

Microscopic pathology in the sex organs of rats treated for 24 months.

Best Possible Copy

Tissue and lesion	Male				Female			
	Control	Low Dose	Mid Dose	High Dose	Control	Low Dose	Mid Dose	High Dose
Seminiferous epithelial degeneration								
moderate	0	7**	5	1				
marked	0	24***	21***	12***				
severe	0	18***	24***	37***				
total incidence	0	49***	50***	50***				
Testes tubular atrophy	8	0**	0**	0**				
Testes tubular mineralisation	7	0*	0*	1				
Epididymis no abnormality detected	44	0***	0***	0***				
Epididymis atrophy								
moderate	1	0	0	0				
marked	0	1	0	1				
severe	1	48***	50***	48***				
total incidence	2	49***	50***	49***				
Prostate no abnormality detected	40	0***	0***	0***				
Prostate Atrophy								
mild	0	0	0	1				
marked	0	1	0	0				
severe	0	46***	46***	46***				
total incidence	0	47***	46***	47***				
Seminal vesicles no abnormality found	40	0***	0***	0***				
Seminal vesicles atrophy								
mild	1	0	0	1				
marked	0	4*	1	0				
severe	0	42***	45***	45***				
total incidence	1	46***	46***	46***				
Preputial gland no abnormality found	18	15	10	8*	28	20	19	18
Preputial gland atrophy								
mild	14	15	18	22	3	4	11*	8
moderate	0	8**	11***	7*	0	1	1	3
marked	0	2	1	0	2	0	0	4
total incidence	18	30*	36***	37***	10	10	25**	22**
Ovaries No abnormality found					7	0*	0*	0*
Ovaries Atrophy								
mild					4	1	2	1
moderate					1	7*	14***	3
marked					0	28***	20***	32***
severe					0	13***	13***	13***
total incidence					5	49***	49***	49***
Ovaries interstitial cell increased					33	1***	4***	2***
Ovary pigmented macrophages increased					38	0***	1***	1***
Uterus no abnormality found					9	0**	1*	1*
Uterus stromal polyp (benign)					10	0**	0**	0**
Uterus Atrophy								
marked					1	4	9*	1
severe					0	45***	40***	47***
total incidence					1	49***	49***	49***
Uterus interstitial pigmentation					30	0***	0***	0***
Vagina no abnormality found					42	1***	1***	2***
Vagina epithelial atrophy								
minimal					0	1	0	0
mild					3	1	0	1
moderate					2	0	3	1
marked					2	11*	12**	5
severe					0	36***	32***	41***
total incidence					7	49***	47***	48***

Summary of Tumor findings

The investigators compiled the following table to summarize the tumor findings in this study. Consistent with the histopathological findings, long term treatment with fortnightly degarelix decreased the incidence of some tumors, particularly in organs that became atrophic in response to GnRH inhibition.

Best Possible Copy

GROUP DOSE	TUMOUR TABLE							
	Males				Females			
	Grp 1 0 mg/kg/ 2weeks	Grp 2 2 mg/kg/ 2weeks	Grp 3 10 mg/kg/ 2weeks	Grp 4 25 mg/kg/ 2weeks	Grp 1 0 mg/kg/ 2weeks	Grp 2 2 mg/kg/ 2weeks	Grp 3 10 mg/kg/ 2weeks	Grp 4 25 mg/kg/ 2weeks
NUMBER OF ANIMALS	50	50	50	50	50	50	50	50
NUMBER OF ANIMALS WITH TUMOURS	35	22	21	20	41	24	25	26
NUMBER OF ANIMALS WITH SINGLE TUMOURS	24	20	15	15	13	20	19	20
NUMBER OF ANIMALS WITH MULTIPLE TUMOURS	11	2	6	5	28	4	6	6
NUMBER OF ANIMALS WITH BENIGN TUMOURS	30	16	17	14	39	22	24	18
NUMBER OF ANIMALS WITH MALIGNANT TUMOURS	11	6	7	9	10	4	4	12
NUMBER OF ANIMALS WITH METASTASISING TUMOURS	1		1	2		1		1
TOTAL NUMBER OF TUMOURS	50	24	30	26	88	30	31	34
TOTAL NUMBER BENIGN TUMOURS	38	18	23	17	78	26	27	20
TOTAL NUMBER OF MALIGNANT TUMOURS	12	6	7	9	10	4	4	14
TOTAL NUMBER OF METASTASISING TUMOURS	1		1	2		1		1
% ANIMALS WITH TUMOURS	70	44	42	40	82	48	50	52
% ANIMALS WITH SINGLE TUMOURS	48	40	30	30	26	40	38	40
% ANIMALS WITH MULTIPLE TUMOURS	22	4	12	10	56	8	12	12
% ANIMALS WITH BENIGN TUMOURS	60	32	34	28	78	44	48	36
% ANIMALS WITH MALIGNANT TUMOURS	22	12	14	18	20	8	8	24
% ANIMALS WITH METASTASISING TUMOURS	2		2	4		2		2

The following table shows the incidences of all neoplasms that were significantly different from control calculated either by Peto analysis (one tailed – increased incidence only) or Fisher’s Exact test (two-tailed). The table also presents the range for historical controls for this rat strain.

Control values are from M. Giknis and C Clifford, Spontaneous Neoplasms and survival in Wistar Han Rats: Compilation of Control Group Data, March 2003, Charles River Laboratories,

http://info.criver.com/flex_content_area/documents/rm_rm_r_survival_wistar_han_rats_compilation_data.pdf

Tissue and lesion	Male				Female				Range in Historical controls	
	Control	Low Dose	Mid Dose	High Dose	Control	Low Dose	Mid Dose	High Dose	males	females
Hemangiosarcoma of the mesenteric lymph node - metastatic										
N	1	3	1	0	0	1	1	4	1.8 - 10 %	1.8 - 11 %
p (sponsor's Peto analysis)	0.93	0.34	0.78	1	0.015	0.54	0.55	0.038		
Liver eosinophilic cell focus										
N	43	35	30	31	8	25	15	12	Not Reported	Not Reported
p (sponsor's Peto analysis)	0.99	1	1	1	0.82	< 0.001	0.066	0.19		
Pituitary adenoma benign										
N	9	5	5	4	27	9	10	9	22 - 51%	1.7 - 62 %
p (Fisher's Exact)	0.87	0.9	0.89	0.92	0.01	0.02	0.01			
Fibroadenoma of the mammary gland benign										
N					10	1	0	0	3.3 - 3.6 %	1.8 - 3.6 %
p (Fisher's Exact)					0.024	0.005	0.005	0.005		

In this table, the increase in eosinophilic foci in the liver is not dose dependant. The incidences of benign pituitary adenoma and benign fibroadenoma of the mammary gland decrease.

The following table shows the incidence of all hemangiomas and hemangiosarcomas (benign and malignant) in this study. Malignant tumors are in bold. The combined incidence of benign and malignant hemangiomas and hemangiosarcomas was 16% (total 8 of 50 rats).

Best Possible Copy

	Male				Female			
	Control	Low Dose	Mid Dose	High Dose	Control	Low Dose	Mid Dose	High Dose
Hemangiosarcoma and hemangioma combined from all tissues								
Hemangiosarcoma of the mesenteric lymph node - malignant N	1 50	3 48	1 49	0 50	0 50	1 49	1 50	4 50
Hemangioma of the mesenteric lymph node - benign N	1 50	1 48	1 49	3 50	0 50	0 49	2 50	2 50
Hemangiosarcoma of the Aorta - malignant N	0 49	1 50	0 50	0 50	0 50	0 50	0 50	0 49
Hemangiosarcoma of the abdominal cavity - malignant N	0 50	0 50	0 50	1 50	0 50	0 50	0 50	0 50
Hemangiosarcoma of the skin - malignant N	0 50	0 50	1 50	0 50	0 50	0 50	1 50	2 50
Hemangioma of the skin - benign N	0 50	0 50	0 50	0 50	0 50	1 50	0 50	0 50
Hemangiosarcoma of the skeletal muscle - benign N	1 50	0 50	0 50	0 50	0 50	0 50	0 50	0 50
Total number of tumors combined p-value	3	5	3	4	0	2	4	8
Incidence %	6%	10%	6%	8%	0%	4%	8%	16%

The following table shows the tumor incidence stratified for time of necropsy according to FDA guidelines. The combined incidence of hemangiosarcoma and hemangioma remains increased in the high dose group by pairwise comparison. This data was presented to the ExecCAC on December 9, 2008.

Dose group	control				Low				Mid				High			
	HMG	HMS	Total deaths	Total incidence	HMG	HMS	Total deaths	Total incidence	HMG	HMS	Total deaths	Total incidence	HMG	HMS	Total deaths	Total incidence
male																
before day 665	0	0	5	0	0	0	2	0	0	0	2	0	0	0	3	0
day 665 to termination	0	0	4	0	0	0	3	0	0	0	1	0	0	0	3	0
Terminal necropsy	1	2	41	0.073	1	4	45	0.111	1	2	47	0.064	3	1	44	0.091
Total	1	2	50	0.073	1	4	50	0.111	1	2	50	0.064	3	1	50	0.091
Fisher's Exact for total one tail								0.150				0.360				0.220
Female																
before day 665	0	0	8	0	0	0	3	0	0	0	1	0.000	0	0	5	0.000
day 665 to termination	0	0	3	0	0	0	2	0	0	0	0	0.000	0	2	3	0.67
Terminal necropsy	0	0	39	0.000	1	1	45	0.044	2	2	49	0.082	2	4	42	0.143
Total	0	0	50	0.000	1	1	50	0.044	2	2	50	0.082	2	6	50	0.160
Fisher's Exact for total one tail								0.300				0.100				0.009

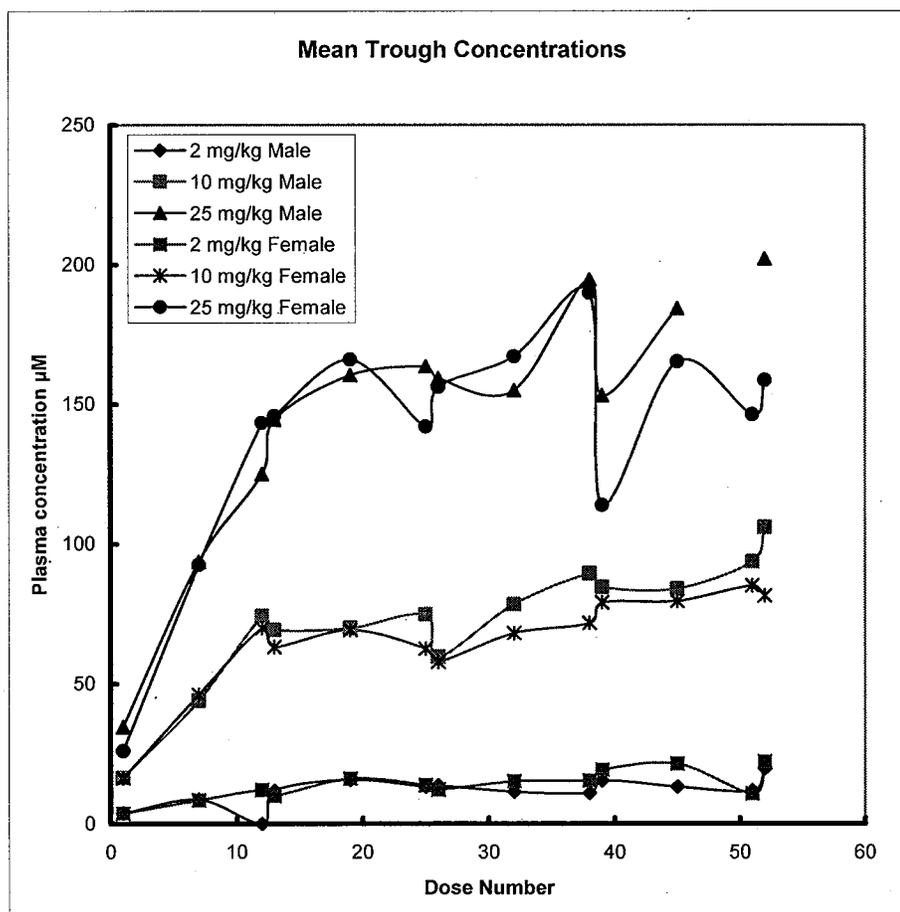
HMG = hemangioma
HMS = hemangiosarcoma

Dr. Karl Lin analyzed the data above and determined that the increased incidence in combined hemangiomas plus hemangiosarcomas was statistically significant in female rats. Dr. Paul Brown and Dr. Abby Jacobs of the ExecCAC concurred by email that the result was significant and should be described in the product label.

Species	Sex	Combined Tumor Type	One-sided P-Values	
			Asymptotic	Exact
Rat	M	Hemangioma + Hemangiosarcoma	0.51	0.50
Rat	F	Hemangioma + Hemangiosarcoma	0.0008	0.0013

Toxicokinetics

The following graph shows the trough concentrations of degarelix, that is, the plasma concentrations from blood taken just prior to dosing. The graph demonstrates slow accumulation and with steady state reached only after about the 12th dose or about 168 days into the experiment. After this, the plateau concentration stays relatively constant throughout the rest of the experiment. Comparing the averages of the trough values from dose 19 through dose 52, the increase in the steady state trough concentration is roughly dose proportional in both males and females.



All mean plasma concentration vs time profiles contained sufficient data to be evaluable for pharmacokinetic purposes. Profiles were generally consistent with extravascular dosing of drug. There was no evidence of a lag phase following administration of degarelix (i.e. there was quantifiable drug in the first plasma sample taken after dosing in all animals).

T_{max} was highly variable ranging from 2 to 48 hours. The estimates of the terminal elimination half-life were also highly variable ranging from 132 to 750 hours. This variability is due to the fact that

many of the half-life values exceeded the dosing interval, and in no case did the elimination curve span the usual requisite of 5 half-lives necessary for reliable determination.

The following tables from the study report show the toxicokinetic parameters determined after the first dose. The increase in AUC and particularly C_{max} is much less than dose proportional again suggesting saturation of the subcutaneous absorption process. There are no differences between males and females.

Best Possible Copy

Table A - Parameters Relating to Systemic Exposure to FE200486: Effect of Increasing Dose in Males and Females in Relation to Dose 1

Sex	Dose Level (mg/kg/2weeks) [Ratio]	AUC (ng.h/mL) [Ratio]	AUCt (ng.h/mL) [Ratio]	C_{max} (ng/mL) [Ratio]
Male	2 [1]	7445 [1]	5912 [1]	143 [1]
	10 [5]	29039* [3.90]	15532 [2.63]	169 [1.11]
	25 [12.5]	58744 [7.89]	27605 [4.67]	212 [1.48]
	2 [1]	7183 [1]	5912 [1]	119 [1]
Female	10 [5]	23874 [3.33]	17936 [3.03]	128 [1.10]
	25 [12.5]	40282 [5.62]	24344 [4.12]	221 [1.86]

* Parameter estimate derived from unreliable determination of λ_{da-z} .

Table B - Toxicokinetic Parameters: Effect of Increasing Dose in Males and Females in Relation to Dose 1

Sex	Dose Level (mg/kg/2weeks)	t_{max} (h)	t_w (h)	CL/F (mL/h/kg)	Vd/F (mL/kg)
Male	2	2.00	169.10	268.6	65537
	10	2.00	348.05*	344.4*	172912*
	25	2.00	382.02	425.6	234549
Female	2	2.00	142.63	278.2	57454
	10	2.00	154.74	418.9	93506
	25	2.00	259.93	620.6	232735

* Parameter estimate derived from unreliable determination of λ_{da-z} .

Estimates of half-life, clearance and volume increased with increasing dose, suggesting saturation of the subcutaneous process. The volume is unusually large as one would expect for a drug given as a subcutaneous depot.

The following tables from the study report show the changes in AUC and C_{max} with repeated dosing.

Appears This Way
 On Original

Best Possible Copy

Table E - Parameters Relating to Systemic Exposure: Effect of Repeat Dosing in Males

Dose Level (mg/kg/2weeks) [Group]	Dose	AUC (ng.h/mL)	AUCt (ng.h/mL)	C _{max} (ng/mL)
2 [2]	1	7445	5912	143
	13	20389	13849	186
	26	21651	14438	124
	39	23650	16797	137
	52	29400	19340	184
10 [3]	1	29039*	15532	159
	13	130739	53590	299
	26	93712	51415	318
	39	189049	62315	371
	52	256240	76537	370
25 [4]	1	58744	27605	212
	13	511448*	92615	553
	26	397263	116438	689
	39	239476	114764	672
	52	336654*	140557	621

* Parameter estimate derived from unreliable determination of lambda-z.

Best Possible Copy

Table F - Parameters Relating to Systemic Exposure: Effect of Repeat Dosing in Females

Dose Level (mg/kg/2weeks) [Group]	Dose	AUC (ng.h/mL)	AUCt (ng.h/mL)	C _{max} (ng/mL)
2 [2]	1	7163	5912	119
	13	18020	14932	168
	26	20062	15633	132
	39	32995	17292	154
	52	33506	21008	202
10 [3]	1	23874	17936	128
	13	120078	49634	355
	26	93881	45892	277
	39	169977*	54725	334
	52	159779	89222	396
25 [4]	1	40282	24344	221
	13	342592	113451	558
	26	276864	134027	666
	39	173071	106148	539
	52	303022	116640	587

* Parameter estimate derived from unreliable determination of lambda-z.

The AUC for males from the table above the average increase across all determinations from the low dose to the middle dose (an increase in dose of 5 fold) was 6.3 fold and the average increase from low dose to high dose (an increase in dose of 12.5 fold) was 11.9 across all the different time points measured. The values in females were similar. Thus, the increase in exposure roughly dose proportional. Nevertheless, the increase in C_{max} was considerably less than dose proportional, again suggesting a limitation on the subcutaneous absorption process.

The mean AUC from 14 prostate cancer patients receiving a dose of 240 mg of degarelix in study FE200486CS07 was 24624 hr*ng/mL. In this study, the AUC at steady state in the high dose group is about 300000 hr*ng/mL. Thus, exposure in rats in this study is about 12 times higher than it is in patients receiving a single loading dose. In the sponsors simulation of the proposed clinical schedule the AUC for degarelix at steady state after a loading dose of 240 mg and a maintenance dose of 80 mg is 15939 hr*ng/mL or about 18 fold greater than the steady state exposure in the high dose group.

