post-dosing period

- Body weights: weekly during acclimation period, on the day of dasage, weekly prior to first cohabitation and on gestation days 0, 7, 14 and 20.

- Food consumption: weekly prior to first cohabitation and then on gestation days 0, 7, 14 and 20.

- Toxicokinetics: none

- Other: Estrous cycling was evaluated by examination of vaginal cytology for 7 days before assignment to the first cohabitation period and daily during each 5 day cohabitation period until spermatozoa were observed in a smear of the vaginal contents or a copulatory plug was observed in situ (gestation day 0). Rats that were found not pregnant were maintained on the study and entered in the following cohabitation period

Gross necropsy: All female rats except those in the ninth cohabitation period were sacrificed on gestation day 20, caesarian sectioned and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Ovaries were weighed and preserved. Tissues with gross lesions were preserved. Number of corpora lutea in each ovary was recorded. Uterus was examined for pregnancy, number and distribution of implantations, early and late resorptions and live and dead fetuses

Fetuses were weighed, examined for sex and gross external alterations.

Results:

- Clinical signs: no dose-related clinical observations were recorded. Clinical findings consisted of localized alopecia, lesions/abrasions, dental problems observed in some control and treated rats. However, chromodacryorrhea and chromorhinorrhea was observed in few treated animals. The incidence of swollen and purple ears was seen only in the high dose females on 5th cohabitation when 4/10 rats were affected. One high dose rat had a red substance in the vagina on gestation days 14 to 20; the litter consisted of 7 viable fetuses, 2 early resorptions and 10 late resorptions. One control rat had a firm cyst on the right oviduct.

- Body weights: body weight was significantly higher for groups 4 and 5 on day 8 and 15 after dosing.

- Food consumption: food consumption was increased for groups 4 and 5 during the first 2 weeks after dosing.
- Organ Weights: Ovaries from rats sacrificed after the third cohabitation were heavier than those from rats sacrificed after the first and second cohabitation periods.

Estrous cycling, mating and fertility parameters are summarized in the following table:

Table 41

Dosage	Days postdosage on first day of cohabitation	0	0.3	1	3	10
Cohabitation period		Mated/pregnant				
1	14	21/20	24/21	24/23	9/16	0/0
2	28	1/1	0/0	0/0	4/3	0/0
3	42	4/4	4/4	2/2	4/3	2/0
4	70	-	-	-	3/2	13/11
5	98	-	-	-	1/1	6/3
6	126	-	-	-	-	1/0
7	154	-	-	-	-	8/8
8	182	-	-	-	-	1/0
9	217	-	-	-	-	0/0

There were 25 rats in each group

As shown in the table, all rats in the vehicle, 0.3 and 1 mg/kg dosage groups had mated by the third cohabitation period. There were no pregnant dams in the 10 mg/kg group after these 3 cohabitations. No fetal gross external alterations related to treatment were reported. The effect of treatment primarily in the 10 mg/kg group and to small extent in the 3 mg/kg groups was reduction of corpora lutea and total liter size. Also the percent of resorbed conceptuses/litter (early and late resorptions) was increased in the 10 mg/kg dose group compared to historical control values for the laboratory. One of the 3 rats in the 10 mg/kg group that had not mated after the ninth cohabitation period had cystic endometrial glands and mild squamous metaplasia in the uterus.

Key Study Findings: Fertility was returned after cessation of treatment.

Overall Toxicology Summary: Maternal NOEL for PPI-149-Depot was determined to be less than 0.3 mg/kg as dose of 0.3 and 1 mg caused reduced gestation body weight gain on gestation days 0-7 and 3 and 10 mg/kg increased body weight and food consumption for 2 weeks postdosage. The NOEL for mating and fertility was determined to be 1 mg/kg. Dose levels of 3 and 10 altered estrous cycles and the 10 mg/kg decreased numbers of corpora lutea and litter size and caused increased resorption.

Study Title: **Subcutaneous development toxicity study of PPI-149-Depot in female rats. Final report**

Study No: Protocol 1316-005
Amendment #, Vol #, and page #: vol.24 p. 159
Conducting laboratory and location:
Date of study initiation: 3-31-98
GLP compliance: yes
QA- Report Yes (*) No ()
Methods:
Dosing:

- species/strain: rat/Crl:CD VAF/Plus (Sprague-Dawley)
- #/sex/group or time point: as shown in table below

Table 42

Dosage group	# of rats	Dosage mg/kg	Concentration mg/ml	Dosage volume ml/kg
1	25	0	0	0.3
2	25	0.3	1.0	0.3
3	25	1.0	3.33	0.3
4	25	2.0	6.67	0.3

It was stated that the dosage formulations administered to approximately 40% of the rats were 70% of the targeted concentration.
Doses levels of 0.3, 1.0 and 2.0 mg/kg represent 0.034, 0.114 and 0.228 multiples of the human therapeutic dose (HTD) of 100 mg/70 kg person.

