VI. CARCINOGENICITY:

Study title: A 2 YEAR INTRAMUSCULAR CARCINOGENICITY STUDY OF ICI 182,780 IN THE ALBINO RAT.

Key study findings:

- ICI 182,780 increases the incidence of ovarian granulosa cell tumors and testicular interstital Leydig ademomas.
- ICI 182,780 decreases the incidence of uterine endometrial stromal polyps, mammary tumors (adenoma, fibroadenoma, adenocarcinoma) in females, and pituitary adenomas in females. Reductions in mammary gland and pituitary tumors may have contributed to the increase in longevity of animals administered ICI 182,780.

Study number: TCR/2683
Volume #, and page #: \N_000\2001-10-29\TCR2683 Complete Report

Conducting laboratory and location:

Test Facility:	Test Site:	Test Site:
(*in vivo* study)	(Pharmacokinetics)	(Electron Microscopy)
[redacted]	AstraZeneca UK Ltd	AstraZeneca UK Ltd
	DMPK Mereside Alderley Park	Safety Assessment Alderley
	Macclesfield, Cheshire	Mereside Alderley Park
	England	Macclesfield,
		Cheshire England

Date of study initiation: 10 November 1998
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity:

ICI 182,780	Bulk Drug	Bulk Drug	LA (IM) Injection	LA (IM) Injection	Vehicle	Vehicle
Batch #	C169/4	C177/2	P/1465/22A	P/1359/4	P/1465/19	P/1359/17
ADM #	00193A98	39679H96	62181D99	39454G97	62077F99	01184F98
Date of Manufacture	12/18/97	11/11/1996	5/19/1999	11/12/1997	5/12/1999	3/11/1998
Date of Analysis	2/18/98	1/13/1997	7/8/1999	2/5/1998	6/25/1999	6/3/1998
Strengh (HPLC)					-	-

CAC concurrence: Yes

Study Type: 104 weeks in albino rats; IM administration to the lateral compartment of the thigh (dose was divided in two parts (up to 0.1 mL) and administered on the right and left side).
Species/strain: male and female Sprague Dawley (Crl:CD(SD)BR) rats (Rattus norvegicus) from [redacted]

Number/sex/group; age at start of study: 50/sex; 28±1 days old

Animal housing: Individual

Formulation/vehicle: Long acting formulation

Ingredients	Drug %w/v
ICI 182,780	5
Ethanol	10
Benzyl Alcohol	10
Benzyl Benzoate	15
Castor Oil	to 100

Drug stability/homogeneity:

Data from the primary stability studies indicate that fulvestrant is stable at the proposed long term storage condition of [redacted]. In addition no significant change (as defined by ICH guideline Q1A) has been observed after 6 months storage at the accelerated storage condition of [redacted] nor after 12 months storage at the intermediate accelerated storage conditions of [redacted] and [redacted].

Methods:

Doses:

Group No. Identification	Dose Levels	Dose Volume	Animal number	
			Males	Females
1 Vehicle control	0 mg/kg/15 days	0.2 mL/rat	1001-1029, 1031-1051	1501-1550
2 Vehicle control	0 mg/kg/30 days	0.2 mL/rat	2001-2050	2501-2550
3 Saline control	0 mg/kg/15 days	0.2 mL/rat	3001-3050	3501-3550
4 ICI 182,780	15 mg/kg/30 days[a]	0.3 mL/kg	4001-4050	4501-4550
5 ICI 182,780	10 mg/rat/30 days	0.2 mL/rat	5001-5050	5501-5550
6 ICI 182,780	10 mg/rat/15 days	0.2 mL/rat	6001-6050	6501-6550
7 Health screen	-	-	7001-7010	7501-7510

a Dosage limited by maximum injection volume of 0.2 mL/rat

* Control Male 1030 replaced by Male 1051 following mortality during replacement period

The following shows the ~ actual dose (mg/kg) administered to Groups V (10 mg/rat/30days) and Group VI (10 mg/rat/15 days).

	Male					Female				
	BW (g)	~Actual dose	BW (g)	~Actual dose	~Actual dose	BW (g)	~Actual dose	BW (g)	~Actual dose	~Actual dose
Week	10 mg/rat/ 30 days	mg/kg/ 30 days	10 mg/rat/ 15 days	mg/kg/ 15 days	mg/kg/ 30 days	10 mg/rat/ 30 days	mg/kg/ 30 days	10 mg/rat/ 15 days	mg/kg/ 15 days	mg/kg/ 30 days
0	192.3	-	187.5	-	-	152.9	-	153.2	-	-
1	262.9	38	257.8	39	78	184.7	54	185.7	54	108
48	830.5	12	787.3	13	26	487.8	21	507.3	20	40
96	793	13	781.5	13	26	580.3	17	574.4	17	34
104	795.8	-	782.3	-	-	559.6	-	577.4	-	-

Basis of dose selection: According to the Sponsor, the dose levels selected represent the maximum possible doses by the intramuscular (IM) route based on strength of the formulation and injection volumes.