2) 104 Week Carcinogenicity Study in Mice with Subcutaneous Administration

Major findings

Treatment with degarelix at doses of 6, 30 and 150 mg/m² fortnightly for two years caused an increase in benign hepatocellular adenoma of the liver (p = 0.015) in high dose females and benign bronchio-alveolar adenoma in all groups of treated females (p = 0.006, 0.039 and 0.01 for low mid and high dose animals respectively) when analyzed by pairwise comparison with control. By trend analysis the increase in benign hepatocellular adenoma of the liver reached significance in both males (p = 0.024) and females (p = 0.037).

When the incidence of benign bronchio-alveolar adenoma was combined with that of malignant bronchio-alveolar carcinoma the result was not statistically different from controls by pairwise comparison. The incidence of benign bronchio-alveolar adenoma in male CD-1 mice ranges from 11 to 36 %, in females it ranges from 3 to 16%.

Dosing in this study also caused an increase in benign hepatocellular adenoma of the liver (p = 0.015) in high dose females. By trend analysis the increase in benign hepatocellular adenoma of the liver reached significance in both males (p = 0.03) and females (p < 0.04). When the incidence of benign hepatocellular adenoma was combined with that of malignant hepatocellular carcinoma the total incidence was between 28 and 32% in all males groups including controls and 2, 14, 14 and 13% in female controls, low dose, mid dose and high dose groups respectively. These results were not significantly different from controls in males or females (p < 0.09) by pairwise comparison. The combined incidence of these tumors was also not statistically different from controls by asymptotic trend test (p < 0.09). The normal incidence of hepatocellular adenoma of the liver ranges from 2 to 33 % in male CD-1 mice and from 0 to 4% in females. The normal range for hepatocellular carcinoma ranges from 0 to 1.7 % in females and 0 to 6% in males.

Dosing was also associated with an unusual, but not statistically significant, incidence of sarcomas in the spinal cord. This finding is possibly related to dosing.

Dosing in this study was adequately high and was associated with increased mortality in males and females in the high dose groups as demonstrated by Kaplan-Meyer analysis. Exposure was of the same order as k_i at C_{trough} in the low dose group and substantially higher in mid and high dose groups.

Study number 455832
 Ferring reference number FE200486DSCAR0102
 EDR filename car0102-nonclinical-data.pdf
 Conducting laboratory _____
 Date of study initiation October 2002
 GLP compliance Yes
 QA reports Yes
 Drug Degarelix, Batches listed in the table below from the study report

Batch Number	Weight (g)	Date Received	Peptide Content (%)	Correction Factor	Expiry Date
0048262#PPL-FE4860001	22.73	03 July 2002	91.77	1.00	02 July 2004
PPL-FE4860002*	13.74	28 January 2003	89.50		31 May 2004
PPL-FE4860002*	7.14	03 February 2003	89.50		31 May 2004
0051981#PPL-FE4860002*	25.00	05 March 2003	89.50		25 May 2004
PPL-FE4860201A	84.39	10 July 2003	91.40		31 March 2005

*The same batch, although the complete batch number was not given on the first 2 batches.

Methods

b(4)

Animal Male and female CD-1 mice — CD-1™(ICR)BR
 four to five weeks old
 Males 20-24 g
 Females 16-20 g

Doses 0, 2, 10 or 50 mg/kg, (0, 6, 30, 150 mg/m²)
 Dose levels are expressed as 100% peptide content
 Dose levels are based on a 13 Week Toxicity Study in Mice with Subcutaneous Administration conducted at _____

N _____ Study No. 455827, Ferring No. FE200486DSTOX0111, see above).

50 per sex per dose level for control, low and mid dose groups
 60 per sex for the high dose group

Schedule Once fortnightly
 Route Subcutaneous injection rotated over two different sites
 Dose volume 5 mL/kg
 Formulation Mannitol_{aq} 5% w/v

b(4)

Observations

Mortality Twice daily
 Clinical signs Twice daily
 body weight Weekly
 Ophthalmoscopy Every 6 months (20 animals per sex only)
 Hematology End of study
 Toxicokinetics Samples taken in relation to doses 1, 8, 13, 20, 26, 33, 39, 46 and 52 as follows (0.25 mL blood samples)

Best Possible Copy

Dose No.	Timepoints relative to dosing									
	Predose	+2 h	+4 h	+8 h	+12 h	+24 h [Day 2]	+48 h [Day 3]	+96 h [Day 5]	+192 h [Day 9]	+336 h [Day 15]*
1	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
8	(10)	-	-	-	-	-	-	-	-	-
13	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
20	(10)	-	-	-	-	-	-	-	-	-
26	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
33	(10)	-	-	-	-	-	-	-	-	-
39	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
46	(10)	-	-	-	-	-	-	-	-	-
52	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)

() number of animals per sex per treatment group sampled.
 *On Day 15, the sample was taken prior to administration of the next dose.

Necropsy Week 104 for treatment animals, week 102 for controls
 Histopathology Adequate battery

Results

Toxicokinetics

The following table from the study report demonstrates that AUC did not increase linearly with dose on day one of dosing; the AUC at the high dose (25 fold greater than the low dose) was only 10 fold greater than the low dose AUC in males and 8 fold higher in females. This indicates that a significant amount of drug was either metabolized prior to entry into the plasma or much of the dose remained within the subdermal depot or both. C_{max} was strikingly less than dose proportional, the high dose value being only 2.1 and 1.6 fold greater than that of the low dose for females and males respectively. This suggests that the absorption process from the subdermal depot was rate limiting.

Best Possible Copy

Table A - Parameters Relating to Systemic Exposure to FE200486: Effect of Increasing Dose in Males and Females in Relation to Dose 1

Sex	Dose Level (mg/kg/2weeks) [Ratio]	AUC (ng.h/mL) [Ratio]	AUCt (ng.h/mL) [Ratio]	C _{max} (ng/mL) [Ratio]
Male	2 [1]	4290* [1]	4271 [1]	294 [1]
	10 [5]	27099* [6.32]	12874 [3.01]	408 [1.39]
	50 [25]	43487 [10.14]	29107 [6.82]	482 [1.64]
Female	2 [1]	4177 [1]	4141 [1]	292 [1]
	10 [5]	16265 [3.89]	13165 [3.18]	652 [2.23]
	50 [25]	33403 [8.00]	23286 [5.62]	614 [2.10]

* Parameter estimate derived from unreliable determination of lambda-z.

The following table from the study report demonstrates that t_{max} did not vary significantly with dose but that $t_{1/2}$ was highly variable and quite long. This variability is a result of small sample sizes at each time point and the fact that half-life was almost as long as the sampling time. This means that the terminal elimination rate constant, λ_z , is overestimated thus $t_{1/2}$ is actually considerably longer than the values shown in the table and plasma clearance is overestimated.

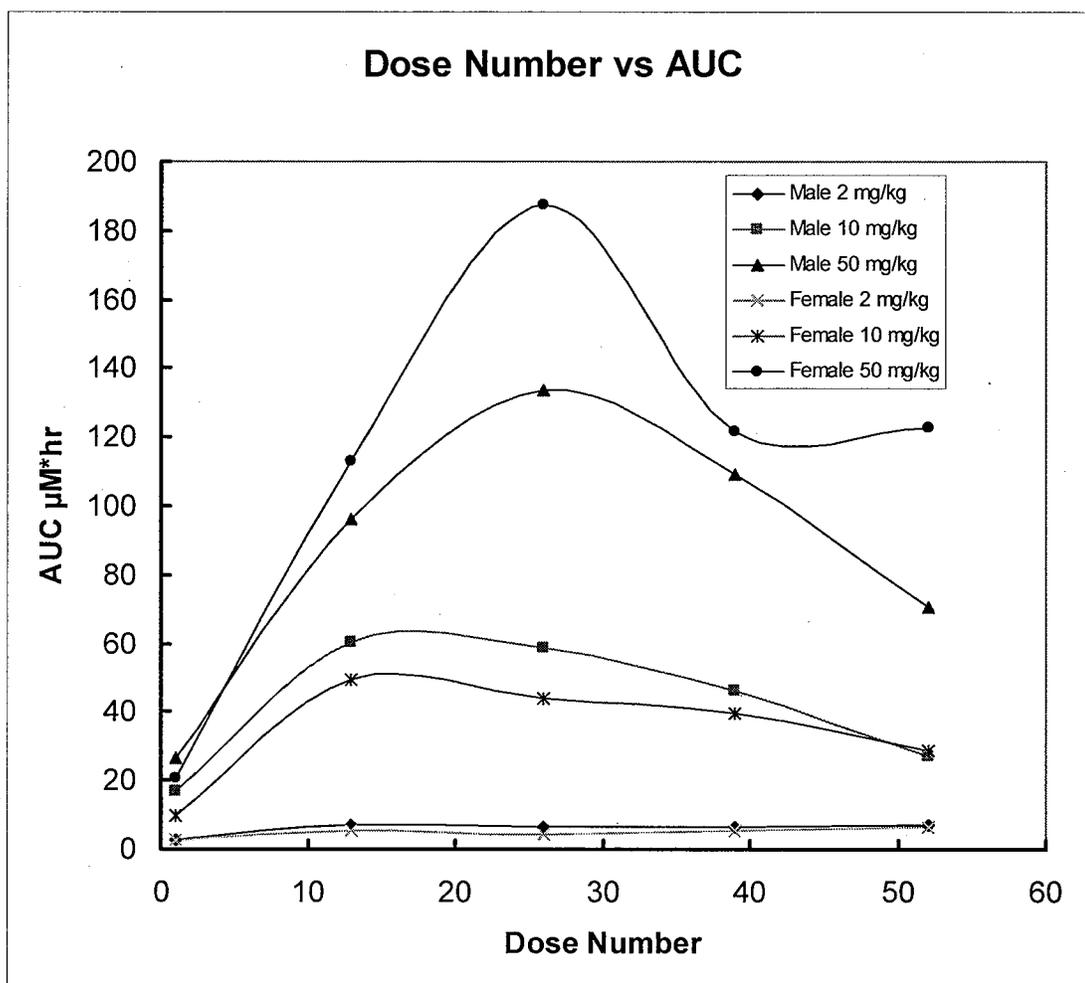
Best Possible Copy

Table B - Toxicokinetic Parameters: Effect of Increasing Dose in Males and Females in Relation to Dose 1

Sex	Dose Level (mg/kg/2weeks)	t_{max} (h)	$t_{1/2}$ (h)	CL/F (mL/h/kg)	V _z /F (mL)
Male	2	2.00	26.40*	466.2*	17752*
	10	4.00	456.48*	369.0*	243025*
	50	2.00	274.60	1150	455486
Female	2	2.00	50.56	478.8	34924
	10	2.00	190.64	614.8	169095
	50	2.00	208.39	1497	450019

* Parameter estimate derived from unreliable determination of lambda-z.

The following graph shows that AUC increased to a maximum at about dose 15 (week 30) in mid dose animals and at about dose 25 (week 50) in high dose animals. AUC then decreased in both dose groups. The investigators do not mention this decrease in exposure over the course of the experiment. The time frame of the decrease in AUC is too long to suggest induction of metabolism. One possible explanation for this unusual finding is that repeat dosing causes changes in the basal membrane below the skin that result in decreased absorption of the drug. The decrease in exposure at the end of the experiment was not sufficient to affect the integrity of the experiment.

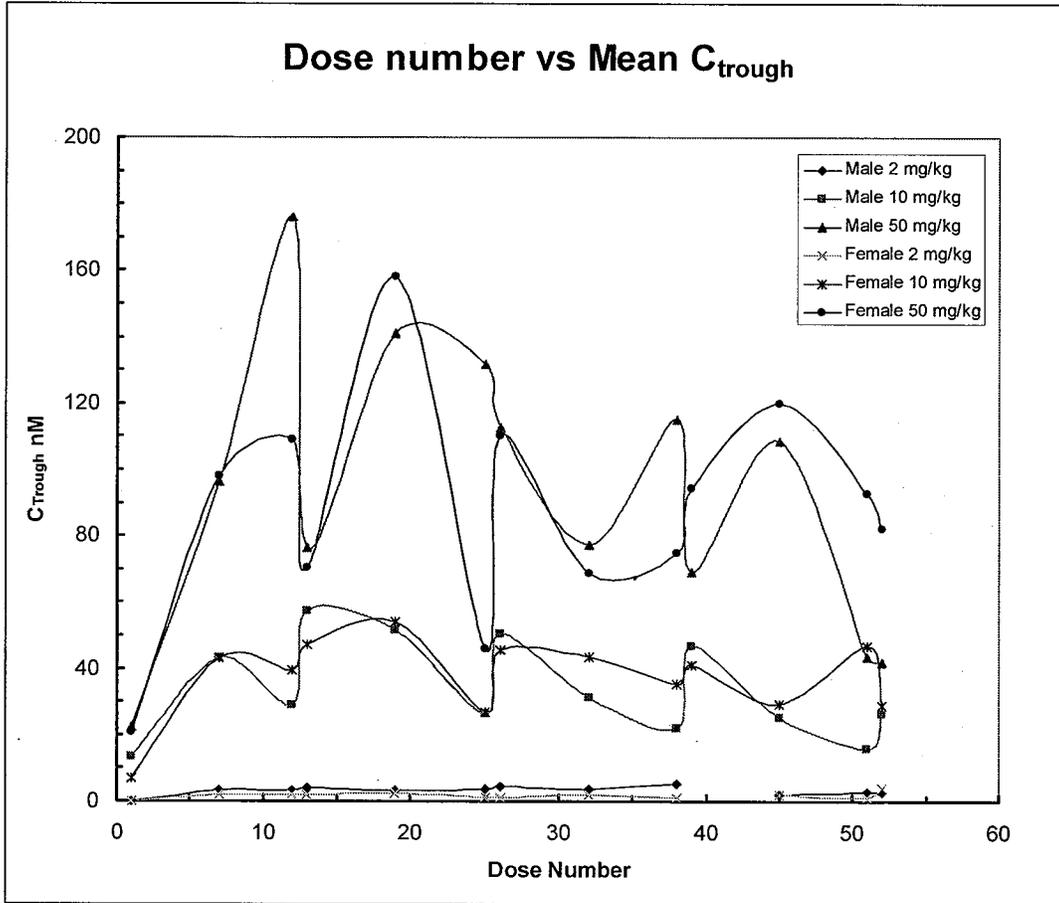


The following table shows the fold increase in AUC over the AUC for the low dose at the different pharmacokinetic assay times in males. As AUC increases to its maximum around week 26 the fold increase is greater than dose proportional in the mid dose group, that is an 8.8 fold increase in AUC compared to a 5 fold increase in dose. The AUC at the high dose is 20 fold greater than that of the low dose animals compared to a 25 fold increase in dose; for high dose females the increase is greater still. This finding illustrates the adequacy of exposure.

Dose Proportionality Expressed as a Multiple of the 2 mg/kg dose.

Dose Number	Mid Dose	High Dose
1	6.3	10.1
13	8.6	13.7
26	8.8	20.1
39	7.2	17.1
52	3.9	10.2

The following graph shows that the concentration at trough (just prior to the next dose) increased with increasing dose but again not in a linear manner. Here the increase in C_{trough} is greater than dose proportional at almost all time points in the mid dose animals (mean 10, range 4.1 to 15.5 fold increases). In high dose animals, the increase is close to dose proportional (mean 30, range 15 to 56 fold increases). The values were on the order of k_i (1.7 nM) in the low dose group and considerably greater than k_i in the mid and high dose groups, thus confirming the adequacy of exposure.



The graph also shows a periodicity in the data for both males and females and at both mid and high doses. This periodicity is very regular in the mid dose animals but less so in the high dose animals probably due to increased toxicity. Since it occurs in two different dose groups and in both males and females, it is unlikely to be an artifact. It does not relate to the varying sample size at the different data points.

Mortality

The following table from the study report shows that unscheduled mortality was high in this study.

Best Possible Copy

Dose Group (mg/kg/2weeks)	Number of Premature Decedents	
	Males	Females
1 (0)	25	33#
2 (2)	22	20
3 (10)	20	20
4 (50)	43	37

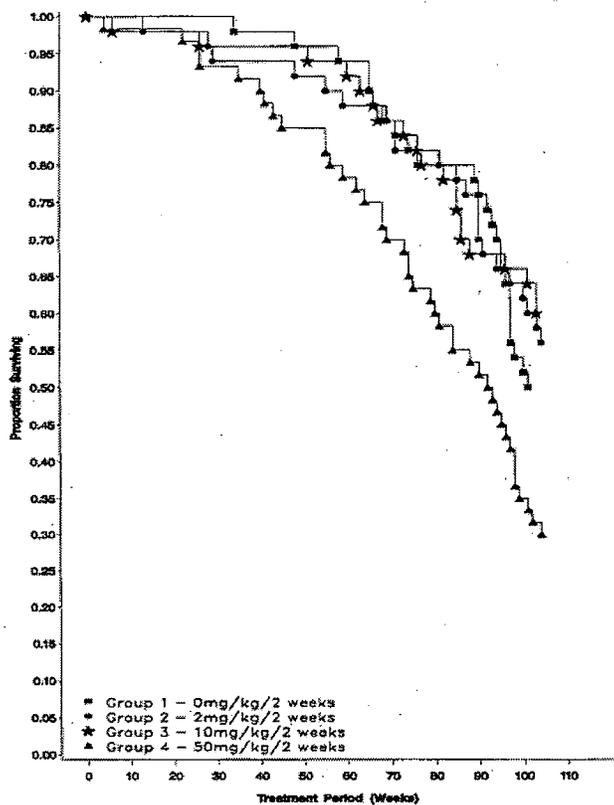
-Group 1 females were terminated during Week 102.

Mortality was somewhat high in control females so the investigators decided to terminate this group in week 102. Because of this high mortality in controls, there was a statistically significant difference in mortality in low ($p = 0.03$) and mid dose ($p = 0.026$) female mice but not in the high dose group. In males the mortality in the high dose group was higher than in controls and this difference was significant ($p = 0.008$).