Dosage was selected on the basis of study 1316-004. In that study single SC dose of PPI-149-Depot was administered on gestation day 7. A dose of 1 mg/kg increased body weight during lactation and dosage of 3 and 30 mg/kg reduced body weight and feed consumption during gestation. The dosage of 3 and 30 mg/kg decreased litter sizes and increased resorptions. Maternal NOAEL was determined to be 0.3 mg/kg and for the development NOAEL was 1 mg/kg.

- age: 66 days for females. males 40 days old
- weight: Day after arrival females weighed 180- 231 and males 130- 169. Weight at study assignment for females 222- 254 and for males on cohabitation 470- 879
- satellite groups used for toxicokinetics or recovery: none
- dosage groups in administered units: as shown in table above
- route, form, volume, and infusion rate: SC, Depot, 0.3 ml/kg, single administration

Drug, lot#, radiolabel, and % purity: 112697
Formulation/vehicle: depot/saline
Observations and times:
- Clinical signs: observed daily for mortality. Clinical observation noted for effects of PPI-149, abortions, premature deliveries and deaths before and 60 minutes after injection (DG 7). These observations were also made daily during the postdosage period (DGs 8 through 20).
- Body weights: recorded during acclimation period, on DGs 0, 7, 14, 18 and 20.
- Food consumption: same time points as for body weight

Gross necropsy: was performed when on gestation day 20, female rats were sacrificed and caesarian-sectioned. To confirm pregnancy status, uteri from rats that appeared nonpregnant were stained with 10% ammonium sulfide to confirm absence of implantation sites. Number of corpora lutea in each ovary was recorded. Uterus was examined for pregnancy, number and distribution of implantations, early and late resorptions and live and dead fetuses. Each fetus was weighed, sexed, and examined for gross external alterations. Half of the fetuses in each litter were examined for soft tissue alterations and other half for skeletal alterations.

- Other: To determine statistical significance of various parameters, analysis of variance and t-test were used.

Results:

- Clinical signs: no mortality or dose-related clinical or necropsy observations reported. A few observations recorded consisted of localized alopecia, an abrasion on the back, misaligned incisors, chrmodacryorrhea and in one high dose rat hydrometra.
- Body weights: body weight was sig reduced in the 1 mg/kg dose group on DG 14 and in the 2 mg/kg dose group on DG 14, 18 and 20. On DG 20, body weights were 99.8, 90.3 and 74.2% of the control group value in the 0.3, 1.0 and 2.0 mg/kg dose groups, respectively. The reduced values were attributed to reduced live litter sizes and increased resorption incidences in the 1 and 2 mg/kg groups.
- Food consumption: Food consumption was significantly decreased for the 1 mg/kg and 2 mg/kg groups during gestation.

Gross pathology: Significant caesarian-sectioning and litter observations are given in table below. Values are expressed as mean +/- SD (not shown)

Table 43

Dosage group Dosage mg/kg	1 0 (vehicle)	2 0.3	3 1.0	4 2.0	
Rats tested N	25	25	25	25	
Pregnant N (%) Cesarean-sectioned	23 (92.0)	25 (100.0)	23 (92.0)	24 (96.0)	
Corpora lutea	17.6	17.2	17.0	17.5	
Implantations	15.6	15.5	15.5	15.5	
Litter size	14.8	14.4	9.4	2.5**	
Live fetuses N Mean	341 14.8	359 14.4	216 9.4	61 2.5**	
Dead fetuses N	0	0	0	0	
Resorptions	0.8	1.2	6.1*	13.0**	
Early resorptions N Mean	18 0.8	29 1.2	140 6.1*	312 13.0**	
Late resorptions N Mean	1 0.0	0 0.0	0 0.0	0 0.0	
Dams with any resorptions N (%)	13 (56.5)	15 (60)	19 (82.6)**	22 (91.7)**	
Dams with all conceptuses resorbed N (%)	0 (0.0)	1 (4.0)	8 (34.8)	20 (83.3)**	
Dams with viable fetuses N (%)	23 (100)	24 (96.0)	15 (65.2)	4 (16.7)**	
Placenta appeared normal N (%)	23 (100.0)	25 (100.0)	23 (100.0)	24 (100.0)	
Live male fetuses N	183	174	114	28	