The high mortality in controls (50% for males and 66% in females) is not unusual. Paul Baldrick and Lesley Reeve (Toxicol. Path. 2007. 35(4):562-575) have shown that mortality in this strain of mice ranged from 27 to 73 % in males and 25 to 78 % in females between 1991 and 2004 (meta-analysis of 10 studies). The investigators consider the increase in mortality in HD males related to the increased incidence in injection site damage. The Kaplan-Meier curve from the study report suggests that the excess mortality in the HD males was treatment related.

Appears This Way
On Original

FE200486
104 Week Carcinogenicity Study in Mice with Subcutaneous Administration
Figure 1 Kaplan Meier Survival Curve
Main Study: Males

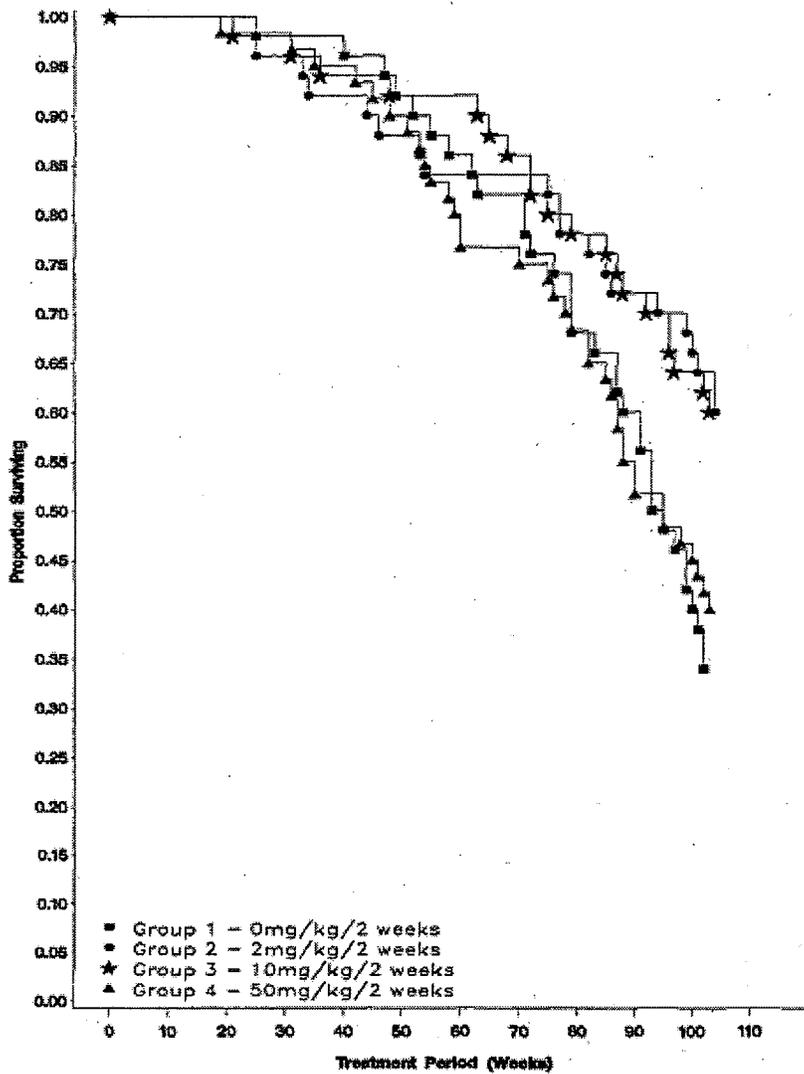


Best Possible Copy

As in other studies (above) degarelix treatment may actually be protective in female mice at lower doses due to the ablation of hormonal ablation and atrophy of the sex organs. The Kaplan-Meier curve from the study report suggests that this is the case here.

Appears This Way
On Original

FE200486
104 Week Carcinogenicity Study in Mice with Subcutaneous Administration
Figure 2 Kaplan Meier Survival Curve
Main Study: Females



Sarcoma (NOS) was only a cause of death in treated animals. Non-neoplastic causes of death that were seen mostly in high dose animals included pyogranuloma, hyperplastic and ulcerative dermatitis, renal disease and arteritis or periarteritis. Other causes of death seen only in treated animals included spinal inflammation, renal hemorrhage and renal abscess.

Clinical Observations

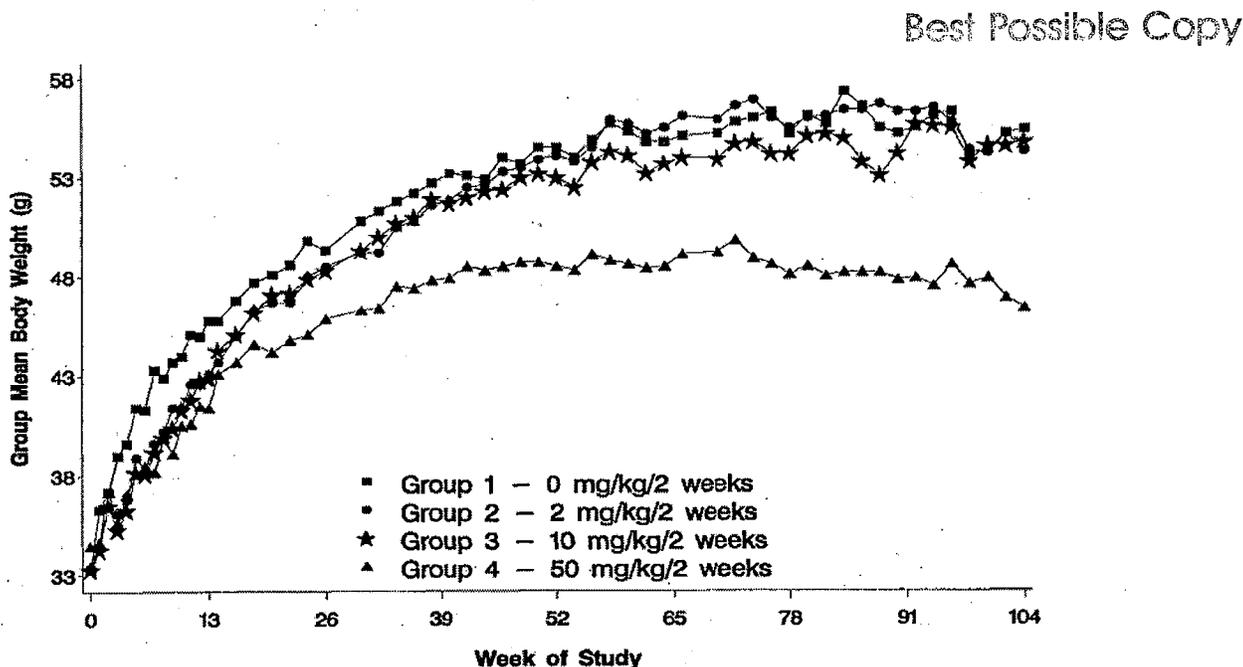
Clinical observations of toxicity were relatively few but included injection site damage in HD males and females with swelling, scabbing and skin thickening. Similar damage was less severe and less frequent in low and mid dose animals.

Body weight

High dose male mice weighed significantly less than controls through out the study (about 18% at termination). Low and mid dose animals weighed less than controls from about week 1 to about week 40 but than low dose animals appeared to weigh about the same as controls. Mid dose animals continued to appear to weigh somewhat less until about week 90. These differences only reached significance between week 1 and week 13 and are somewhat confounded by the fact that all treated animals weighed less than controls in week, but not week 0 (about 6%). The following graph from the study report demonstrates these differences.

FE200486
104 Week Carcinogenicity Study in Mice with Subcutaneous Administration
Group Mean Body Weights: Main Study: Males

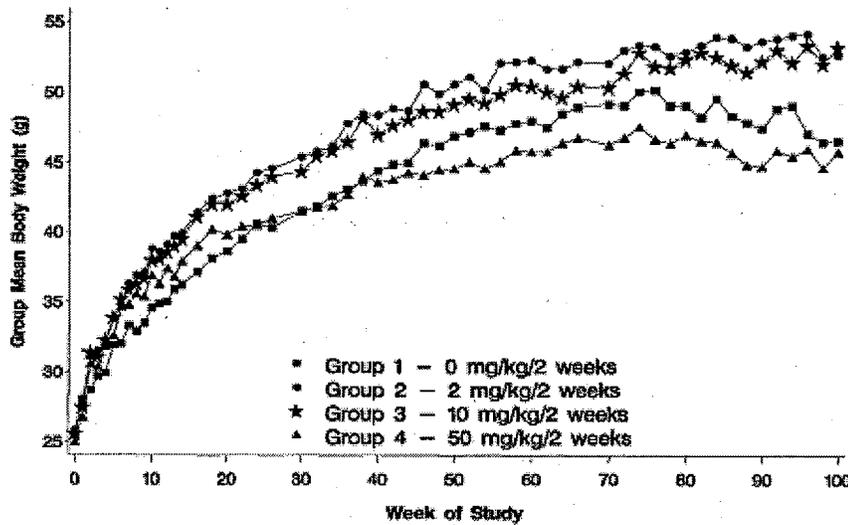
Figure 3



High dose female consistently weighed less than controls from about week 45 onward. Low and mid dose females weighed consistently more than controls.

FE200486
104 Week Carcinogenicity Study in Mice with Subcutaneous Administration
Group Mean Body Weights: Main Study: Females

Figure 4



Best Possible Copy

Food consumption

All treated groups ate less than controls. These differences were frequently statistically significant.

Ophthalmology

No toxicologically significant changes

Hematology

The following table from the study report shows that at termination red cell parameters were somewhat lower than controls in the HD mice. WBC and lymphocytes were also decreased.

FE200486
104 Week Carcinogenicity Study in Mice with Subcutaneous Administration
Haematology: Termination
Group Mean Values: Males

Table 7

Best Possible Copy

Group/Dose Level (mg/kg/2weeks)		Hb	RBC	Hct	MCH	MCV	MCHC	Retl	WBC	Neut	Lymp	Mono	Eos	Baso	LUC	Plat
1 (0)	Number	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	Mean	12.7	8.53	0.428	14.9	50.3	29.6	3.2	8.05	1.76	5.38	0.52	0.25	0.02	0.12	1807
	SD	0.8	0.60	0.023	0.6	1.9	0.9	0.7	3.21	0.89	2.55	0.71	0.17	0.01	0.18	397
2 (2)	Number	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14
	Mean	12.5	8.63	0.432	14.5	50.1	29.0	3.4	9.89	1.98	7.09	0.41	0.28	0.03	0.10	1791
	SD	0.7	0.60	0.022	0.5	1.8	0.7	1.0	3.43	1.89	2.15	0.25	0.17	0.02	0.06	651
3 (10)	Number	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
	Mean	12.5	8.83	0.427	14.2	48.5	29.4	3.3	11.74	2.33	8.48	0.43	0.29	0.03	0.19	1971
	SD	1.1	0.80	0.033	0.8	2.7	0.7	0.5	4.33	1.33	3.15	0.25	0.32	0.02	0.21	469
4 (50)	Number	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
	Mean	10.9	7.78	0.381	14.0	49.2	28.4	4.1	13.33	2.70	9.81	0.34	0.24	0.04	0.20	2280
	SD	1.4	0.98	0.041	0.7	2.4	0.9	0.7	5.30	1.02	4.67	0.18	0.20	0.03	0.12	1452
	Prob.	***	*	**	**	2.4	**	**	**	**	**	**	**	**	*	*

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

The following table from the study report shows that in females at termination, white cell parameters and platelets were mildly elevated.

FE200486
104 Week Carcinogenicity Study in Mice with Subcutaneous Administration
Haematology: Termination
Group Mean Values: Females

Best Possible Copy

Table 8

Group/Dose Level (mg/kg/2weeks)		Hb	RBC	Hct	MCH	MCV	MCHC	Reti	WBC	Neut	Lymp	Mono	Eos	Baso	LUC	Plat
1 (0)	Number	9	9	9	9	9	9	0	9	9	9	9	9	9	9	9
	Mean	12.7	8.28	0.418	15.4	50.8	30.4		4.92	1.57	2.72	0.20	0.33	0.02	0.05	1278
	SD	2.3	1.48	0.072	0.6	1.6	0.9		2.51	0.85	1.59	0.14	0.14	0.03	0.09	397
2 (2)	Number	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
	Mean	12.6	8.44	0.425	14.9	50.4	29.5	3.4	8.01	1.86	5.61	0.39	0.24	0.02	0.08	1680
	SD	1.2	0.87	0.042	0.7	2.2	0.5	0.9	3.01	0.89	2.48	0.17	0.16	0.02	0.04	267
3 (10)	Number	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
	Mean	12.4	8.33	0.424	14.9	51.1	29.3	3.5	9.09	1.59	6.71	0.31	0.23	0.04	0.21	1542
	SD	1.1	0.94	0.040	0.8	3.5	0.9	1.4	4.64	0.87	3.92	0.16	0.19	0.03	0.31	518
4 (50)	Number	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Mean	11.7	8.11	0.407	14.6	50.8	28.9	5.3	11.18	3.30	7.00	0.33	0.32	0.04	0.19	1946
	SD	0.9	0.86	0.033	1.4	5.9	0.9	4.2	4.97	2.30	3.94	0.17	0.24	0.02	0.10	685

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

Clinical Chemistry

The following table from the study report shows that glucose was elevated in all treated males. Changes in globulin and A/G ratio suggest the possibility of mild hepatic toxicity.

FE200486
104 Week Carcinogenicity Study in Mice with Subcutaneous Administration
Clinical Chemistry: Termination
Group Mean Values: Males

Best Possible Copy

Table 9

Group/Dose Level (mg/kg/2weeks)		Urea	Glu	AST	ALT	AP	Na	K	Cl	TP	Alb	Glob	AG-R	Crea	Ca	Phos
1 (0)	Number	12	12	11	11	8	12	11	12	11	11	11	11	9	11	11
	Mean	9.4	5.58	97	99	132	156	4.2	113	58	31	26	1.2	34	2.43	1.91
	SD	1.2	1.41	65	69	50	2	0.4	3	5	4	4	0.2	3	0.08	0.24
2 (2)	Number	14	13	12	13	14	13	12	12	13	11	11	11	11	14	13
	Mean	10.0	7.31	193	129	303	155	4.2	113	55	32	24	1.4	35	2.44	1.68
	SD	1.4	1.43	181	112	199	2	0.7	2	5	3	4	0.3	5	0.10	0.16
3 (10)	Number	15	15	14	12	14	15	15	15	14	12	12	12	11	14	14
	Mean	8.9	7.47	180	111	228	156	4.2	114	55	33	22	1.5	35	2.43	1.61
	SD	1.8	1.44	99	74	242	3	0.4	3	5	3	3	0.2	8	0.11	0.15
4 (50)	Number	8	8	8	8	4	8	8	8	8	6	8	6	6	8	8
	Mean	9.5	7.66	194	153	83	156	4.4	115	63	33	37	1.1	29	2.54	1.71
	SD	2.2	2.00	196	179	10	1	0.7	1	6	5	14	0.1	3	0.08	0.36

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

The following table from the study report shows that glucose actually decreased in all dosed females while sodium increased.

Best Possible Copy

FE200486
104 Week Carcinogenicity Study in Mice with Subcutaneous Administration
Clinical Chemistry: Termination
Group Mean Values: Females

Table 10

Group/Dose Level (mg/kg/2weeks)		Urea	Glu	AST	ALT	AP	Na	K	Cl	TP	Alb	Glob	AG-R	Crea	Ca	Phos
1 (0)	Number	8	7	7	6	6	8	8	5	6	3	3	3	2	4	3
	Mean	8.7	12.17	93	57	128	147	4.6	116	50	24	28	0.9	23	2.32	1.65
	SD	1.6	2.12	42	30	51	3	0.2	2	7	4	10	0.4	0	0.05	0.12
2 (2)	Number	15	15	15	15	15	15	15	15	15	11	11	11	9	13	12
	Mean	9.7	7.78	207	98	152	154	4.0	114	57	33	25	1.6	39	2.40	1.83
	SD	1.2	1.50	215	112	55	2	0.4	2	10	4	14	0.5	5	0.06	0.40
	Prob.		***				***	**		**	**		**	**		
3 (10)	Number	15	15	14	14	15	15	15	14	15	12	12	12	13	15	15
	Mean	8.8	9.02	158	82	186	166	4.2	114	55	33	22	1.5	35	2.44	1.72
	SD	1.7	1.72	76	49	79	2	0.4	2	4	4	0.3	5	0.08	0.24	
	Prob.		***				***	*		*	**		*	*		
4 (50)	Number	13	12	12	13	12	13	13	12	12	9	9	9	10	13	13
	Mean	11.3	8.65	116	66	158	155	4.7	115	54	30	25	1.3	34	2.46	1.77
	SD	4.1	1.92	102	58	99	1	0.6	3	10	4	9	0.4	5	0.20	0.46
	Prob.		***				***				*					

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

Organ Weights

Best Possible Copy

The following table shows that sex organ weight decreased substantially in both males and females. Kidney weights were decreased in both males and females and heart weight was decreased in males. Adrenals decreased in females and increased in females.

Organ weights	Absolute Change				Relative Change *		
	Control value	Low Dose	Mid Dose	High Dose	Low Dose	Mid Dose	High Dose
male body weight	53	51	55	44			
Heart male	0.32	-21.9%	-25.0%	-28.1%	0.812	0.723	0.866
Brain male	0.51	5.9%	3.9%	3.9%	1.100	1.001	1.252
Kidney male	0.879	-37.1%	-36.7%	-38.8%	0.654	0.610	0.737
Adrenals male	0.0058	34.5%	41.4%	19.0%	1.398	1.362	1.433
Liver male	3.07	-17.3%	5.9%	-16.6%	0.860	1.020	1.004
Epididymides	0.1305	-79.4%	-84.4%	-87.0%	0.214	0.150	0.157
Prostate	0.06	-86.7%	-93.3%	-86.7%	0.139	0.064	0.161
Seminal Vesicles	2.2672	-98.5%	-99.0%	-99.2%	0.016	0.010	0.010
Testes	0.23	-91.3%	-91.3%	-87.0%	0.090	0.084	0.157
Pituitary male	0.0019	-21.1%	21.1%	-26.3%	0.820	1.167	0.888
Spleen male	0.124	33.1%	56.5%	118.5%	1.383	1.508	2.633
Female body weight	43	51	55	44			
Adrenals female	0.0113	-19.5%	-41.6%	-53.1%	0.679	0.457	0.458
Brain female	0.53	-1.9%	-1.9%	-1.9%	0.827	0.767	0.959
Heart female	0.22	-9.1%	-9.1%	-9.1%	0.766	0.711	0.888
Kidneys female	0.568	-7.0%	-20.1%	-11.8%	0.784	0.625	0.862
Liver female	2.29	-1.3%	-7.4%	-8.7%	0.832	0.724	0.892
Lung female	0.31	38.7%	0.0%	0.0%	1.170	0.782	0.977
Ovaries	0.662	-97.6%	-99.3%	-99.6%	0.020	0.005	0.004
Uterus	0.62	-85.5%	-95.2%	-95.2%	0.122	0.038	0.047
Pituitary female	0.0032	-12.5%	-53.1%	-50.0%	0.738	0.366	0.489
Spleen female	0.252	-13.5%	-39.3%	-16.7%	0.729	0.475	0.814

(organ weight in control ÷ body weight of control) ÷ (organ weight in treated group) ÷ (body weight in treated group);
Values close to 1 show no change in organ weight relative to body weight
Values shown in bold were significantly different based on the investigators analysis.

Some changes in organ weights, such as the increase in the weights of the adrenals in males, did not reach significance because while inhibition of secretion of gonadotropins caused hyperplasia the general decrease in weight contravened the effect in HD animals resulting in a bell shaped dose response

curve. Standard statistical analysis is thwarted by bell shaped dose response curves. The change in adrenal weights may be significant because of the pharmacological mechanism of the degarelix.

Gross findings – Atrophic sex organs and injection site damage

Microscopic findings

The following table lists the toxicologically significant microscopic lesions found in these animals at necropsy. It does not include injection site damage, masses or some redundant findings. The table shows the number of animals with a particular finding in each dose group. The almost complete incidence of atrophic findings in the sex organs of both males and females demonstrates the primary pharmacology of this treatment even at the lowest dose.

*Appears This Way
On Original*

Tissue and lesion	Male				Female			
	Control	Low Dose	Mid Dose	High Dose	Control	Low Dose	Mid Dose	High Dose
N for most tissues	41	46	49	57	47	45	46	59
Trachea - Infiltration by lymphoma cells	1	1	2	1	4	3	0	0*
Lung - infiltration by lymphoma cells	4	8	5	8	17	11	4**	8*
Lung - lymphocytic infiltration	9	8	15	23**	12	13	11	24
Lung - agonal congestion or hemorrhage	1	6	7	11**	4	5	7	11
Mandibular lymph node infiltration by lymphoma cells	3	5	5	9	14	12	6	7*
Mesenteric lymph node infiltration by lymphoma cells	4	7	5	9	19	13	7*	10*
Renal lymph node - infiltration by lymphoma cells	2	3	3	1	5	2	1	2*
Lumbar lymph node - infiltration by lymphoma cells	2	5	6	8	12	6	3	4*
Spleen - infiltration by lymphoma cells	4	6	6	9	19	15	9*	9**
Spleen - no abnormality found	19	21	17	8*	6	19*	22**	13
Spleen - Capsular fibrosis	0	0	1	7*	0	0	0	1
Spleen - extramedullary hematopoiesis	25	15	22	39	29	16**	16*	25
Thymus - infiltration by lymphoma cells	3	7	6	7	18	13	7*	9*
Thymus - lymphoid hyperplasia	16	20*	12	11	5	17**	15*	18**
Heart - no abnormalities found	6	15*	28*	32	31	36	37	38
Heart - progressive cardiomyopathy	30	16**	13**	16**	8	4	1*	7
Thyroid - lymphocytic infiltration	0	0	0	0	5	1	2	0*
Adrenal - no abnormality found	15	19	26*	24	5	5	9	13
Adrenal - focal cortical cell hypertrophy	9	1**	1**	0***	0	2	1	1
Adrenal - cortical atrophy	5	0*	0*	0*	0	0	1	0
Pituitary - focal hyperplasia	1	11*	10**	13**	0	2	2	5
Endocrine Pancreas - No abnormalities found	41	45	47	56	45	49	46	57*
Testis - No abnormality found	25	0***	0***	1***				
Testis - Seminiferous epithelial degeneration	0	45***	45***	45***				
Testis - pigmented interstitial cell	0	43**	45**	50***				
Testis - tubular atrophy	20	3***	2***	8***				
Testis - tubular mineralisation	17	0***	2***	2***				
Prostate - No abnormality found	34	0***	0***	0***				
Prostate - atrophy	0	41***	29***	38***				
Seminal vesicles - atrophy	0	48***	42***	42***				
Seminal vesicles - lymphocytic infiltration	1	13***	12***	7				
Seminal vesicles - Distension	28	0***	0***	0***				
Preputial gland - No abnormalities found	14	2**	2**	1**				
Preputial gland - atrophy	9	45***	42***	56***				
Ovary - No abnormality found					4	6	13**	12
Ovary - Atrophy					1	15***	23***	35***
Ovary - Cyst					36	16***	3***	5***
Uterus - Atrophy					1	35***	48***	59***
Uterus - Cystic endometrial hyperplasia					25	3***	0***	0***
Vagina - squamous epithelial hyperplasia					7	5	0*	0*
Kidney - Hyaline droplets	0	4	4	8**	12	5	3*	8
Kidney - Chronic progressive nephropathy	36	27	22**	31**	15	22	11	27
Urinary Bladder - No abnormality found	38	39	39	48	35	37	42*	49*
Rectum - No abnormality found	45	44	45	54	39	43	47*	54*
Liver - Eosinophilic cell focus	4	6	5	9	2	2	6	11*
Liver - Inflammatory cell foci	11	21	14	27*	4	11	13*	19**
Liver - Extramedullary hematopoiesis	2	3	6	11*	1	0	0	5
Salivary Gland - No abnormality found	30	8***	12***	12***	20	16	21	25
Salivary Gland - granular ductal atrophy	6	24***	16***	24***	3	5	0	4
Salivary Gland - Lymphocytic infiltration	9	23**	22**	27***	12	18	21	18
Pancreas (exocrine) - lymphocytic infiltration	3	9	8	5	3	8	15**	3
Mammary gland - no abnormality found					35	12***	4***	14***
Mammary gland - Atrophy					0	35***	41***	35***
Mammary gland - Lobular hyperplasia					7	0*	0*	0*
Lacrimal gland - Acinar cell vacuolization	17	2***	2***	4***				
Spinal Cord - No abnormality found	34	33	28	14***	19	22	28	19
Spinal Cord - Increased granulopoiesis in marrow	4	2	7	17	12	8	8	24
Sternum - Increased granulopoiesis in marrow	3	4	7	17	12	6	8	27*
Femur - Increased granulopoiesis in marrow	11	4	2**	13	9	7	1**	13

Significantly different from the Control: * P < 0.05 ** P < 0.01, *** P < 0.001

Appears This Way
 On Original

Best Possible Copy

David McGuinn, Jr., M.S., Ph. D., D.A.B.T.
Robert Dorsam, Ph. D.

NDA No. 22-201

Tumor findings

The following table lists the percentage of animals (incidence) with the finding of a particular type of mass or tumor. The incidence of the tumors in this table decreases with treatment. Most of these tumors are in sex organs so the decrease in incidence is the result of atrophy of these organs. Most of the tumors are benign.

Tissue and tumor	Male				Female			
	Control	Low Dose	Mid Dose	High Dose	Control	Low Dose	Mid Dose	High Dose
Lymphoma - follicular center cell - metastatic	4	12	2	8	24	18	14	10
Uterus - hemangiosarcoma - malignant					2	0	0	0
Preputial Gland - squamous cell carcinoma - malignant	2	0	0	0				
Kidney - carcinoma - malignant	2	0	0	0	0	0	0	0
Adrenal subcapsular cell - benign	11	0	0	0	2	0	0	0
Testis - interstitial cell adenoma - benign	4	0	0	0				
Seminal vesicle - adenoma - benign	2	0	0	0				
Seminal vesicle - granular cell - benign	2	0	0	0				
Preputial Gland - adenoma - benign	2	0	0	0				
Ovary - Hemangioma - benign					2	0	0	0
Ovary - granulosa cell tumor - benign					2	0	0	0
Ovary - luteoma - benign					2	0	0	0
Ovary - Cystadenoma - benign					2	0	0	0
Uterus - Stromal Polyp - benign					4	0	0	0
Uterus - Leiomyoma - benign					2	0	0	0
Uterus - hemangiosarcoma - benign					2	0	0	0

The following table lists the percentage of animals with the finding of a particular tumor type where the incidence of that tumor increased (percent) in treated animals.

Tissue and tumor	Male				Female			
	Control	Low Dose	Mid Dose	High Dose	Control	Low Dose	Mid Dose	High Dose
Spinal cord - histocytic sarcoma - malignant	0	2	0	2	0	0	0	0
Spinal cord - osteosarcoma - malignant	0	2	0	2	0	0	0	0
Spinal cord - hemangiosarcoma - malignant	0	2	0	0	0	0	0	0
Spinal cord - schwannoma - benign	0	0	0	0	0	0	0	2
Liver - hepatocellular adenoma - benign	21	20	16	25	2	10	14	13
Bronchio-alveolar adenoma - benign	38	22	20	23	8	28	22	20

By the analysis of the study investigators, the increased incidence reached significance by pairwise comparison in two tumor types, benign hepatocellular adenoma of the liver in high dose females ($p = 0.015$) and benign bronchio-alveolar adenoma in all groups of treated females ($p = 0.006$, 0.039 and 0.01 for low, mid and high dose animals respectively). These groups are in bold text in the table above. By trend analysis the increase in benign hepatocellular adenoma of the liver reached significance in both males ($p = 0.024$) and females ($p = 0.037$). In the Peto analysis of the benign bronchio-alveolar adenoma the result of the trend test was not statistically significant. The following table shows the results of combining benign bronchio-alveolar adenoma with malignant bronchio-alveolar carcinoma. None of the control females survived to scheduled necropsy. None of the differences in tumor incidence were different from control (see Dr. Karl Lin's analysis below).

*Appears This Way
On Original*

Male	control				Low dose				Mid dose				High dose			
	BAA	BAC	tumors	dead at time point	BAA	BAC	tumors	dead at time point	BAA	BAC	tumors	dead at time point	BAA	BAC	tumors	dead at time point
< 595 days	2	1	3	10	1		1	10			0	11	3		3	27
< 665 days	2	2	4	5	1		1	7		1	1	5	1		1	5
<735 days	3	2	5	10	1	2	3	5	1		1	4	2	1	3	10
Terminal necropsy	12	0	12	25	8	0	8	28	9	4	13	30	8		8	18
Percentage (total)			48%				26%				30%				25%	
totals	19	5	24	50	11	2	13	50	10	5	15	50	14	1	15	60

Female	control				Low dose				Mid dose				High dose			
	BAA	BAC	tumors	dead at time point	BAA	BAC	tumors	dead at time point	BAA	BAC	tumors	dead at time point	BAA	BAC	tumors	dead at time point
< 595 days		2	2	17	1		1	12	1		1	11	1	1	2	21
< 665 days	1	1	2	8	1	1	2	3			0	4	1	1	2	8
<735 days	3	1	4	25	1		1	5		2	2	5	4		4	7
Terminal necropsy*			0		11	1	12	30	10		10	30	6		6	24
Percentage (total)			16%				32%				26%				23%	
totals	4	4	8	50	14	2	16	50	11	2	13	50	12	2	14	60

BAA = broncio-alveolar adenoma
 BAC = broncio-alveolar carcinoma

When the incidence of benign hepatocellular adenoma was combined with that of malignant hepatocellular carcinoma the total incidence was between 28 and 32% in all males groups including controls and 2, 14, 14 and 13% in female controls, low dose, mid dose and high dose groups respectively. These results were not significantly different from controls in males or females ($p < 0.09$) by pairwise comparison. The combined incidence of these tumors was also not statistically different from controls by asymptotic trend test ($p < 0.09$). The following table shows these results.

Male	control				Low dose				Mid dose				High dose			
	HCA	HCC	tumors	dead at time point	HCA	HCC	tumors	dead at time point	HCA	HCC	tumors	dead at time point	HCA	HCC	tumors	dead at time point
< 595 days				10				10				11	2			27
< 665 days	1		1	5	1		1	7	0		0	5	1		1	5
<735 days	3		3	10			0	5	3	1	4	4	4		4	10
Terminal necropsy	8	2	10	25	12	3	15	28	8	2	10	30	9	1	10	18
Percentage (total)			28%				32%				28%				28%	
totals	12	2	14	50	13	3	16	50	11	3	14	50	16	1	17	60

Female	control				Low dose				Mid dose				High dose			
	HCA	HCC	tumors	dead at time point	HCA	HCC	tumors	dead at time point	HCA	HCC	tumors	dead at time point	HCA	HCC	tumors	dead at time point
< 595 days				17				12				11				21
< 665 days	0		0	8	0		0	3			0	4	1		1	8
<735 days	1		1	25	1		1	5			0	5	2		2	7
Terminal necropsy*	0		0	0	5	1	6	30	7		7	30	5		5	24
Percentage (total)			2%				14%				14%				13%	
totals	1	0	1	50	6	1	7	50	7	0	7	50	8	0	8	60

The normal incidence of hepatocellular adenoma of the liver ranges from 2 to 33 % in male CD-1 mice and from 0 to 4% in females. The normal range for hepatocellular carcinoma ranges from 0 to 1.7 % in females and 0 to 6% in males (P Baldrick and L Reeve, Toxicol. Path. 2007. 35(4):562-575).

This information was presented to the ExecCAC on December 9, 2008. Dr. Karl Lin subsequently did a statistical analysis of this data. The following table shows his results. Dr. Paul Brown and Dr. Abby Jacobs concurred by email that the results were not significant and need not be reported in the product label.

Best Possible Copy

Mouse	M	Bronchio-alveolar adenoma + Carcinoma	0.76	0.76
Mouse	F	Bronchio-alveolar adenoma + Carcinoma	0.44	0.44
Mouse	M	Hepatocellular Adenoma + Carcinoma	0.087	0.09
Mouse	F	Hepatocellular Adenoma + Carcinoma	0.086	0.089

The sarcomas in the spinal cord are unusual; Baldrick and Reeve do not list this lesion among common or less common spontaneous neoplasms. The incidence in the spinal cord did not reach significance but it is possibly related to dosing. This finding correlated with spinal inflammation noted as a cause of death above.

The following table illustrates the increase in the sarcomas in male and female mice. Sarcoma (NOS) occurred in the hind limb of one low dose male and in the cervix of one control female. These tumors were probably incidental.

Number of tumors	Control male	LD male	MD male	HD male	Control female	LD female	MD female	HD female
Subcutaneous sarcoma near the injection site		1	3				1	2
Subcutaneous sarcoma in the injection site				1				1
Sarcomas at other sites		1 (hind limb)			1 (cervix)			
Sarcomas total	0	2	3	1	1		1	3
N	50	50	49	60	50	50	49	59

The incidence of sarcoma of the skin ranges from 0 to 5% in males and 0 to 7% in females (Baldrick and Reeve, 2007). The incidence of sarcomas near the injection site is within these normal ranges but may have some association with injection.

*Appears This Way
 On Original*

2.6.6.6 Reproductive and Developmental Toxicology

1) Subcutaneous developmental Toxicity Study in the Rat - WDM

Major findings

Doses of 0.072 mg/m²/day from day 6 through day 12 followed by doses of 0.18 mg/m²/day on days 13 through 17 of gestation caused significant post-implantation loss in pregnant rats (23.6 %) and a concomitant decrease in the number of live fetuses/dam. This dose caused no significant maternal toxicity and is only about 0.13% of the proposed clinical loading dose. Dosing was associated with an increase in the number of major abnormalities in the fetuses in the high dose group (p < 0.05) but most of these abnormalities occurred in a single litter (4 of 6). In fetuses in the mid dose group (0.054 mg/m²/day from days 6 to 12 and 0.54 mg/m²/d on days 13 to 17 of gestation) there was a statistically significant increase in a number of minor skeletal abnormalities and variants. These were findings generally associated with the state of ossification and were considered to be related to maternal treatment with degarelix.

Study number: ADR0011
 Sponsor reference number: FE200486DSTOX0121
 EDR filename: tox0121-nonclinical-data.pdf
 Laboratory: _____
 Study date: January 2002
 GLP: Yes
 Audited: Yes
 Drug: Degarelix, Batch 0048262#PPL-FE4860001
 Purity 99.66%, Peptide content 87.99%

b(4)

Method

Animals: sexually mature timed-mated Sprague-Dawley rats

Doses and N

Group	N	Dose (mg/kg/day) *		Dose (mg/m ² /day)		Dose (days 13 to 17) = proposed human loading dose
		Days 6 to 12	Days 13 to 17	Days 6 to 12	Days 13 to 17	
1	24	0	0	0	0	
2	24	0.003	0.03	0.018	0.18	0.13%
3	24	0.009	0.03	0.054	0.18	0.13%
4	24	0.009	0.09	0.054	0.54	0.40%
5	30	0.012	0.03	0.072	0.18	0.13%

Dose selection: In the dose-ranging study (ADR0007), dosing with 0.03 mg/kg/day during Days 6 to 12 of gestation resulted in termination of pregnancy in all treated dams. Doses up to 0.009 mg/kg/day when dosed between Days 6 and 12 of gestation had no effect on the pregnancy rate.

Dose volume: 0.4 mL/kg
 Vehicle: 5% Mannitol_{aq}
 Route: Subcutaneous injection
 Necropsy: Day 28 of gestation

Exposure A single blood sample was collected from all females prior to dose administration on Days 7 and 14 of gestation.

Observations

Mortality Twice daily

Clinical signs daily

Body weight Day 0 and days 4 through 28

Food consumption Days 5 to 6, 6 to 9, 9 to 12, 12 to 17 and 17 to 20 of gestation.