Live fetuses body wt Male fetuses Female fetuses	 3.78 3.56	 3.78 3.51	 3.93 3.74*	 4.02 3.85*	

* significantly different from the vehicle control group value ($p < 0.05$) ** significantly different from the vehicle control group value ($p < 0.01$)

Caesarean-sectioning and litter observations: Embryo-fetal viability was reduced in the 1 and 2 mg/kg dose groups based on significant increases in the litter means for early resorptions and significantly increased numbers of dams with all conceptuses resorbed as compared to the control group values, resulting in significantly reduced live litter sizes.
No treatment-related soft-tissue fetal malformations or variations were reported.
Fetal skeletal malformation was observed in one 0.3 mg/kg dosage group fetus that had fused thoracic vertebral arches, not ossified thoracic vertebral centrum, absent 2nd and 4th ribs, 6th to 8th fused ribs and short 6th rib.
Fetal skeletal variations consisted of significantly increased litter incidence of incomplete ossified pelvis in high dosage group.
The average number of metacarpal/fetus/litter was significantly increased ($p<0.05$) in the 1.0 and 2.0 mg/kg dose groups. However, sponsor stated that this was not considered related to the treatment because the expected toxic effect is a decrease rather than increase in this average.

Key studies findings: PPI-149-Depot caused embryo-fetal lethality but no teratogenicity.

Overall Toxicology Summary: Since there was no dose-response relationship observed for any finding, it was concluded that although doses of 1 mg/kg and 2 mg/kg proved to be highly embryolethal, these were not teratogenic.

Study Title: **Subcutaneous development toxicity of PPI-149-Depot in rabbits. Final report**
Study No: Protocol 1316-002
Amendment #, Vol #, and page #: vol. 25, p. 1
Conducting laboratory and location:
Date of study initiation: 2-8-1998 (replicate A), 3-9-1998 (replicate B)
GLP compliance: yes
QA- Report Yes (*) No ()
Methods:
Dosing:

- species/strain: rabbit/ New Zealand White
- #/sex/group or time point: as shown in table below:

Table 44

Group	# of female rabbits	Dosagea mg/kg	Concentration mg/ml	Dosage volume ml/kg
1	20b	0 (vehicle)	0	1
2	20b	0.1	2	0.05
3	10c	0.3	6	0.05
4	10c	1	20	0.05
5	10c	3	30	0.1
6	10c	30	30	1
7	20d	0.01	0.4	0.025
8	20d	0.03	1.2	0.025

a. The test article was considered 78.3% active for the purpose of the dosage calculations.
b. rabbits were evaluated in 2 replicates, each replicate consisting of 10 rabbits/dose group
c. rabbits were evaluated during the first replicate only
d. rabbits were evaluated during the second replicate only.

Dose levels of 0.01,0.03, 0.1, 0.3. 1.0, 3.0, and 30.0 mg/kg are equivalent to 0.12, 0.36, 1.2, 3.6, 12.0, 36.0 and 360.0 mg/m^2, respectively and represent 0.0023, 0.0068, 0.0227, 0.0681, 0.227o, 0.6810, and 6.810 multiples of the human therapeutic dose of 100 mg/70 kg person.

Rationale for dose selection: Based on the outcome of the first replicate, dosage of 0.3, 1, 3 and 30 mg/kg were not administered during the second replicate. These doses produced a high incidence of fetal resorption. Dosage of 0.01, and 0.03 mg/kg were added to the study in the second replicate.

- age: 7-8 months
- weight: 3.34 – 4.35 kg
- satellite groups used for toxicokinetics or recovery: none
- dosage groups in administered units: as shown in table above
- route, form, volume, and infusion rate: as shown in table above

Drug, lot#, radiolabel, and % purity: SCI-10V1g, no, not given
Formulation/vehicle: Depot/saline
Observations and times:
- Clinical signs: twice daily for viability and once for general appearance. Also observed for effects of drug, abortions, premature deliveries and deaths prior to dosage (DG 7) and 60 minutes after SC injection.
- Body weights: recorded on DG 0 and DGs 7 through 29
- Food consumption: given 180 grams of feed each day

- Other: All rabbits were sacrificed on DG 29 and grossly examined. Gross lesions were preserved with the exception of parovarian cysts, which are mentioned as common and spontaneous lesions in rabbits.
- Number of corpora lutea in each ovary was recorded. The uterus was examined for pregnancy, number and distribution of implantations, early and late resorptions and live and dead fetuses. Uteri of non-pregnant rabbits were stained with 10$ ammonium sulfide to confirm absence of implantation sites.
- Fetuses were weighed, examined for gross external alterations. Fetuses were examined internally to identify sex and visceral alterations. All fetuses were eviscerated, stained with Alizarin red S and evaluated for skeletal alterations.
- It was stated that 33% of the rabbits in replicate B received dose, which was 75% of the target concentration. For all other rabbits it was 80-120% of targeted concentration.