At necropsy Number of corpora lutea, live and dead implantations, Live fetuses (weight and gender), External, visceral and skeletal abnormalities, Placental weights, Number and distribution of implantations in uterine horns, classified as early resorptions, late resorptions

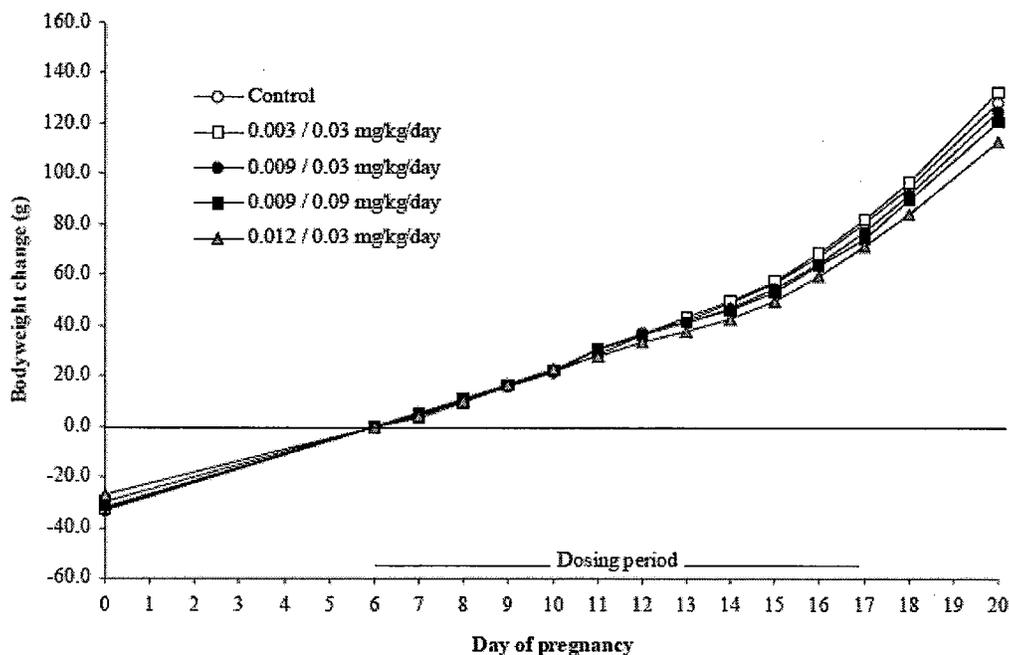
Results

Mortality All dams survived to scheduled necropsy

Clinical signs One Group 3, two Group 4 and three Group 5 dams had red colored discharge from the vulva on Days 11, 12 or 13 of gestation. Most these animals had lower bodyweight on the day of the discharge. In three animals this bodyweight reduction continued for up to five days after the discharge was observed. One of the high dose animals was not pregnant at necropsy and the other four had total resorption.

Body Wt. Small differences in body weight gain in group 5, while statistically significant, were not toxicologically significant. The following table from the report demonstrates the comparability of body weight in all animals throughout the study. The small difference in the maternal weight in Group 5 is possibly due to post-implantation loss.

Figure 1 - Group mean maternal bodyweight change (g)



Food cons. All treated dams ate amounts comparable to controls.

Pregnancy The following table from the report shows that dosing did not diminish the pregnancy rate but the rate of total resorption did increase with dosing. Pre-implantation loss was higher in Group 5 than in controls but this increase did not reach statistical significance. Post implantation loss increased with increasing dose and was statistically significant in Group 5; consequently the mean number of live fetuses per dam was significantly lower in Group 5. The investigators classified all post-implantation losses as early embryo/fetal deaths.

Table 5 - Group mean uterine / implanon data

(Page 1 of 1)

Best Possible Copy

Group	1	2	3	4	5
Treatment	Control		FE200486		
Dose (mg/kg/day) - Days 6-12	0	0.003	0.009	0.009	0.012
Dose (mg/kg/day) - Days 13-17	0	0.03	0.03	0.09	0.03

	Group 1	Group 2	Group 3	Group 4	Group 5
Number of females with implantations at scheduled kill	24	24	24	23	26
Number of corpora lutea #	356	362	371	329	401
Mean number per female	14.8	15.1	15.5	14.3	15.4
Standard deviation	2.1	2.1	2.3	1.9	2.5
Number of implantations #	331	337	346	308	353
Mean number per female	13.8	14.0	14.4	13.4	13.6
Standard deviation	2.3	1.7	1.5	1.6	3.0
Mean % pre-implantation loss #	6.8	6.3	5.8	5.7	11.0
Number of early embryo/foetal deaths	17	16	42	47	85
Number of late embryo/foetal deaths	0	0	0	0	0
Number of dead fetuses	0	0	0	0	0
Mean % post-implantation loss #	5.5	4.6	12.4	14.9	23.6+
Number of live foetuses #	314	321	304	261	268
Mean number per female	13.1	13.4	12.7	11.3	10.3+
Standard deviation	2.6	1.6	4.2	4.8	5.4
Mean % of implantations	94.5	95.4	87.6	85.1	76.4

- Statistically analysed

+ = significantly different from Controls, p<0.05 (Shirleys test)

Fetal weight Fetal and placental weights were comparable to controls in all dosed groups.

Fetal abnormalities The following table from the study report shows that the incidence of major abnormalities in group 5 was greater than that of controls. These abnormalities included imperforate anus, absent or filamentous tail, absent or fused ribs and absent neural arches and centra. This increase was statistically significant by pairwise analysis and may be dose related. In Group 4 there was a statistically significant increase in a number of minor skeletal abnormalities. The incidences of minor and variant abnormalities in Groups 2 and 3 were comparable with the Controls.

Best Possible Copy

Table 7 - Foetal examination : summary of group mean data

(Page 1 of 1)

Group	1	2	3	4	5
Treatment	Control		FE200486		
Dose (mg/kg/day) - Days 6-12	0	0.003	0.009	0.009	0.012
Dose (mg/kg/day) - Days 13-17	0	0.03	0.03	0.09	0.03

	Group 1	Group 2	Group 3	Group 4	Group 5
Combined examination (external/visceral/skeletal)					
Total number of litters examined	24	24	22	20	22
Total number of fetuses examined	314	321	304	261	268
Number with major abnormalities #	1	2	0	0	6*
Mean % of fetuses examined	0.3	0.6	0.0	0.0	2.3
Number of litters affected	1	2	0	0	3
Number with minor abnormalities #	87	93	87	85	66
Mean % of fetuses examined	29.0	29.4	27.7	33.0	26.8
Number of litters affected	24	22	21	20	18
Number with variations #	182	173	175	151	153
Mean % of fetuses examined	58.4	54.4	57.3	57.3	57.9
Number of litters affected	24	24	22	20	22

- Statistically analysed *p<0.05

2) Subcutaneous developmental Toxicity Study in the Rabbit – WDM

Major findings

A daily dose of 0.024 mg/mg² on days 6 through 14 followed by doses of 0.072 mg/m² from days 15 through 27 of gestation was associated with a decrease in the number of does with implantations, the number of corpora lutea per female, the number of implantations and the number of live fetuses per female. Some of these decreases reached statistical significance in the mid-high (0.012 mg/mg² on days 6 through 14 followed by doses of 0.036 mg/m² from days 15 through 27) and high dose groups particularly the number of live fetuses. Dosing was also associated with an increase in mean post-implantation loss. There was an increase in the number of fetuses with minor abnormalities in the high dose group and an increase in the incidence of major abnormalities in the mid dose group (5 in three litters) but the number of fetuses in the high dose group was so diminished as to render any determination of teratogenicity equivocal. The high dose caused only minimal toxicity in the does (minimal decreased body weight gain). Thus, a daily dose of degarelix that was just 0.05% that of the proposed loading dose was a potent abortifacient in rabbits.

Study number: ADR0009
 Sponsor reference number: FE200486DSTOX0201
 EDR filename: tox0201-nonclinical-data.pdf
 Laboratory: _____
 Study date: February 2002

b(4)

GLP Yes
 Audited Yes
 Drug Degarelix, Batch 0048262#PPL-FE4860001
 Purity 99.66%, Peptide content 87.99%

Method
 Animals Timed-mated sexually mature female New Zealand White rabbits approximately 4 months of age and weighing between 3 and 4 kg

Doses and N

Group	N	Animal Identification	Dose (mg/kg/day) *		Dose (mg/m ² /day)		Dose (days 15 to 27) + proposed human loading dose
			Days 6 to 14	Days 15 to 27	Days 6 to 14	Days 15 to 27	
1	20	1-20	0	0	0	0	
2	20	21-40	0.0003	0.001	0.0036	0.012	8.96E-05
3	20	41-60	0.001	0.001	0.012	0.012	8.96E-05
4	20	61-80	0.001	0.003	0.012	0.036	2.69E-04
5	30	81-110	0.002	0.006	0.024	0.072	5.37E-04

Additional animals were allocated to the high dose group to allow for lower numbers of pregnant females at term
 All doses are expressed as mg of peptide (free base)

Dose selection On the basis of — Study numbers ADR0008 and ADR0015, not reviewed. These earlier range finding studies determined that rabbits were very sensitive to the effects of degarelix during the early phase of gestation (days 6 through 14). These studies determined that the dose could be increased after this period.

Schedule Daily from day 6 of gestation to day 27

Dose volume 0.04 mL/kg

Vehicle 5% Mannitol_{aq}

Route Subcutaneous injection

Necropsy Day 28 of gestation

Exposure A single blood sample of approximately 1.0 mL was collected from the marginal ear vein of 2 females per group per time-point on each of Days 6 and 15 of gestation. The time-points were: - 2, 4, 7, 12 and 24 hours after dosing. In addition a single blood sample was taken from 5 females per group on Day 28 of gestation. Results not reported.

b(4)

Observations

Mortality Twice daily

Clinical signs Twice daily

Body weight Day 0 and days 4 through 28

Food cons Days 4 through 6 then every two days

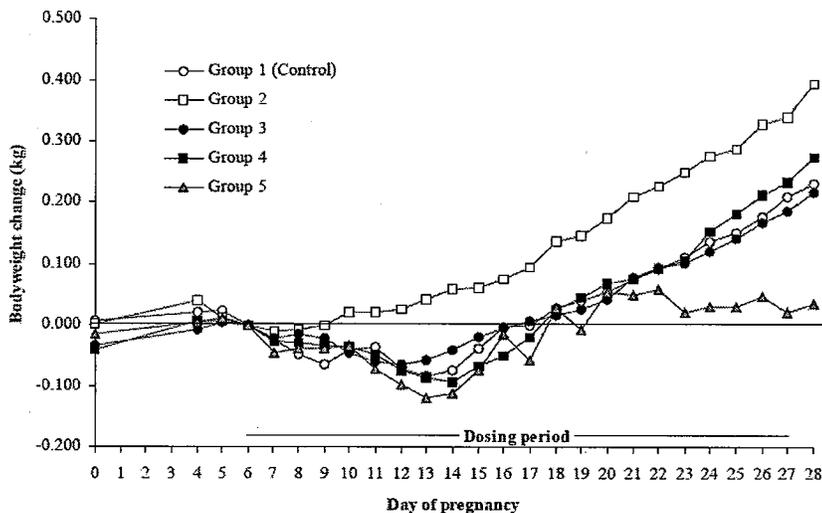
At necropsy Number of corpora lutea, live and dead implantations, Live fetuses (weight and gender), Ecternal, visceral and skeletal abnormalities, Placental weights, Number and distribution of implantations in uterine horns, classified as early resorptions, late resorptions

Results

Mortality All dams survived to scheduled necropsy

Clinical signs Five dams in Group 5, one in Group 4 and one in Group 3 had red staining on the tray liner during mid-gestation (sporadically between Days 11 – 28). All these animals were pregnant at scheduled necropsy. One doe in Group 4 aborted on Day 17 of gestation. Seven dams in Group 4 and twelve in Group 5 aborted or delivered prematurely between Day 22 and 28 of gestation.

Body wt All treated animals gained less weight than controls after day 9 of gestation. The investigators constructed the following graph to illustrate the decrease in weight gain in groups 2, 3, 4 and 5. All treated animals resumed a normal pattern of weight gain after about day 14 but group 5 animals again stopped gaining weight after day 20.



Food cons. From about day 22 through the end of the experiment all treated animals ate less than controls.

Gross path. There were no gross changes in the treated dams.

Pregnancy The investigators constructed the following table to show that dosing was associated with a decrease in the number of does with implantations, the number of corpora lutea, the number of implantations and the number of live fetuses. Some of these decreases reached statistical significance in the mid-high and high dose groups particularly the number of live fetuses. Dosing was also associated with an increase in mean post-implantation loss. Thus, degarelix had a profound effect on pregnancy even at these very low doses.

Appears This Way
On Original

Table 5 - Group mean uterine / implantation data

Best Possible Copy

(Page 1 of 1)

Group	1	2	3	4	5
Treatment	Control		FE200486		
Dosage (mg/kg/day) : Days 6 - 14 of gestation	0	0.0003	0.001	0.001	0.00
Dosage (mg/kg/day) : Days 15 - 27 of gestation	0	0.001	0.001	0.003	0.00
	Group 1	Group 2	Group 3	Group 4	Group 5
Number of females with implantations at scheduled kill	19	18	18	11	10
Number of corpora lutea	184	183	162	102	77
Mean number per female	9.7	10.2	9.0	9.3	7.7*
Standard deviation	2.2	2.4	1.7	2.7	2.7
Number of implantations	156	153	143	85	75
Mean number per female	8.2	8.5	7.9	7.7	7.5
Standard deviation	2.4	2.6	2.5	3.3	2.6
Mean % pre-implantation loss	14.6	16.4	11.5	18.1	2.0*
Number of early embryo/foetal deaths	11	8	9	20	40
Number of late embryo/foetal deaths	9	2	8	8	3
Number of dead fetuses	1	0	0	0	0
Mean % post-implantation loss	12.9	6.7	16.0	27.8	64.9*
Number of live fetuses	135	143	126	57	32
Mean number per female	7.1	7.9	7.0	5.2*	3.2***
Standard deviation	2.2	2.5	2.8	2.9	4.2
Mean % of implantations	87.1	93.3	84.0	72.2	35.1

* = statistically analysed p<0.05
 *** = statistically analysed p<0.001

Fetal health

Treatment did not affect the male to female ratio of the fetuses compared to controls. Group 5 female fetuses weighed somewhat more than controls. Mean fetal weight in other groups was comparable to controls. Placental weight was increased in all treated groups and reached significance in groups 4 and 5. The following table from the study report shows these changes.

Appears This Way
 On Original

Table 6 - Group mean litter weights (g) / foetal data

Best Possible Copy

(Page 1 of 1)

Group	1	2	3	4	5
Treatment	Control			FR200486	
Dosage (mg/kg/day) : Days 6 - 14 of gestation	0	0.0003	0.001	0.001	0.002
Dosage (mg/kg/day) : Days 15 - 27 of gestation	0	0.001	0.001	0.003	0.006
	Group 1	Group 2	Group 3	Group 4	Group 5
Number of females with live foetuses at scheduled kill	19	18	17	10	4
Number of live foetuses	135	143	126	57	32
Mean number per female	7.1	7.9	7.4	5.7	8.0
Standard deviation	2.2	2.5	2.3	2.5	1.2
Number of male foetuses	52	71	54	31	16
Number of female foetuses	73	72	72	26	16
Mean # male foetuses	44.0	47.8	45.1	52.7	48.4
Mean litter weight	266.6	322.0	287.8	222.6	338.2
Standard deviation	67.6	93.3	89.1	71.0	54.7
Mean foetal weight	36.5	41.0	39.6	40.8	42.2
Standard deviation	4.2	2.6	4.0	4.9	1.9
Mean foetal weight - males only	36.5	41.3	39.5	41.5	42.5
Standard deviation	5.5	3.2	4.1	5.0	1.4
Mean foetal weight - females only	36.2	41.3	39.0	40.3	42.1
Standard deviation	4.0	2.7	3.1	5.7	2.2
Mean placental weight	3.95	4.51	4.24	5.43	5.55
Standard deviation	0.90	0.90	0.85	1.04	0.75

statistically analysed

Fetal abnormalities

The interpretation of the fetal pathology in the high dose group is rendered difficult by the fact that only four does had live litters at scheduled necropsy leaving only 32 fetuses available for examination. There was an increase in the incidences of an additional aortic blood vessel and 27 pre-sacral vertebrae and fused sternbrae in this high dose group. These findings may be related to degarelix treatment. Tissue distribution studies (above) showed that degarelix accumulates in the aorta; GnRH may be involved in aortic development.

The number of major abnormalities was increased in group 4 in comparison with controls. The investigators dismiss this finding as incidental because there was no dose related trend, but this conclusion is not justified since the low number of fetuses obviates the ability to observe a dose effect. Nevertheless, the findings were dissimilar in nature and the majority of the abnormalities were present in isolation in different fetuses. Attribution of these abnormalities to degarelix treatment remains equivocal. There was an increase in the number of fetuses with minor abnormalities in the high dose group. There were no treatment-related differences in the incidence of any minor abnormality or variations observed in Groups 2, 3 or 4. The investigators constructed the following table to demonstrate these findings.

Appears This Way
On Original

Table 7 - Foetal examination : summary of group mean data

Best Possible Copy

(Page 1 of 1)

Group	1	2	3	4	5
Treatment	Control		FE200486		
Dosage (mg/kg/day) : Days 6 - 14 of gestation	0	0.0003	0.001	0.001	0.002
Dosage (mg/kg/day) : Days 15 - 27 of gestation	0	0.001	0.001	0.003	0.006

	Group 1	Group 2	Group 3	Group 4	Group 5
Combined examination (external/visceral/skeletal)					
Total number of litters examined	19	18	17	10	4
Total number of fetuses examined	136	143	126	57	32
Number with major abnormalities	2	3	3	5*	1
Mean % of fetuses examined	1.3	2.1	2.3	7.3	2.8
Number of litters affected	2	3	2	3	1
Number with minor abnormalities	40	45	36	22	16*
Mean % of fetuses examined	30.9	33.7	27.4	33.9	50.0
Number of litters affected	16	16	12	7	4
Number with variations	129	132	122	53	30
Mean % of fetuses examined	93.5	93.2	92.2	91.7	94.4
Number of litters affected	19	18	16	10	4

* = statistically analysed p<0.05

3) Subcutaneous Return to Fertility Study in the Male Rat – WDM

Major Findings

Single doses of 1 mg/kg or five fortnightly doses of degarelix caused complete loss of fertility in male rats. Fertility returned in most animals between 10 to 14 weeks after the end of dosing demonstrating pharmacological reversibility of the effects of degarelix.

Study number: ADR0019
 Sponsor reference no: FE200486DSTOX0205
 EDR filename: tox0205-nonclinical-data.pdf
 Laboratory: _____
 Study date: November 2002
 GLP: Yes
 Audited: Yes
 Drug: Degarelix, Batch 0048262#PPL-FE4860001
 Peptide content 91.77 %

Method

Animals: Sprague-Dawley derived rats of the ~~CD~~ CD (SD) IGS BR VAF PLUS
 Doses and N: 0, 0.5 or 1 mg/kg (0, 3 or 6 mg/m²) according to the following schedule (from the study report)
 Groups 1 and 2 received a single dose
 Groups 3 and 4 received 5 fortnightly doses

b(4)

b(4)

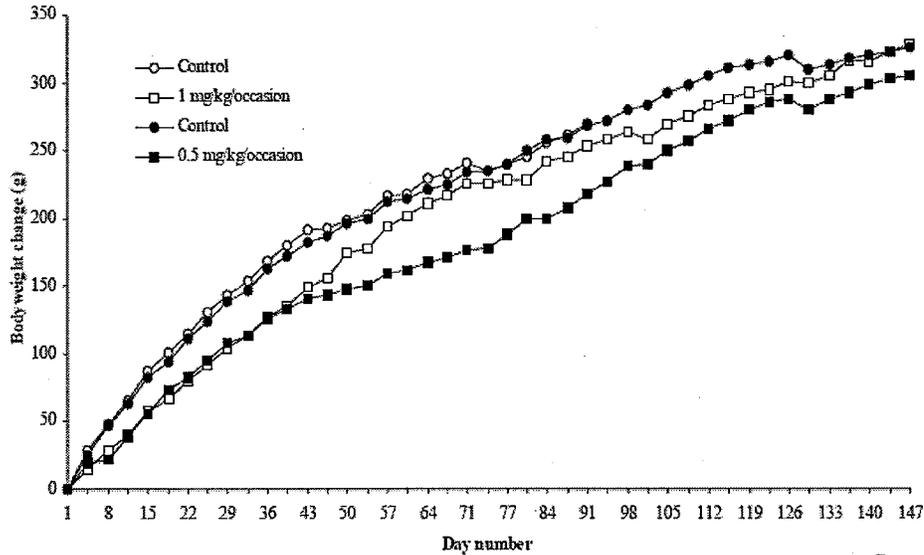
Group Number	Colour code	Number of animals		Animal identification numbers		Day of Dosing	Male dose level (mg/kg/occasion) FE200486
		Males	Females	Males	Females		
1	White	20	40	1-20	81-90 101-120 121-130*	1	Vehicle control
2	Green	20	62	21-40	91-100 131-160* 261-282	1	1.0
3	Yellow	20	23	41-60	161-183	1, 15, 29, 43, 57	Vehicle control
4	Pink	20	41	61-80	201-241	1, 15, 29, 43, 57	0.5

* Females 121-140 were also used for the first pairing with Group 2 males. As none of the females were mated, they were retained for the second pairing; females 121-130 for pairing with Group 1 males and females 131-140 for pairing with Group 2 males.

Route	Subcutaneous injection
Dose volume	1 mg/kg
Mortality	Twice daily
Clinical observations	Daily
Bodyweight	Twice weekly
Mating	Group 1 males – paired in weeks 6 and 10 after the last dose Group 2 males – paired in weeks 2, 6, 10 and 14 after the last dose. Three males were also paired in week 22. Group 3 males – paired in weeks 10 and 14 after the last dose Group 4 males – paired in 10, 14, 18 and 22 after the last dose
Male necropsy	After individual fertility was established
Female necropsy	Mated females – gestation day 13 Non-mated females – 14 days after pairing
Female observations	Pregnancy status, Number of corpora lutea, Number and distribution of implantations in uterine horns, classified as early resorptions, dead embryos or live embryos
Results	
Plasma concentration	In control males, the concentration of degarelix was below the limit of quantitation. In Group 2 males, the plasma concentration of degarelix was 2.4 (range 1.8 to 3.1) ng/mL and in Group 4 males 1.7 (range 1 to 3.5) ng/mL at the time of the first pairing 2 weeks after the (last) dose.
Mortality	None
Clinical signs	No dose related signs
Body weight	The following graph from the study report shows that Group 2 males weighed slightly less then controls as a result of treatment while Group 4 males weighed

significantly less than controls and did not completely recover the weight after the end of treatment.

Figure 1 - Group mean male bodyweight change (g)



Best Possible Copy

The following table shows that Group 2 males were completely infertile after a single dose of 1 mg/kg. The fertility of these animals slowly returned and was 90% complete 14 weeks after treatment. Animals treated with five fortnightly doses were also completely infertile 2 weeks after the end of dosing. Fertility returned in these animals within ten weeks after the end of dosing.

	Group 1		Group 2				Group 3			Group 4			
	6 weeks	10 weeks	2 weeks	6 weeks	10 weeks	14 weeks	22 weeks	10 weeks	14 weeks	2 weeks	6 weeks	10 weeks	14 weeks
Pairing (time post dose)	20	20	20	20	20	20	3	20	3	20	20	20	1
N	20	20	0	0	18	20	1	19	3	0	2	19	1
Sperm found in vaginal smear	20	20	0	4	18	20	2	19	3	0	15	20	1
Copulation plugs	20	20	0	0	9	18	1	19	3	0	0	19	1
Pregnant females	20	20	0	0	9	18	1	19	3	0	0	19	1
Females with live fetuses	20	20	0	0	9	18	1	19	3	0	0	19	1

Gross findings – No toxicologically significant changes in females. One male that had not mated successfully had small testicles

Organ Weights – low relative weight in testicles and epididymides in some males that had not completely recovered in Group 2. Statistically significant reduction in testicles and epididymides weights in Group 4.

4) Subcutaneous Fertility and Early Embryonic Development Study in the Female Rat – RTD

Major Findings

- Pregnant females receiving 0.3 mg/kg degarelix gained less weight than the control-treated pregnant females.
- The HD group (0.3 mg/kg) remained in estrus over a 21 day period, whereas control and lower dose groups completed 1 or 2 estrus cycles in 7 days.

- 0.1 (0.6 mg/m²) and 0.3 mg/kg degarelix (1.8 mg/m²) had decreased fertility rate (72 and 80%, respectively) when compared with control and 0.01 mg/kg dosing groups, both of which had 100% fertility.
- 0.1 (0.6 mg/m²) and 0.3 mg/kg degarelix (1.8 mg/m²) had less implantations at gestational day 13 and lower number of corpora lutea compared with control. These rats had a decreased number of implantations per female and the percentage of pre-implantation loss was elevated in these groups. Lastly, these two dosing groups had reduced number of live embryos per female (12.8 embryos and 9.5 embryos) when compared with the control group (15.1 embryos).

Study number: ADR0014
Sponsor reference no: FE200486DSTOX0202
EDR filename: tox0202-nonclinical-data.pdf
Laboratory _____
Study Date: April 26, 2002
GLP Yes
Audited Yes
Drug Degarelix, Batch 0048262#/PPL-FE4860001

b(4)

Method

Animals	Sprague-Dawley derived rats of the — CD (SD) IGS BR VAF PLUS		
Doses	degarelix dose (mg/kg)	Group	Mating after dose
	Vehicle	1	Paired 1 week post-dose
	0.03 (0.18 mg/m ²)	2	Paired 1 week post-dose
	0.1 (0.6 mg/m ²)	3	Paired 1 week post-dose
	0.3 (1.8 mg/m ²)	4	Paired 3 weeks post-dose
N	25 females in each group		
Age	8-10 weeks of age		
Route	Subcutaneous injection 0.1 and 0.3 mg/kg		
Dose volume	1 mL/kg		
Mortality	Twice daily		
Clinical Observations	Daily		
Bodyweight	Twice weekly		
Male necropsy	Not conducted		
Female necropsy	Mated females – gestation day 13		
Female observations	Pregnancy status, number of corpora lutea, Number and distribution of implantations in uterine horns, classified as early resorptions, dead embryos or live embryos		

b(4)

Results

Mortalities and Clinical Observations –

No mortalities occurred in this study. Observations of hair loss and scabbing were evenly distributed throughout dosing groups, including control, and therefore are not drug-related.

Body Weight

The body weights of female rats that received 0.1 or 0.3 mg/kg degarelix were slightly elevated in Days - 1 through Day 7 of the study. During gestation, pregnant females dosed with 0.3 mg/kg gained less weight than the control group. (both graphs excerpted from the study report)

Best Possible Copy

Figure 1 - Group Mean Female Bodyweight Change (g) : Pre-pairing

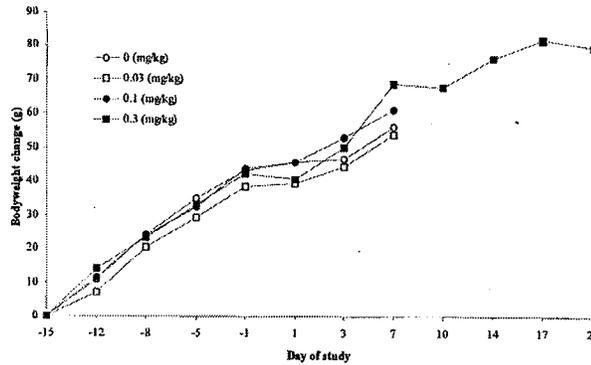
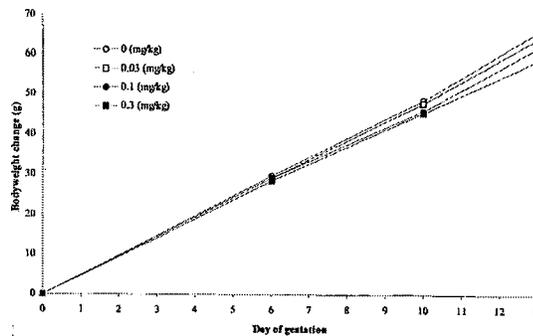


Figure 2 - Group Mean Female Maternal Bodyweight Change (g) : Gestation



Estrus cycling

Estrus cycling was slightly reduced in females of Group 3 (0.1 mg/kg degarelix) compared with control or Group 2 during the 7 days pre-pairing. Females dosed with 0.3 mg/kg were allowed 21 days before pairing, yet remained in estrus through this extended period of time.

Group	N	Mean number of estrus cycles	
		Pre-dosing 18 days	Pre-pairing 7 days
1	25	3.5	1.3
2	25	3.2	1.5
3	25	3.4	0.9
4	25	3.5	0.9 (given 21 days)

Treatment did not affect the time course of mating. All females in all groups (except one in the high dose) mated within 4 days of pairing. The final high-dose female mated on day 7. Females receiving 0.1 or 0.3 mg/kg degarelix had reduced fertility compared to the control group. (Table excerpted from study report).

Appears This Way
 On Original

Table 9 - Group Mean Fertility and Mating Data

(Page 1 of 1)

Group	Sex	Number paired	Number mated	Number fertile	Copulation index#	Fertility index#
1	F	25	25	25	100.0	100.0
2	F	25	25	25	100.0	100.0
3	F	25	25	18	100.0	72.0**
4	F	25	25	20	100.0	80.0*

= Statistically analysed * = p<0.05 ** = p<0.01

Best Possible Copy

Implantation data

Females dosed with 0.1 or 0.3 mg/kg degarelix had fewer implantations at gestational day 13 and lower number of corpora lutea compared with control. In addition, these rats had a decreased number of implantations per female and the percentage of pre-implantation loss was elevated in these groups. Lastly, the number of live embryos per female in control group was 15.1 embryos, whereas the groups that received 0.1 and 0.3 mg/kg degarelix had 12.8 and 9.5 embryos, respectively.

Implantation data	Dose Degarelix (mg/kg)			
	Vehicle	0.03	0.1	0.3
Females with implantations at gest. day 13	25	25	18	20
Number of corpora lutea	409	415	259	273
Mean number per female (#)	16.4	16.6	14.4**	13.7***
Standard deviation	1.6	1.6	3.3	3.3
Number of implantations	399	387	239	206
Mean number per female (#)	16	15.5	13.3*	10.3***
Standard deviation	1.7	2.6	4.5	4.1
Mean % pre-implantation loss (#)	2.5	6.7	10.7	25.2***
Number of early embryo/fetal deaths	21	15	9	17
Number of dead embryos	0	0	0	0
Mean % post-implantation loss (#)	5.1	3.9	3.5	7.3
Number of live embryos	378	372	230	189
Mean number per female (#)	15.1	14.9	12.8	9.5***
Standard deviation	1.6	2.6	4.3	3.8
Mean % of implantations	94.9	96.1	96.5	92.7

(#) = sponsor statistically analyzed,
* = p<0.05, ** = p<0.01, *** = p<0.001

Females that did not become pregnant in the course of the study had no visible signs of early resorptions in the uterus, a determination made from ammonium sulphide staining.

2.6.6.7 Local tolerance

Not reviewed

2.6.6.8 Special toxicology studies

Not reviewed

2.6.6.9 Discussion and Conclusions

See below

2.6.6.10 Tables and Figures

Appears This Way
On Original

2.6.7 TOXICOLOGY TABULATED SUMMARY

Acute Toxicity

Study Number, Date and Species	GLP	Route	Doses		N	Mortality	Toxicity
			mg/kg	mg/m ²			
FE200486DSTOX9902 October 2001	Yes	IV	0	0	5/5	0	
Mouse			30	90	5/5	0	distress including partially closed eyelids, cold extremities and hunched posture
			100	300	5/5	3/5 m, 3/3 f	distress including partially closed eyelids, cold extremities and hunched posture. Dose dependant decrease in size of sex organs
FE200486DSTOX9903 July 2001	Yes	SC	0	0	5/5	0	
Rat			30	180	5/5	0	
			100	600	5/5	0	In male rats, dose dependant decreases in kidney, liver, prostate, seminal vesicles, testes and epididymus weight, thymus increased in weight by 68% compared to control. In female rats, the liver, lungs, splcen and thymus grew larger, while the ovaries an
FE 200486DSTOX9905 July 1999	Yes	SC	0	0	6/6	0	
Cynomolgus Monkey			0.03	0.36	3/3	0	decreased ovary weight
			0.3	3.6	3/3	0	decreased ovary weight
			3	36	6/6	0	Splenomegaly & decreased body weight in males. The testes, prostate and epididymus decreased, reversible. In females, decreased uterine weight. Decreased ovary weight reversible.
			30	360	6/6	0	Splenomegaly & decreased body weight in males. The testes, prostate and epididymus decreased at Week 2. Decreased uterus and ovary weight by week 2 not reversible at wk 39.
FE200486DSTOX9901 May 2000		IV	0	0	5/5	0	
Rat	Yes		30	180	5/5	0	Respiratory distress and partially closed eyelids, lower body wt. Decreased sex organ wt.
			100	600	5/5	2/2 m, 2/2 f	died within minutes probably neurological toxicity. No more animals were treated with this dose
FE 200486 DS TOX 9904 May-99		SC	0	0	20/20	1 m, 1 f incidental	
Rat	Yes		0.03	0.18	10/10		Dose related inhibition of LH, FSH, Testosterone & estradiol.
			0.3	1.8	10/10		Dose related inhibition of LH, FSH, Testosterone & estradiol. Recovery 16 weeks. Dose dependant wt loss. Sex organ atrophy
			3	18	20/20	1 f pneumonitis	Dose related inhibition of LH, FSH, Testosterone & estradiol. Recovery 16 weeks. Dose dependant wt loss. Sex organ atrophy. Decreased red cell parameters in males.
			30	180	20/20	1 male injection site damage, 1 male injury	Dose related inhibition of LH, FSH, Testosterone & estradiol. Recovery 28 weeks. Dose dependent wt. loss. Sex organ atrophy. Red cell parameters decreased in males increased in females. Increased WBC in females.