Results:

- Clinical signs: All does survived to scheduled sacrifice on DG 29. Clinical observations included soft and liquid feces, localized alopecia, ungroomed coat, red substance in cage pan associated with resorption of the litter and a uterus in which right horn contained red and green

viscous fluid and the left horn contained amniotic sacs in which there was yellow and green caseous material, which was attributed to uterine infection. None of these were dose-dependent.

- Body weights: body weight was decreased though not statistically in 0.3, 3 and 30 mg/kg groups and were suggested to reflect reduced live litter sizes and increased resorption incidence in the 0.1 mg/kg and higher dose groups.
- Food consumption: Absolute food consumption was increased in the 0.3, 1 and 30 mg/kg dosage groups.

Gross pathology:

Caesarean-sectioning and litter observations: are shown in table below:

Table 45

Dosage group Dosage mg/kg	1 0	2 0.1	3 0.3	4 1	5 3	6 30	7 0.01	8 0.03
Rabbits tested	20	20	10	10	10	10	20	20
Pregnant N (%)	20 (100)	19 (95)	10 (100)	8 (80)	8 (80)	10 (100)	19 (95)	19 (95)
Rabbits pregnant and caesarean sectioned on DG 29 N	20	19	10	8	8	10	19	19
Corpora lutea means	9.8	8.5	9.6	8.7	7.8	8.3	9.1	8.3
Implantations means	9.4	7.8	8.9	8.0	7.2	8.2	8.2	7.9
Litter sizes means	9.0	3.6**	2.8**	1.1**	0.0**	0.0**	7.3	6.2
Live fetuses N Means	179 9.0	69 3.6**	28 2.8*	9 1.1**	0 0.0**	0 0.0**	138 7.3	118 6.2
Dead fetuse	0	0	0	0	0	0	0	0
Resorptions mean	0.4	4.2**	6.1**	6.9**	7.2**	8.2**	0.9*	1.7*
Early resorptions N Mean	2 0.1	77 4.0**	60 6.0**	55 6.9**	58 7.2**	82 8.2**	10 0.5	31 1.6
Late resorptions N Mean	7 0.4	2 0.1	1 0.1	0 0.0	0 0.0	0 0.0	7 0.4	2 0.1
Does with any resorptions N (%)	7 (35.0)	14 (73.7)	8 (80.0)	7 (87.5)	8 (100.0)	10 (100)	6 (31.6)	10 (52.6)
Does with all conceptuses resorbed N (%)	0 (0.0)	10 (52.6)	7 (70.0)	7 (87.5)	8 (100.0)	10 (100.0)	2 (10.5)	3 (15.8)
Does with viable fetuses n (%)	20 (100.0)	9 (47.4)	3 (30.0)	1 (12.5)	0 (0.0)	0 (0.0)	17 (89.5)	16 (84.2)
Placenta appeared normal %	100	95	100	80	80	100	95	95
Litters with one or more fetuses N	20	9	3	1	0	0	17	16
Live male fetuses N	90	33	13	5			70	53
Litters with fetuses with any alterations N (%)	14 (70.0)	5 (55.6)	2 (66.7)	1 (100.0)			10 (58.8)	10 (62.5)
Fetuses with nay alteration observed N (%)	17 (9.5)	7 (10.1)	3 (10.7)	2 (22.2)			14 (10.1)	19 (16.1)

Results showed that embryo-fetal viability was reduced as is evident with significant increases in the litter means for resorptions and increased number of does with all conceptuses resorbed at does of 0.01 mg/kg and higher, as compared to control group values. This reflected in significantly reduced live litter sizes. Fetal weight for all groups with live litters did not significantly differ. No placental abnormalities were observed.

The average number of litters with fetuses with any alterations, the number of fetuses with any alteration observed and the average % of fetuses with any alteration did not differ significantly among the groups. There were no live fetuses in the 3 and 30 mg/kg dose groups.

No fetal malformations were attributable to depot at doses as high as 1 mg/kg. No fetuses were available at higher doses.

At 0.3 and 1 mg/kg doses there were increases in the litter averages for thoracic ribs (supernumerary ribs) with associated increases for thoracic and decreases for lumbar vertebrae. This was stated as reversible developmental variation.

On fetal gross examination following external alterations were reported:

Malformations: One control fetus had a short tail. Skeletal examination of this fetus revealed associated malformations, fused and misaligned caudal vertebrae.