Appears This Way
 On Original

Repeat Dose Toxicology

Study Number, Date and Species	GLP	Dose Frequency Study Duration	Route	Doses (mg/kg)	Doses (mg/m ²)	N	Mortality	Toxicity
FE200486TOX0111 10/18/2001 CD-1 Mice	Yes	Fortnightly 13 weeks	SC	0 1 10 100	0 3 30 300	10 per sex 10 per sex 10 per sex 10 per sex		Decreased heart and kidney weight. Decreased epididymus, testes, prostate and ovary weight and male splenomegaly. Increased female body weight and splenomegaly. Decreased female pituitary, salivary gland and uterus weight. ↓ seminal vesicle size. Swelling at injection site. Increased WBC populations, female body weight, spleen size, hemopoiesis and lymphoid hyperplasia. Decreased male body weight.
FE200486TOX0112 10/4/2001 Wistar Rats	Yes	Fortnightly 13 weeks	SC	0 0.5 5 50	0 3 30 300	10 per sex 10 per sex 10 per sex 10 per sex	No mortality	Decreased kidney and liver weight, ↑ female body weight, WBC, alk. phos, cholesterol and thymus weight. Splenomegaly. ↓ prostate, epididymus, and testes weight, ↓ female adrenals, ovary and pituitary weight, ↓ Female lung weight Swelling at site of injection, ↑ female glucose, and hemopoiesis, ↑ liver weight.
FE200486TOX0403 11/30/2004 Rat/ Wistar :WI (Han)	Yes	Fortnightly 13 weeks	SC	0 0+ castration 0.5 50	0 0 0 3 300	10 males 10 males 10 males 10 males	No mortality	Decreased heart, liver, kidney and body weight. Decreased testicular size and increased brain, adrenal and thymus weight Decreased body weight, testicular size, urine volume, and food consump., ↓ heart, liver, kidneys, and reproductive organs weight. ↑ adrenal and thymus weight Injection site sores, ↑ platelets and WBC ↓ APTT, atrophy of reproductive organs
FE200486TOX0101 4/3/2001 CD Rats	Yes	Fortnightly 26 weeks	SC	0 0.5 2 10	0 3 12 60	20 per sex 20 per sex 20 per sex 20 per sex		Decreased kidney, liver, pituitary and male and female reproductive organs. Small testes size was reversible at lowest dose. ↓ male and ↑ female weight was reversible. ↑ female WBCs, ↑ male urea and creatinine, ↓ urine volume ↑ monocytes and Ca ²⁺ in males, ↓ Ca ²⁺ in females. Increased Cl ⁻ in both sexes Injection site swelling. ↑ neutrophil, ↓ male urine, K ⁺ , Na ⁺ , and Cl ⁻ , ↑ hemopoiesis
FE2004860401 6/22/2004 Rat/HsdBrlHan:WIST	Yes	Fortnightly 26 weeks	SC	0 10 50 100	0 60 300 600	30 per sex 30 per sex 30 per sex 30 per sex		Swelling at injection site, ↓ male and ↑ female weight. Decreased testes, prostate, epididymus, seminal vesicle, uterus and ovaries. ↓ female pituitary weight, ↑ female alk. phos., ↓ albumin, liver, pituitary, and kidney weight, ↑ thymus, cholesterol. ↓ male and ↑ female RBCs, ↑ leukocytes. ↑ male urine vol., ↑ platelets, ↓ APTT,

b(4)

Appears This Way
On Original

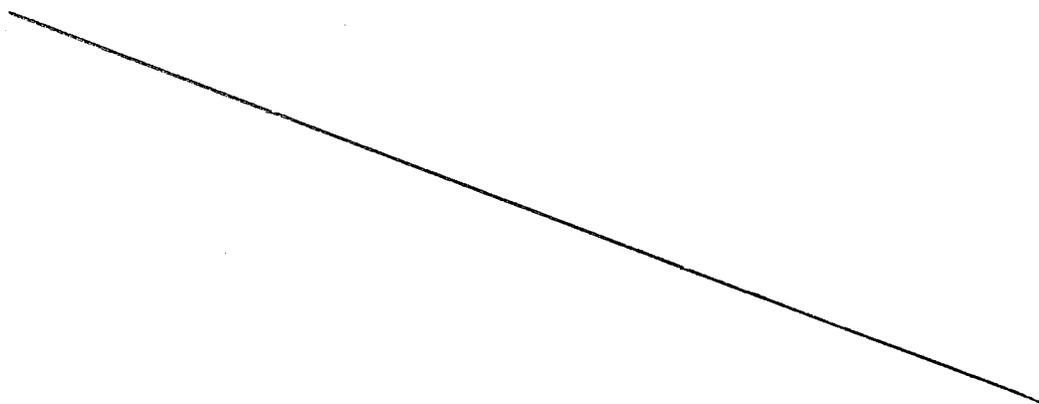
Tabulated Summary - Repeat dose studies (continued)

Study Number, Date and Species	GLP	Route	Doses (mg/kg)	Doses (mg/m ²)	N	Mortality	Toxicity
FE200486ROX0109 9/18/2001 SPF Sprague Dawley Rats	Yes	Daily 14 days	IV	0 0.05 0.35 2.5	0 0.3 2.1 15	10 per sex 10 per sex 10 per sex 10 per sex	No mortality ↓ male and ↑ female weight, ↑ cholesterol ↓ testes, seminal vesicle, prostate, epididymus, ovary and uterus weights, ↑ thymus, histopath effects in reproductive organs. ↑ male hemoglobin, RBC and hematocrit, ↑ male urea, urinary GGT, ↑ kidney and liver weights, ↓ female liver enzymes, total protein.
FE200486ROX0122 12/13/2001 Sprague Dawley Rats	Yes	Daily 28 days	IV	0 0.03 0.3 3	0 0.18 1.8 18	10 per sex 10 per sex 10 per sex 10 per sex	No mortality No remarkable toxicologic effect ↓ LH, estradiol, and testosterone levels, ↓ testes, prostate, epididymus, seminal vesicles, ovaries and uterus, ↑ thymus, ↓ male and ↑ female weight, ↓ water intake, RBC, hemoglobin, hematocrit, male APTT and urine pH, ↑ WBC, urea, creatinine and cholesterol and urine volume. ↑ spleen and adrenals. Deposition of degeralix in liver, spleen and kidneys
FE200486TOX0126 6/26/2001 Cynomolgus Monkeys	Yes	Monthly 12 months (13 doses)	SC	0 0.5 5 50	0 6 60 600	8 per sex 8 per sex 8 per sex 8 per sex	↓ testosterone and estradiol levels, ↓ testes, epididymus, seminal vesicles, prostate, ovary and uterus weight, ↑ thymus weight. ↓ male body weight, injection site swelling at MD and HD which recovered, ↓ male APTT, ↓ male red cell parameters, ↑ female GGT, low sperm count and reproductive organ atrophy Effects at this dose were similar to those at lower doses.
FE200486TOX0115 10/4/2001 Cynomolgus Monkeys	Yes	Daily 14 days	IV	0 0.025 0.175 1.25	0 0.3 2.1 15	3 per sex 3 per sex 3 per sex 3 per sex	No mortality Individual incidences of 20-35% reduction in blood pressure of female monkeys in all doses ↓ WBC Injection site swelling, ↓ female heart rate (1/3), ↑ heart rate, ↓ weight of sex organs, ↑ ALT
FE200486TOX0120 11/21/2001 Cynomolgus Monkeys	Yes	Daily 28 days	IV	0 0.25 0.8 2.5	0 3 9.6 30	3 per sex 3 per sex 3 per sex 3 per sex	No mortality ↓ male neutrophils, ↑ ALP, ↓ testosterone and estradiol levels and epididymus, testes, seminal vesicles, prostate and uterus weights ↓ ovary weight ↓ blood pressure (effect 30 min. post drug, recovered by 4 hours), marginal ↑ heart rate, ↑ ALT, cholesterol and urine volume.

Appears This Way
 On Original

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The following table from the product label lists the toxicities noted in the clinical study submitted in support of this NDA and correlates them with signs and symptoms seen in the non-clinical studies reviewed above.



b(4)

Degarelix binds to the isolated human GnRH receptor with an affinity (K_i) of about 1.7 nM. It displaces human GnRH from the isolated receptor with a pA_2 of 9.1. The inhibition of the GnRH receptor prevents the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary gland so these hormonal signals never reach the gonads in either males or females. Pulses of circulating LH and FSH are necessary for the stimulated release of testosterone from the testes. The effect of this inhibition is rapid. Single subcutaneous doses of degarelix as low as 1- μ g/kg caused a significant decrease in plasma testosterone in male rats six hours after the injection. The degree of testosterone suppression increases with increasing dose. Degarelix also suppressed plasma testosterone in mice, dogs, rats and monkeys demonstrating that the structure of the active site of the GnRH receptor is highly conserved across species and that all these species are appropriate models for degarelix toxicology and pharmacology. This decrease in testosterone was accompanied by aspermia, derangement of sperm morphology and loss of fertility. The lack of stimulation by circulating testosterone results in atrophy in the prostate, testes and epididymides that increased in severity with increasing dose. The time to recovery of reproductive function in males also increased with increasing dose and total time of exposure. This decrease in testosterone is the desired clinical effect and the primary endpoint of the clinical study. Treatment with degarelix also caused a rapid decrease in plasma concentrations of estradiol in all species studied again secondary to the inhibition of the release of LH and FSH. This decrease resulted in sporadic or infrequent estrus or complete amenorrhea depending on the dose and the total time of exposure. Recovery of reproductive function in females was somewhat faster than it was in males probably because testicular atrophy was somewhat worse than atrophy in the female sex organs after treatment.

The oophorectomized rat is well established as a non-clinical model for osteoporosis associated with menopause in women (JP Bonjuor *et al.* (*Osteoporos Int.* 1999;9(5):379-93). Oophorectomy in the rat causes increased body weight, hyperphagia and decreased bone strength. Thus the dose dependent weight gain seen in female animals in all the studies in this review is consistent with estradiol deprivation. This effect also correlates with the weight gain seen in some women post-menopause (A Samat *et al.* *Menopause Int.* 2008 Jun;14(2):57-62). In all but one toxicology study reviewed here (13 week dosing in rats) animals either ate less than controls or there was no effect on food consumption

because the dose was relatively low. This finding is not paradoxical. Anorexia nervosa is associated with a suppression of hypothalamic secretion of GnRH and the resultant decrease in pulsatile LH secretion (NH Golden and JL Carlson *Ann N Y Acad Sci.* 2008;1135:163-78). Thus the absence of LH results in mild anorexia in most of the animal studies as opposed to the hyperphagia seen in post-menopausal women.

JMY Jiang *et al.* (*J. Nutr.* 138:2106-2110, November 2008) have shown that the increased stress on bones caused by increased body weight does not compensate for the demineralization and loss of strength associated with oophorectomy in rats. None of the non-clinical studies submitted to this NDA directly measured bone strength but there was evidence in some of the studies of a shift in bone homeostasis. In two studies (the two week study in rats and the two week study in monkeys) plasma concentrations of deoxyypyridinoline, a marker of bone resorption, increased with increasing dose of degarelix. In both these studies the high dose was significantly less than the clinical dose on a mg/m^2 basis and the studies were much shorter than the clinical course of treatment; they may not adequately predict the clinical extent of the toxicity to bone. From 12 month monkey study, the epiphyseal plates of three males from the control group were not found, in addition, three females from the control group also had slight or severe decrease in the size of their epiphyseal plates. This indicated the completion (or near completion) of the maturation process of this growing structure. Conversely, the majority of treated monkey from both sexes had normal epiphyseal plates in the stifle joint which indicates that closure of the growth plates was possibly delayed. Clinically, patients complained of back pain and arthralgia. It is likely that degarelix decreases bone remodeling consistent with its effects on sex hormone concentrations.

Orchiectomized rats weigh less than controls and androgen hormone replacement prevents this decrease in weight gain and also prevents bone loss (N. Gaumet-Meunier *et al. Calcif Tissue Int* (2000) 66:470-475). These findings are consistent with the results of the non-clinical studies in this submission. In all species studied, male body weight increases less than that of controls as a function of increasing dose. Contrary to these results, nine percent of patients in the clinical study submitted in support of this NDA gained weight while on study. Decreases in testosterone are related to weight gain in older men (Harrison's Online Edition, Disorders of the Male Reproductive Axis During Adulthood). This decrease in testosterone is compounded as the amount of adipose tissue increases causing a concomitant increase in the aromatization of testosterone to estradiol. The difference in the effect on weight is because the animals in the non-clinical experiments were all exposed at a relatively young age and were effectively castrated prior to reaching full maturity. All the patients on the study were mature (range 47 to 98 years of age). Also, as mentioned above, all the animals were anorexic due to disruption of hypothalamic control of appetite through the pulsatile release of LH.

Single SC doses as high as $300 \text{ mg}/\text{m}^2$ caused no neurological or behavioral changes in mice, but the same dose given to mice IV rapidly led to death preceded by signs of neurological toxicity including unsteady gate, hyperactivity and pallid brain tissue on gross examination. If one assumes a blood volume of 1.6 mL for the average 20 g mouse, this dose results in a maximum peak blood concentration of over $700 \mu\text{M}$ of peptide so this acute lethality is probably not pharmacological but rather physiological.

At a relatively low dose of $3 \text{ mg}/\text{kg}$ ($\sim 18 \text{ mg}/\text{m}^2$), degarelix caused a $\sim 10 \text{ mmHg}$ left shift in the baroreceptor reflex in rats. This dose was too low to allow a determination of the significance of this finding, but there was a dose response. This was accompanied by a decrease in the maximum attainable heart rate suggesting a decrease in cardiac responsiveness and function. Radiolabeled tissue distribution studies showed that degarelix accumulates in the aorta, the location of the baroreceptors, of monkeys at concentrations greater than twice that in the plasma. It clears from this tissue much more slowly than it does from plasma. Also, in the developmental study in rabbits, pups had additional aortic blood vessels. GnRH may be involved in aortic development and regulation.

In the only dog given a IV bolus of about $3 \text{ mg}/\text{kg}$ ($60 \text{ mg}/\text{m}^2$), arterial pressure increased transiently at 30 minutes post dosing to 180/95 (baseline 145/55) then fell steadily over the next 10 minutes to a nadir of 45/20 at 42 minutes. This hypertension was preceded by an increase in the ST interval between 10 and 30 minutes and a T-wave inversion at 10 minutes. At most time points after dosing the T-wave was prolonged (maximum 26 ms above baseline). Pressure then rose steadily over the

next four hours to a maximum of 200/105 at the end of the experiment. This toxicity correlates with the hypertension seen clinically. The transient hypotension may correlate with the infrequent (<5%) report of dizziness. Two minutes after the pressure zenith, heart rate spiked to 170 (baseline 60) suggesting a transient derangement of the baroreceptor reflex or vagal control. Respiration was rapid and highly variable. The remaining dogs in this experiment received only about 1 mg/kg (~20 mg/m²) IV, so this toxicity remains incompletely characterized. These dogs also showed an unusual but less severe pattern of pressure changes. At 35 minutes post dosing, mean arterial systolic and diastolic pressure reached a zenith of 163/93 (baseline 130/77). Pressure then fell slowly over the next four hours to 142/85. Wave forms, heart rate and respiration were normal. When dogs were given 1 or 3 mg/kg as a 15 minute infusion, graphs of PR, RR, QT, QT_cF, QT_cV and heart rate all suggested a dose effect but these changes did not reach statistical significance. In standard *in vitro* assays, degarelix did not affect the hERG current or Purkinje fiber conduction. Taken together with the effect on baroreceptor reflex this body of evidence suggests that degarelix may directly affect dynamic pressure feed back control or repolarization or both.

In a 12 month repeat dose study in monkeys, systolic and diastolic pressure decreased with dose level on day 1 (24 hours post-dosing). On day 163 systolic and diastolic pressures increased with dose to a maximum at 5 mg/kg then decreased at 50 mg/kg to define a bell shaped dose response curve. The pressure decrease in the 50 mg/kg dose after the maximum at 5 mg/kg may result from chronic cardiac toxicity causing damage to the heart at the higher dose. In the mice, but not rats, absolute and relative heart weight decreases with long term dosing. RD Patten and RH Karas (*Trends in Cardiovascular Medicine*, 16;3, April 2006, Pages 69-75) have shown that estradiol replacement in oophorectomized rats reduced infarction size and reduced cardiomyocytes apoptosis after an induced infarction. They postulate that hormonal stimulation is necessary for efficient cardiac remodeling.

The cardiac toxicity of degarelix is not completely characterized. This acute toxicity seen in the dog given an IV bolus may only be clinically relevant only in the case of overdose. Nevertheless, the implications for patients with congestive failure, frequent orthostatic hypotension, chronic renal failure or other heart related conditions remain unknown.

In a standard safety pharmacology study, a single subcutaneous dose of degarelix increased urine output in rats between 2.5 and 5 hours after a single dose (as much as 38% in females given 1.8 mg/m²). This effect was about 80% as great as that seen in the Furosemide positive control. This relatively small dose did not affect urinary pH, PO₄, Ca⁺⁺ and osmolarity nor did it affect plasma Na⁺⁺, K⁺, Cl⁻ or creatinine. This appears to be a direct sodium sparing hormonal effect at the level of the pituitary in some way effecting ADH. An effect on aldosterone at the level of the adrenals would have increased urinary K⁺. It is possible that this effect is due to an as yet uncharacterized cross reactivity with GnRH-II or GnRH-III. Increased urinary output occurred in rodents in several of the repeat dose toxicity studies but with more chronic dosing this increase was accompanied by increased serum K⁺, decreased Na⁺ and decreased adrenal weights strongly suggesting that this effect was the result of direct toxicity at the adrenals leading to a decrease in aldosterone secretion. In many non-clinical studies this adrenal toxicity was accompanied by increased serum creatinine and decreased relative kidney weight, suggesting mild chronic renal failure in rodents. Males and females in the HD group of the 4 week IV bolus study in rats consumed significantly more water than controls throughout the study. This sign also suggests a disruption in the adrenal-renal-hypothalamic axis. Clinically, several patients had transient minor increases in creatinine and potassium. The implications of this mild renal toxicity for patients with chronic renal failure, congestive heart failure or patient taking diuretics remain unknown.

A relatively low dose of 3 mg/kg (18 mg/m²) caused a 17% increase in intestinal transit time in female rats; male rats were unaffected. This toxicity may correlate with the occasional report of constipation in the clinical study.

Degarelix caused a smaller increase in histamine release from rat mast cells than several other decapeptide GnRH-receptor inhibitors, but the concentration of degarelix required to cause an increase in histamine release of 40% above control *in vitro* was about 180 μM. This stimulation of mast cells may be due to a cross-reaction with an unknown cell surface receptor, but at such high concentrations it is more

likely simple disruption of signaling at the mast cell surface that the mast cell interprets as injury or irritation which initiates degranulation. While the concentrations required for histamine release are far above concentrations measured in the plasma in any experiment in this submission, clinical or non-clinical, they are far less than the concentrations of the formulations used in most of the toxicology studies in this submission where degarelix was given subcutaneously. A typical example is the 26 week study of subcutaneous degarelix in rats (DSTOX0401, above) where the nominal dosing concentrations were 1.22, 6.13 and 12.2 mM. These high concentrations at the subcutaneous injection site almost certainly cause a rapid localized histamine release from the mast cells in the vicinity of the injection. Other hormones associated with mast cell degranulation, heparin, serine proteases, prostaglandin D2, leukotriene C4 and other cytokines, are almost certainly released as well as the mast cell degranulates. This stimulation of mast cells leads to irritation at the injection site. Injection site damage was seen in all the animal studies where degarelix was given subcutaneously and in the 28 day study of IV (tail vein) administration in rats. This non-clinical toxicity correlates with the 35% incidence of "injection site adverse events" seen clinically. There were no incidences of anaphylaxis reported in the clinical trial and no toxicities that would appear to be related to acute histamine release other than injection site damage.

Red and white cell counts varied considerably in treated animals depending on dose, schedule and species. The following table compares end-of-dosing values for major hematological parameters in the highest dose group from all the multiple dose toxicology studies considered in this review. Values that decreased relative to control are in italics and those that increased are in bold.

Species	Duration	Dose	Red Cell Count		MCV		WBC		Eosinophils	
			male	female	male	female	male	female	male	female
mouse	13 week sc	300 mg/m ² /fortnight	2.4%	2.4%	<i>-4.8%</i>	<i>-4.6%</i>	137.2%	108.7%	263.6%	68.4%
rat	13 week sc	300 mg/m ² /fortnight	<i>-4.6%</i>	6.7%	1.9%	<i>-3.7%</i>	21.9%	123.3%	63.2%	138.5%
rat	2 week sc	18 mg/m ² /day	<i>-4.4%</i>	<i>-6.2%</i>	1.8%	3.6%	1.2%	66.7%	100.0%	0.0%
monkey	2 week sc	38 mg/m ² /day	<i>-3.1%</i>	4.8%	0.0%	<i>-1.6%</i>	<i>-15.4%</i>	47.3%	100.0%	0.0%
rat	26 week sc	600 mg/m ² /fortnight	<i>-5.7%</i>	6.2%	<i>-2.8%</i>	<i>-5.0%</i>	28.2%	138.9%	25.0%	80.0%
monkey	12 month sc	600 mg/m ² /monthly	<i>-8.8%</i>	8.5%	2.7%	<i>-3.1%</i>	<i>-34.9%</i>	50.3%	<i>-12.5%</i>	<i>-11.8%</i>
rat	4 week	30 mg/m ² /day	<i>-13.3%</i>	<i>-5.8%</i>	0.0%	1.3%	53.8%	66.8%	52.6%	73.3%
rat	104 week	150 mg/m ² /fortnight	<i>-1.3%</i>	7.3%	0.2%	<i>-2.7%</i>	<i>-11.4%</i>	48.3%	0.0%	62.5%
mouse	104 week	150 mg/m ² /fortnight	<i>-8.8%</i>	<i>-2.1%</i>	<i>-2.2%</i>	0.0%	65.2%	126.8%	<i>-4.0%</i>	0.0%

White cell count increased significantly in every study in females and in most studies in males but the increase was much less, sometimes not significant and in three cases actually decreased; there is definitely a sex difference in white cell response. Because of this difference in response between sexes and because white cell count increased significantly in the rat IV study it is unlikely that this neutrophilia is related to the irritation and inflammation at the injection site. Nevertheless, ET Keller et al (*J Reprod Immunol*, 50:1, April 2001, Pages 41-55) have shown that six months after ovariectomy older female monkeys had normal white cell counts ($4.4 \pm 0.7 \times 10^3/\text{mm}^3$) and a normal differential. There was also no difference in leukocyte subset concentrations (as measured by surface markers) in peripheral blood when compared to ovariectomized monkeys that received supplemental estradiol. Natural killer cell activity was decreased in the ovariectomized animals without supplementation and there was only a small increase in pro-inflammatory cytokines as measured by gp130. In a similar set of experiments, G Kuhn and W Hardegg (*Laboratory Animals* (1991) 25, 40-45) observed a no difference in white cell parameter between ovariectomized and sham surgery Lewis (Han) rats. Degarelix accumulates in concentrations significantly higher than those in plasma in liver, spleen, thymus, and kidney. Most of the drug recovered in the urine and feces is intact. Clearance is slow. In the 28 day IV study in rats, immunohistochemical with antibodies to degarelix and PAS staining showed the presence of drug inside Kupffer cells and splenic macrophages suggesting phagocytic elimination. There was vacuolization in the Kupffer cells and macrophage aggregation in the spleen suggesting organ specific immunostimulation. In this study splenomegaly was somewhat greater in females. This difference in splenomegaly occurred in other studies. In most cases where AUC was measured, female animals cleared degarelix more efficiently than males. It is possible that more effective clearance in the spleen and liver in females leading greater

irritation and a greater cytokine response with a concomitant increase in white cell parameters. The studies in the present submission do not provide the information necessary to establish the mechanism for such a heightened response in female.

The table above shows that with the exception of the 13 week study in mice, male animals were consistently anemic after prolonged treatment with degarelix. There was no consistent change in females so it is unlikely that this anemia is associated with injection site inflammation. Several studies showed decreased erythropoiesis in the marrow. Male puberty is normally associated with a 2 g/dL increase in blood hemoglobin concentration. Low dose testosterone given to boys with delayed puberty increased blood hemoglobin by 0.96 g/dL while treatment with testosterone plus Letrozole, an aromatase inhibitor that prevents the conversion of testosterone to estradiol, caused an increase in hemoglobin of 1.6 g/dL (M Hero *et al*, *The Journal of Pediatrics*, 146:2, February 2005, Pages 245-252). As this anemia was not consistently microcytic and not seen in females it is unlikely secondary to chronic inflammation. This toxicity is probably a direct effect of diminished testosterone. Anemia was not noted clinically but some patients complained of fatigue which can be an indicator of mild anemia.

Serum cholesterol was consistently elevated in males and females in these toxicology studies. This is a well established consequence of hormone deprivation and probably results from feedback stimulation of cholesterol synthesis as cholesterol is the precursor for testosterone and ultimately estradiol (see Goodman and Gilman). Serum glucose showed mild increases in the toxicology studies and again the link between increased glucose and hormone deprivation is well established. In some studies there was a concomitant increase in triglycerides.

Some studies showed vacuolization in the liver accompanied by Kupffer cell infiltration and some studies showed a mild decrease in the concentrations of liver enzymes and plasma proteins suggesting decreased hepatic function. In some studies clotting parameters were mildly affected. As noted above, this toxicity is probably related to mild inflammation associated with the clearance of the drug from liver by Kupffer cells. No hepatic toxicity was noted clinically but again the implications for patients with preexisting liver disease remains unknown.

Degarelix did not cause increases in bacterial mutations in six separate Ames assays either with or without metabolic activation. In six separate studies in L5178Y mouse lymphoma cells, degarelix caused no increase in mutations at the TK locus. In two separate *in vivo* studies, degarelix caused no increase in micronucleated immature rat erythrocytes. Thus, degarelix is not genotoxic under the conditions of standard *in vitro* or *in vivo* assays.

In a standard 24 month carcinogenicity study in rats where degarelix was given fortnightly (52 subcutaneous doses), the high dose of 150 mg/m² was about the same as the proposed clinical loading dose and about 3 times greater than the proposed monthly maintenance dose on a mg/m² basis. The mid dose was 60 mg/m² and the low dose was 12 mg/m². A parallel toxicokinetic study demonstrated that degarelix accumulated to steady state values about week 24 and that exposure was roughly dose proportional. Overall mortality of treated animals was less than that of controls. The high dose males weighed 24% less than controls at the end of the study. Treated females weighed significantly more than controls throughout the study. The incidence of benign adenoma of the pituitary gland decreased in all groups of treated females ($p < 0.02$). The incidence of benign fibroadenoma of the breast decreased in all groups of treated females ($p < 0.024$). These decreases were related to decreased stimulation of the pituitary and atrophy of both the pituitary and the mammary glands. The incidence of eosinophilic cell foci in the liver increased in low dose females ($p < 0.001$). This preneoplastic finding is consistent with mild hepatic damage seen in males and females. Lastly, there was an increase in metastatic hemangiosarcoma of the mesenteric lymph node in HD females ($p < 0.04$, with a positive trend by Peto analysis $p = 0.015$). The incidence of this tumor was 8% which is within the range seen in historical controls. There was no similar finding in males. The combined incidence of all benign and malignant hemangiomas and hemangiosarcomas (16%) was significantly different from controls by pairwise comparison ($p = 0.0013$, Exact test) in the high dose group. This difference remained significant when analyzed by the asymptotic trend test ($p = 0.0008$).

In a standard 24 month carcinogenicity study in mice, treatment with degarelix at doses of 6, 30 and 150 mg/m² fortnightly for two years caused an increase in benign bronchio-alveolar adenoma in all groups of treated females ($p < 0.04$) when analyzed by pairwise comparison with control. When the incidence of benign bronchio-alveolar adenoma was combined with that of malignant bronchio-alveolar carcinoma the result was not statistically different from controls by pairwise comparison. The incidence of benign bronchio-alveolar adenoma in male CD-1 mice ranges from 11 to 36 %, in females it ranges from 3 to 16%. Dosing in this study also caused an increase in benign hepatocellular adenoma of the liver ($p = 0.015$) in high dose females. By trend analysis the increase in benign hepatocellular adenoma of the liver reached significance in both males ($p = 0.03$) and females ($p < 0.04$). When the incidence of benign hepatocellular adenoma was combined with that of malignant hepatocellular carcinoma the result was not significantly different from controls in males or females ($p < 0.09$) by pairwise comparison. The combined incidence of these tumors was also not statistically different from controls by asymptotic trend test ($p < 0.09$). The normal incidence of hepatocellular adenoma of the liver ranges from 2 to 33 % in male CD-1 mice and from 0 to 4% in females. The normal range for hepatocellular carcinoma ranges from 0 to 1.7 % in females and 0 to 6% in males.

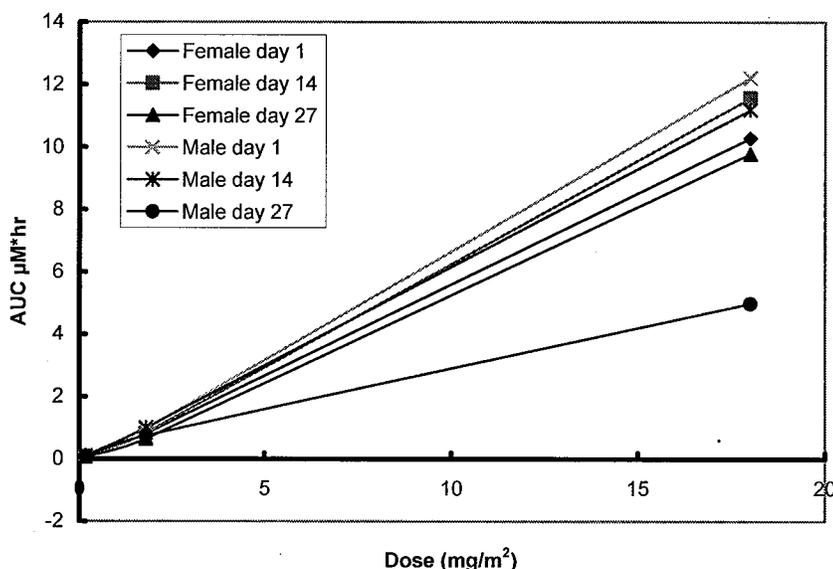
Doses of 0.072 mg/m²/day from day 6 through day 12 followed by doses of 0.18 mg/m²/day caused significant post-implantation loss in pregnant rats (23.6 %) and a concomitant decrease in the number of live fetuses/dam. This dose caused no significant maternal toxicity and is only about 0.13% of the proposed clinical loading dose. Dosing was associated with an increase in the number of major abnormalities in the fetuses in the high dose group ($p < 0.05$) but most of these abnormalities occurred in a single litter (4 of 6). In fetuses in the mid dose group (0.54 mg/m²/day followed by 0.18 mg/m²/day at the schedule above) there was a statistically significant increase in a number of minor skeletal abnormalities and variants observed. These were findings generally associated with the state of ossification and were considered to be related to maternal treatment with Degarelix.

In rabbits, a daily dose of 0.024 mg/mg² on days 6 through 14 followed by doses of 0.072 mg/m² from days 15 through 27 was associated with a decrease in the number of does with implantations, the number of corpora lutea per female, the number of implantations and the number of live fetuses per female. Some of these decreases reached statistical significance in the mid-high (0.12 mg/m²/day followed by 0.36 mg/m²/day at the schedule above) and high dose groups particularly the number of live fetuses. Dosing was also associated with an increase in mean post-implantation loss. There was an increase in the number of fetuses with minor abnormalities in the high dose group and an increase in the incidence of major abnormalities in the mid dose group (5 in three litters) but the number of fetuses in the high dose group was so diminished as to render any determination of teratogenicity equivocal. The high dose caused only minimal toxicity in the does (minimal decreased body weight gain). Thus, a daily dose of degarelix that was just 0.05% that of the proposed loading dose was a potent abortifacient in rabbits.

Single doses of ≥ 6 mg/m² (about 5% of the clinical loading dose on a mg/m² basis) of degarelix caused complete loss of fertility in male rats. Fertility returned in most animals between 10 to 14 weeks after the end of dosing demonstrating pharmacological reversibility of the effects of degarelix in male rats. Single doses of ≥ 0.6 mg/m² (about 0.5% of the clinical loading dose on a mg/m² basis) caused a decrease in fertility in female rats.

The following graph demonstrates that when degarelix is given to rats intravenously as a series of daily doses for 28 days, AUC increases linearly and proportionately with increasing dose except in the high dose male group after the 27th daily dose. The variation in this group is probably just due experimental error. The graph also demonstrates that there is no accumulation of the drug. The doses in this experiment were significantly lower than the clinical dose.

Dose vs AUC after an IV dose in Rats



The terminal elimination half-life in this study did not vary substantially with increasing dose or time on study. The mean value across all times and doses was 3 ± 1.5 hours. Clearance was 0.21 ± 0.04 L/kg/hr, significantly less than portal or renal blood flow; while the volume of distribution was 0.87 ± 0.46 L/kg, significantly greater than plasma volume suggesting cellular uptake.

In monkeys given daily IV doses for 28 days, the increase in AUC was less than dose proportional and nonlinear but there was no accumulation. Again the doses in these IV studies were considerably lower than the proposed clinical dose on a mg/m² basis. Half-life did not vary significantly with increasing dose or time on study. The mean value across all times and doses was 4.8 ± 2.3 . Clearance was substantially less than that seen in the rat; the mean value was 0.070 ± 0.025 L/kg/hr. The volume of distribution was also less than that seen in the rat; the mean value was 0.48 ± 0.26 L/kg.

The following human parameters are from study CS05. When degarelix was given as a slow dose IV infusion to healthy adult men, the terminal elimination half-life increased with increasing infusion time and dose. The half-life was in all cases greater than that seen in monkeys or rats by at least a factor of 2.

	AUC (ng hr/mL)	C _{max} (ng/mL)	T _{1/2} (hr)
6 µg/kg over 15 min (n = 6)	141 ± 34	38.2 ± 6.2	11.6 ± 5.2
15 µg/kg over 45 min (n = 6)	296 ± 82	58 ± 8.7	13.2 ± 1.7
30 µg/kg over 45 min (n = 6)	747 ± 120	160 ± 22	16.5 ± 1.8

Exposure increased proportionally and linearly with dose. In healthy volunteers given a single IV dose of 1 mg of degarelix as a 1 hour infusion, clearance was 3.2 ± 0.5 L/hr and volume of distribution was 79 ± 17 L (about 1 L/kg).

Parameters derived from the toxicokinetic studies of degarelix given subcutaneously are not informative because the absorption of the drug from the subcutaneous depot is rate limiting. The terminal elimination half-life thus reflects the absorption rate constant, but in many cases this could not be determined accurately because the dosing interval was considerably shorter than five half-lives. Thus,

values for clearance and volume were unusually large and variable. In almost all cases, the increase in C_{max} and AUC was non-linear and far less than dose proportional and most repeat dose studies demonstrated significant accumulation. Plots of C_{trough} demonstrated consistent exposure above the value of k_i even at most low doses.

After a single subcutaneous dose of 8.2 $\mu\text{g}/\text{kg}$ (about 98.4 $\mu\text{g}/\text{m}^2$) of radiolabeled degarelix given to monkeys (1 per time point), about 10% of total radioactivity was recovered in the urine after 6 hours. Another 10% was excreted in the next 18 hours; after this urinary excretion was essentially complete with a total of $20 \pm 6\%$. Urinary excretion in humans was $30.7\% \pm 4.3\%$, after 48 hours and was essentially complete. In the monkey study, fecal excretion of total radioactivity was essentially complete after 48 hours; total fecal excretion after 240 hours was $21 \pm 22\%$. Total recovery of radioactivity, including cage wash, urine and feces, ranged from about 40 to 70% at 240 hours, suggesting that a significant amount of the drug remains as body burden. In these monkeys, total radioactivity distributed in highest amounts to excretory organs with the highest concentrations in bile, small intestine, urinary bladder, kidney, and liver respectively at 6 hours. Relatively high concentrations were found in the pituitary, prostate and testes consistent with the drugs pharmacology. Concentrations greater than that found in plasma were found in the aorta, lachrymal gland, lung, skin and vena cava. Elimination from the aorta, bile, pituitary, vena cava, prostate, kidneys and adrenals was slower than elimination from plasma.

In dogs, urinary excretion was about 50% while fecal excretion was about 40% of total radioactivity. Excretion was essentially complete after 48 hours. In this study in dogs, about 90% of plasma degarelix was protein-bound. The following table shows the spectrum of binding in human plasma protein.

TABLE 2: Binding of degarelix to human plasma proteins

Protein	Nominal FE200486 concentration (ng/mL)	Total concentration (C _t) of FE200486 (ng/mL)	binding (%)
Serum albumin	20	17.1	78.1
	60	50.3	77.9
	120	109	72.7
		Overall Mean	76.3
Gamma globulin	20	18.2	48.6
	60	46.7	46.6
	120	114	24.8
		Overall Mean	40.0
α_1 acid glycoprotein	20	19.6	82.5
	60	59.6	79.1
	120	116	73.0
		Overall Mean	78.2
High density lipoprotein	20	18.0	60.6
	60	55.1	56.9
	120	119	56.2
		Overall Mean	57.9
Human plasma	20	17.7	90.7
	60	54.7	90.3
	120	140	90.5
		Overall Mean	90.5

Best Possible Copy

Distribution in dogs was similar to that in monkeys. In the dog study, about 97% of the radioactivity in urine was parent compound. In feces about 45% was parent, 26% was metabolite I-6, and 23% was metabolite M1. Plasma contained parent compound (78%) and metabolite M2a (18%). *In vitro*, human hepatocytes oxidized degarelix to five different metabolites. One metabolite, degarelix (I-9), was the most abundant metabolite and was likely a product of proteolysis. The most abundant oxidative metabolite was degarelix Ox-I, with oxidation occurring at the D-2 Nal amino acid. The second most abundant metabolite was degarelix Ox-II, with oxidation occurring on the D-3Pal amino acid. Total metabolite concentration was less than 1 % of the parent compound in this *in vitro* system. Cytochrome P450s are not likely a major route for degarelix metabolism in humans. Glucuronidated-degarelix was found in the bile (45% of total) of dogs along with metabolites M1 (20.2 %), and degarelix (I-6) (17%).

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

/s/

William McGuinn
12/11/2008 10:47:03 PM
PHARMACOLOGIST

Leigh Verbois
12/12/2008 04:25:10 PM
PHARMACOLOGIST