One 0.03 mg/kg dosage group fetus inn one litter had an umbilical hernia with pertrusion of the intestine.

Another 0.03 mg/kg dose group fetus in one litter had a small head and depressed eye bulges. Associated soft tissue and skeletal malformations in this fetus included small eyes (microphthalmia) and small eye sockets, extreme dilation of the lateral ventricles in the brain (hydrocephalus) with fusion of the right frontal and parietal bones. This fetus also had a variation in skull ossification, an internasal ossification site.

One late absorbed fetus in the 0.3 mg/kg group was examined. This fetus had a short tail and an absent hind limb (no right femur, fibula, tibia, tarsals, metatarsals, digits or phalanges). Skeletal evaluation also revealed an absent pelvis (no aright ilium, ischium or pubis) and fused arches and centra in the lumbar and sacral vertebrae.

Skeletal Variations: One 0.3 mg/kg dose group fetus had a flexed hind paw, considered to be deformation associated with compression in utero.

The incidence of flat ribs was significantly increased in the 1mg/kg dosage group fetuses.

Fetal soft tissue variations: One 0.01 mg/kg group fetus and one 0.1 mg/kg group fetus had a circumcorneal hemorrhage in on eye and was attributed to trauma during processing.

One control group fetus, one 0.01 mg/kg, three 0.03 mg/kg fetuses and two 0.1 mg/kg fetuses had and absent intermediate lobe of he lung, described as a common variation in lung development in NZW rabbits.

One 0.01 mg/kg group fetus had no gall bladder. One 0.03 mg/kg group fetus had a bilobed gall bladder and another fetus of this group had a small gallbladder.

There were increases in the averages for pairs of supernumerary thoracic ribs in the 0.3 and 1 mg/kg dose group as described before.

Key Study Findings: Based on the results of this study, it was concluded that the maternal NOAEL for PPI-149-Depot is greater than 30 mg/kg and the developmental NOAEL is less than 0.01 mg/kg. Although abarelix treatment increased embryo-fetal resorption and does with all

conceptuses resorbed in a dose-related manner, the incidence of fetal malformation though increased in treated groups was not dose-related.

Overall Toxicology Summary: Sponsor has stated that no fetal malformations were attributable to administration of a single SC injection of PPI-149-Depot at dosages as high as 1 mg/kg (no fetuses were available for examination in the 3 and 30 mg/kg dosage groups) and that PPI-149-Depot is selectively toxic to the conceptuses of pregnant rabbits as expected based on its pharmacologic activity.

Comments: Although the incidence of malformations observed was not dose-dependent and occurred mostly in one fetus in one litter in any dosage group, in light of the fact that there were few litters and fetuses available for evaluation and most of the malformation observed occurred in PPI-149 treated groups, suggests that treatment may have some teratogenic effect. This may be supported by the findings on a late resorbed fetus in the 0.3 mg/kg group, which showed skeletal malformations and may suggest that malformed fetuses are preferentially resorbed resulting in false negative findings.

Study Title: **Subcutaneous fertility and general reproductive toxicity of PPI-149-Depot in male rats**
Study No: 1316-001
Amendment #, Vol #, and page #: vol 22 p. 1
Conducting laboratory and location: [redacted]
Date of study initiation: 12-1-1997
GLP compliance: yes
QA- Report Yes (*) No ()
Methods:
Dosing:

- species/strain: rat/Crl: CD VAF/Plus (Sprague-Dawley)
- #/sex/group or time point: 25/male/group
- age: 59 days
- weight: 269 –303 g
- satellite groups used for toxicokinetics or recovery: none
- dosage groups in administered units: as shown in table below:

Table 46

Dosage Group	Dosage mg/kg	Concentratin mg/ml	Volume ml/kg	# of male rats
1	0 (vehicle)	0	0.3	25
2	0.3	1	0.3	25
3	1	3.3	0.3	25
4	3	10	0.3	25
5	10	33.3	0.3	25

Doses were selected on the basis of previous studies conducted with PPI-149-Depot
Dose levels of 0.3, 1.0, 3.0 and 10.0 mg/kg are equivalent to 1.8, 6.0, 18.0 and 60.0 mg/m^2 and are 0.034, 0.114, 0.340 and 1.135 respectively, multiples of the human therapeutic dose of 100 mg/70 kg person.

- route, form, volume, and infusion rate: SC, 0.3 ml/kg, single administration

Drug, lot#, radiolabel, and % purity: 0822797
Formulation/vehicle: Depot/0.9% sodium chloride for injection, USP
Observations and times: Beginning 7 days postdosage, male rats were assigned to 5 consecutive cohabitation periods as shown in table below:

Table 47

Cohabitation Period	Postdosage days	# of female rats	Number of female rats per group per cohabitation period				
			Group 1	Group 2	Group 3	Group 4	Group 5
1	7	125	25	25	25	25	25
2	28	123	25	25	24	25	24
3	56	123	25	25	24	25	24
4	84	121	24	25	23	25	24
5	119	48	24	-	-	-	24

Each cohabitation period consisted of a maximum of 4 days. Female rats with spermatozoa observed in a vaginal smear and/or a copulatory plug observed in situ were considered to be at gestation day 0.

- Clinical signs: twice daily for viability. Clinical observations were recorded before and 60 minutes post-injection and then once daily for male rats. For females clinical observations recorded on DG 0, 6 and 13.
- Body weights: for male on day of injection and daily during post injection period. For females on DGs 0, 6 and on the day sacrificed.
- Food consumption: weekly for males. On DGs 0, 6 and 13 for females.
- Mating performance: daily during cohabitation. Mated females removed from cohabitation

Organ weights: Male rats sacrificed at the completion of last scheduled cohabitation period. Right testis, left testis, left epididymis, right epididymis, seminal vesicles and prostate weighed.

Other: A portion of the left cauda epididymis was used for evaluation of cauda epididymal sperm counts and motility. On DG 13, female rats were sacrificed, caesarean-sectioned and grossly examined. Female rats examined for number of corpora lutea, number and distribution of implantation sites, and live and dead embryos. Gross lesions and ovaries were retained.

Results:

-Mortality: 1, 1, 2 and 1 male rats in the 0, 0.3, 1 and 10 mg/kg dosage groups, respectively died before scheduled sacrifice. These deaths were considered unrelated to treatment with PPI-149-Depot. Control rat had difficulty eating because of an injured palate; associated observations included swollen snout and missing incisors, chromorhinorrhea, chromodactyorrhea and localized alopecia. It successfully impregnated cohort female rat. Rats in the 0.3 and 1 mg/kg groups were moribund sacrificed. They showed same symptoms as the control rat and they also successfully impregnated a cohort female each time. The 10 mg/kg rat was fond dead on day 22. It had pale eyes and labored breathing on day 21. Necropsy revealed distended urinary bladder with thickened walls containing about 80 calculi with extreme dilatation of pelvis of the kidneys.

- Clinical signs: Small testes or no apparent testes occurred in significantly increased number of rats in the 10 mg/kg dosage group only.

- Body weights: was decreased in the 3 and 10 mg/kg dosage groups in dose dependent manner in the first week. After initial reduction in body weight gains, they were comparable in all groups. Terminal body weights did not differ significantly.
Food consumption: was decreased in the 10 mg/kg dosage group.
Organ Weights: The 3 and 10 mg/kg dosage groups had significantly reduced absolute weights of cauda epididymis and the absolute weights of he left epididymis, left testis, right epididymis, right testis and prostate were significantly reduced in the 10 mg/kg dosage group, as compared to control group values.
Gross pathology: No gross lesions attributable PPI-149-Depot at necropsy. Small testes and epididymides were observed. These were observed in 1, 0, 1, 0 and 3 rats in the vehicle, 0.3, 1, 3 and 10 mg/kg groups.
- Histopathology: revealed moderate to marked atrophy of the testes and marked hypospermia. One of the high dose rats did not mate in any of the 5 cohabitation periods.
Cauda epididymal sperm motility, count and density: The averages for sperm motility parameters [motile sperm, % motile sperm, static (non-motile) and total sperm counts] were comparable and did not significantly differ among treatment groups. Averages for caudal epididymal sperm count and density was reduced in the 0.3 mg/kg and higher dosage groups but were not dose-dependent.
Mating and fertility: Results of the 5 cohabitation periods is shown in table below:

Table 48

Group (mg/kg)	0 (vehicle)	0.3	1	3	10
Cohabitation period 1 (7 days postdosage)					
N	25	25	25	25	25
Mated (%)	100	100	84	20**	8**
Days in cohabitation	2.3	2.1	1.9	2.3	2.5
Fertility (%)	100	100	84	12**	0**
Cohbitation period 2 (28 days postdosage)					
N	25	25	24	25	24
Mated (%)	80	84	95.8	80	0**
Days in cohabitation	3.2	2.6	2.5	2.8	-
Fertility (%)	80	84	91.7	52.0	0**
Cohabitation period 3 (56 days postdosage)					
N	25	25	24	25	24
Mated (%)	100	100	100	92	16.7**
Days in cohabitation	1.8	2.0	2.0	2.1	2.2
Fertility (%)	100	100	100	88	8.3**
Cohabitation period 4 (84 days postdosage)					
N	24	25	23	25	24
Mated (%)	95.8	100	95.6	100	50**
Days in cohabitation	1.5	1.8	2.0	1.5	2.0
Fertility (%)	91.7	96	95.6	100	41.7**
Cohabitation period 5 (119 days postdosage)					
N	24	-	-	-	24
Mated (%)	100	-	-	-	91.7
Days in cohabitation	1.6	-	-	-	2.3*
Fertility (%)	100	-	-	-	87.5

Key studies findings: Male fertility was returned on cessation of treatment.

Overall toxicology summary: Results showed that mating rate and fertility were severely affected in the 10 mg/kg dosage groups for the first 3 cohabitation periods. Results of the 4th cohabitation period indicated that these effects were beginning to reverse.
Mating and fertility returned to within control values on 5th cohabitation. The 3 mg/kg dosage group had significantly reduced number of matings in the first cohabitation period but 100% of

the rats mated and impregnated female rats. The 0.3 and 1 mg/kg dosages did not affect mating or fertility of the male rats.

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Histopathology Inventory for IND #

Study	28-day	6 mon	12 mon	
Species	Rat	Rat	Monkey	
Adrenals	*	*	*	
Aorta	*	*	*	
Bone Marrow smear		*	*	
Bone (femur)	*	*	*	
Brain	*	*	*	
Cecum	*	*	*	
Cervix				
Colon	*	*	*	
Duodenum	*	*	*	
Epididymis	*	*	*	
Esophagus	*	*	*	
Eye	*	*	*	
Fallopian tube				
Gall bladder			*	
Gross lesions	*	*	*	
Harderian gland	*	*		
Heart	*	*	*	
Hyphophysis	*	*	*	
Ileum	*	*	*	
Injection site	*	*	*	
Jejunum	*	*	*	
Kidneys	*	*	*	
Lachrymal gland	*		*	
Larynx	*			
Liver	*	*	*	
Lungs	*	*	*	
Lymph nodes, cervical				
Lymph nodes mandibular	*	*	*	
Lymph nodes, mesenteric	*	*	*	
Mammary Gland	*	*	*	
Nasal cavity				
Optic nerves	*	*	*	
Ovaries	*	*	*	
Pancreas	*	*	*	
Parathyroid	*	*	*	
Peripheral nerve	*	*	*	
Pharynx				
Pituitary	*	*	*	
Prostate	*	*	*	
Rectum	*	*	*	
Salivary gland	*	*	*	
Sciatic nerve	*	*	*	
Seminal vesicles	*	*	*	
Skeletal muscle	*	*	*	
Skin	*	*	*	
Spinal cord	*	*	*	
Spleen	*	*	*	
Sternum	*	*	*	

Stomach	*	*	*	
Testes	*	*	*	
Thymus	*	*	*	
Thyroid	*	*	*	
Tongue	*	*	*	
Trachea	*	*	*	
Urinary bladder	*	*	*	
Uterus	*	*	*	
Vagina	*	*	*	
Zymbal gland				

* organ weight obtained

GENETIC TOXICOLOGY:

Study Title: **Evaluation of a test article in the Salmonella typhimurium/Escherichia coli plate incorportion/preincubation mutation assay in the presence and absence of induced rat liver S-9.**
Study No: 0482-2140
Study Type: in vitro mutagenicity assay
Amendment #, Volume # and Page #: vol. 26 p. 74
Conducting Laboratory: _____
Date of Study Initiation/completion: 3-30-1998
GLP Compliance: yes
QA- Reports Yes (*) No ( ):
Drug Lot Number: 2WI
Study Endpoint: number of revertant colonies

Methodology:

- Strains/Species/Cell line: Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 and Escherichia coli strain WP2uvrA.
- Dose Selection Criteria: Toxicity evaluated based on chemical precipitation,
- background lawn evaluation and relative cloning efficiency
    - Basis of dose selection: dose range finding study using S. typhimurium TA100 and E.coli strain WP2uvrA and spontaneous reversion frequency.
    - Range finding studies: yes
- Test Agent Stability:
- Metabolic Activation System: yes
- Controls:
    - Vehicle: DMSO
    - Negative Controls: solvent control cultures
    - Positive Controls: 2-nitrofluorine, 2-aminoanthracene, sodium azide, 9-aminoacridine and methyl methanesulfonate
- Exposure Conditions:
    - Incubation and sampling times: Plates were incubated at 37 +/- 1 ^{0}C for 68 hours
    - Doses used in definitive study: 219, 437, 874, 2186 and 4372 ug/plate
- Study design- Mutation assay:
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- Analysis:
  - No. slides/plates/replicates/animals analyzed: All test concentrations, including the controls, were tested in triplicate.
  - Counting method: automated colony counter and manual when precipitate interfered.
  - Cytotoxic endpoints: background lawn
  - Genetic toxicity endpoints/results: increase in revertant colonies
  - Statistical methods:

- Criteria for Positive Results: A response was considered to be positive if either strain TA98 or TA100 exhibits a mean reversion frequency that is at least double the mean reversion frequency of the corresponding solvent control in at least one doe, or if either strain TA1535, TA 1537 of WP2uvrA exhibits a threefold increase in mean reversion frequency compared to solvent control in at least one dose. In addition, the response must be dose-dependent or increasing concentrations of the test article must show increasing mean reversions frequencies.

Results: In the following table results for S.typhimurium/E.coli plate incorporation mutation assay are shown without S-9 activation and with S-9 activation.

Table 49 Without activation

	Average number of revertant/plate						
S.typhimurium	Positive control	Solvent control	Concentration per plate (ug) 219	437	874	2186	4372
Strain TA98	429	19	16	14	22	18	15
Strain TA100	1140	95	110	108	107	103	106
Strain TA1535	626	11	9	11	8	9	7
Strain TA1537	34	3	3	2	3	3	3
E.coli	833	10	10	9	7	10	0
With activation							
Strain TA98	297	30	31	30	19	29	27
Strain TA100	652	113	103	112	102	85	96
Strain TA1535	95	10	11	12	11	10	9
Strain TA1537	42	5	4	3	6	5	3
E.coli	178	13	7	12	10	8	15

The number of cells seeded ranged from 0.476 x 10^8 to 0.686 x 10^8 for S.typhimurium strains and 1.024 x 10^8 for E. coli.
At all the concentrations, lawn was normal and no precipitate appeared.

Similar results were obtained with pre-incubation mutation assay without and with S-9 activation.

- Study Validity: seems valid
- Study Outcome; negative for Ames test

Study title: **Evaluation of a test article in the L5178Y TK+/_ mouse lymphoma mutagenesis assay in the presence and absence of aroclor-induced rat liver S-9**
Study No: —— study NO. 0461-2400

Study type: in vitro mutagenesis assay
Amendment #, volume # and page #: vol, 26 p.171
Conducting laboratory: ————————
Date of study initiation/completion: 9-30-1997/3-29-1999 Revised study completion 5-11-2000
GLP compliance: yes
QA- Reports Yes (*) No ( ):
Drug Lot number: CIC607020
Study endpoint: potential to induce mutations at the thymidine kinase locus in L5178Y TK+/- mouse lymphoma cells.

Methodology:

Strain/species/cell line: L5178Y TK+/- mouse lymphoma cells, clone 3.7.2C
Dose selection criteria:

Basis of dose selection: Maximum achievable concentration in selected solvent
Range finding studies: were performed to determine test article concentrations that will produce from 0-100% cytotoxicity. The concentrations used with and without exogenus metabolic activation were 0.058, 0.29, 0.58, 2.9, 5.8, 29, 58, 289, 578 and 2891 ug/ml. At 20 hours and 44 hours post treatment, samples are removed from each culture to determine cell population density. Three coincidence-corrected counts are made and the average count used to determine the concentration of cells/ml for each culture. The cultures are adjusted to 0.3×10^6 cell/ml in the combined final volume of 20 ml.

Test agent stability: not given
Metabolic activation system: S-9 metabolic activation mix
Controls:

Vehicle: DMSO
Negative controls: DMSO
Positive controls: Hycanthone methanesulfonate (HYC) in the non-activated system and 7, 12-dimethylbenz(a)anthracene (DMBA) in the S-9 activated system.

Exposure conditions:

Incubation and sampling time: 20 and 44 hours to determine cell population density. After 2-day expression period, cultures selected for cloning based on their suspension growth (SG). After 10 to 12 day incubation period, the number of colonies/TFT (trifluorothymidine) and VC (viable count) plates counted and mutation frequency determined.

Doses used in definitive study:

Without activation (first mutation assay) 289, 578, 867, 1156, 1446, 1735, 2313 and 2891 ug/ml
With activation) 58, 116, 145, 173, 202, 231, 289, and 347 ug/ml
Without activation(second mutation assay)18, 36, 73, 182, 364, 547, 729, 1093 ug/ml
With activation 219, 237, 255, 273, 292, 310, 328, 364 ug/ml

Study design: L