Restriction paradigm for dietary restriction studies: n/a

Route of administration: IM injection
Frequency of drug administration: every 15 or 30 days
Dual controls employed: Vehicle and saline controls included
Interim sacrifices: n/a
Satellite PK or special study group(s): None
Deviations from original study protocol: Occasional minor deviations from the protocol occurred and were documented in the raw data and/or text. The Sponsor reports these deviations had no impact on the outcome of the study or upon the interpretation of the results.

Statistical methods:
First the Levene's test was used to assess the equality of the group variances followed by ANOVA if this test was not significant. If the Levene's test was significant, then the statistical analyses were performed on the ranked transformed data.
Survival Analysis: An overall test for homogeneity was performed on the survival functions of all 6 groups.
Tumor Data: All tests for tumour incidence were one-sided looking for an increase in response/incidence. The Haseman (1983) principle of statistical significance was adopted in the formal assessment of statistically significant effects. One-sided 5% tests for decreasing response/incidence were also performed.
The statistical comparisons of interest were implemented using Peto's survival-adjusted trend test. Statistical comparisons were performed in three phases:
Phase 1: Vehicle effect with dosing every 15 or 30 days: Both the 15-day and 30-day vehicle groups (Groups 1 and 2) were compared to the saline control group (Group 3).
Phase 2: Treatment effect for each dosing frequency: Two vehicle groups (Groups 1 and 2) and the two 10 mg/kg groups at 15 and 30 days frequency (Groups 5 and 6, respectively). Treatment groups were then compared to the appropriate vehicle separately for each dosing frequency.
Phase 3: Dose effect over the 30 day dosing regimen: Group 2 (vehicle/30 days) was compared with the 15 mg/kg/30 days treatment group (Group 4).

Observations and times:

Clinical signs:	Twice daily for mortality and clinical signs. A complete physical examination was performed once during the pretreatment period and weekly during the treatment period. In addition, from Week 26 onwards, all animals were examined for the presence of palpable masses during the detailed examination.
Body weights:	Weekly.
Food consumption:	Weekly for the first 13 weeks of treatment, then monthly, thereafter.
Hematology:	Red blood cell counts and total and differential white blood cell counts were performed at 12 and 18 months and at terminal necropsy.
Clinical chemistry:	Not obtained
Organ weights:	Not obtained

Gross pathology: A gross pathological examination was performed on all animals on this study.

Histopathology: Tissues, as defined in the protocol, were examined histopathologically for all animals on this study. See addendum.

Toxicokinetics:

Groups 4 and 5 (n=3/sex) were bled at 2, 4, 8, 12, 16 and 24 days post dose after the 12th dose. Samples were also taken from 6 rats/sex (Groups 4 and 5) prior to the the 2nd, 4th, 7th and 10th dose.
Group 6 (n=3) were bled at 2, 4, 8, 12, 16 and 24 days after the 23th dose. Samples were also taken from 6 rats/sex from the same group prior to the 3rd, 7th, 13th and 19th dose.

Results:

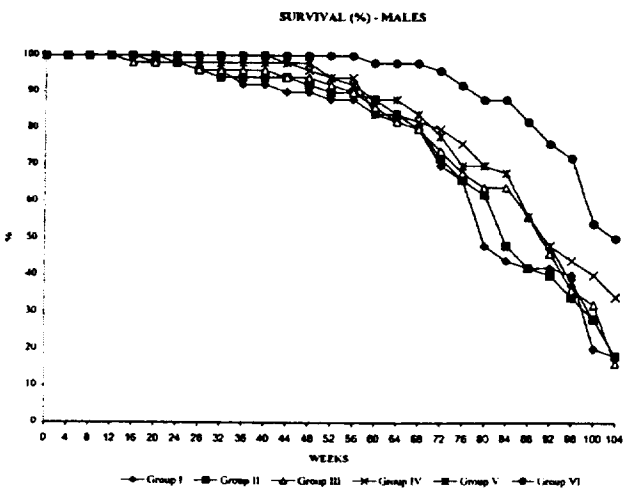
Mortality:

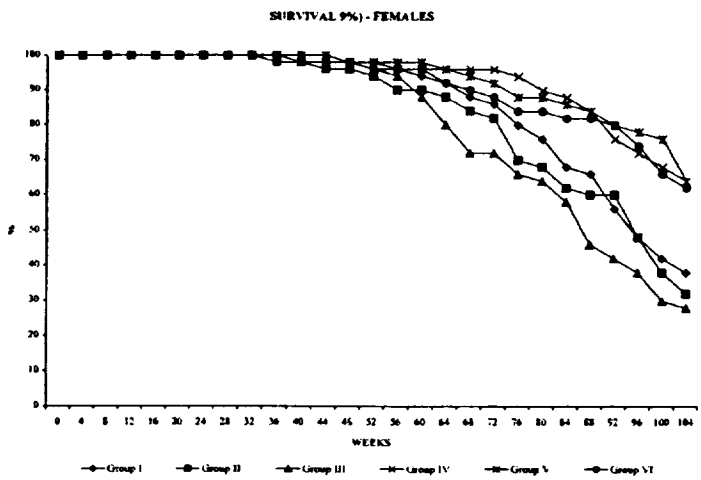
	Group	Dose	Dose * (mg/kg/30 days)	Frequency (days)	Survival			
					Males (n=50)	% Survival	Females (n=50)	% Survival
1	Vehicle Control	0 mg/kg	0	15	9	18	19	38
2	Vehicle Control	0 mg/kg	0	30	9	18	16	32
3	Saline Control	0 mg/kg	0	15	8	16	14	28
4	ICI 182,780	15 mg/kg	15	30	17	34	32	64
5	ICI 182,780	10 mg/rat/	♂:76 ♀:104	30	13	26	32	64
6	ICI 182,780	10 mg/rat/	♂:78 ♀:104	15	25	50	31	62

* The ~ actual dose administered for group V and VI was calculated based on the average weight for males and females on day 1.

For control males, survival rates appear lower than expected. In 1997, according to the [redacted], the % surival for male and female SD rat ranged 31.7-61.9% and 31.7-61.4%, respectively. In this study, the overall survival rate ranges between 17.1-62.9 and 24-61.4% for male and females rats, respectively.

Percent survival as a function of time (weeks) in ♂ and ♀ SD rats.

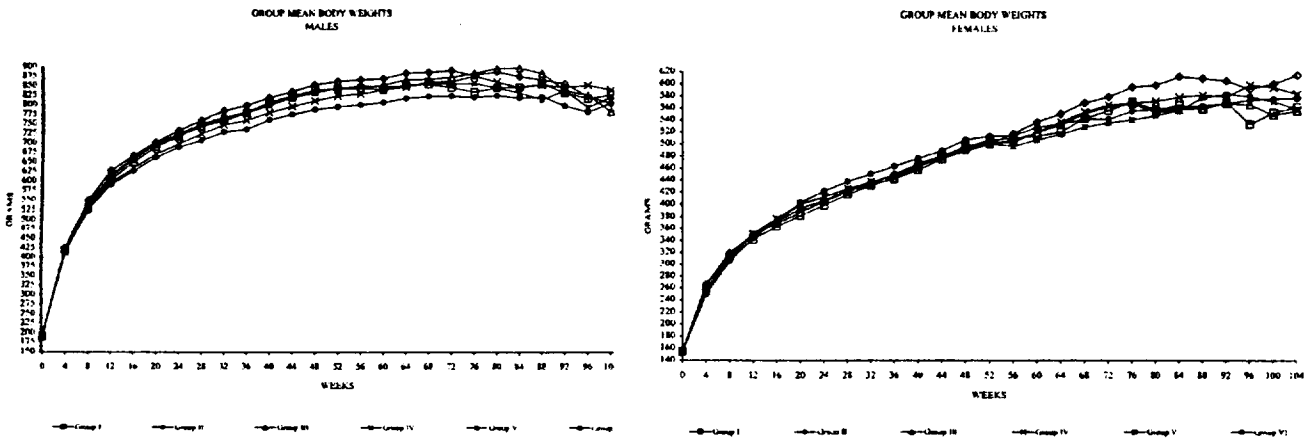




Clinical signs: There were no treatment-related clinical signs, however a number of animals from all treated groups and from control animals in Groups 1 and 2 (vehicle), exhibited cysts at the injection sites.

Body weights: Overall body weights throughout the study were not affected by treatment. Occasional statistically significant variations in body weight gain across all groups (both sexes) were considered incidental.

Body weight (g) as a function of time (weeks) for ♂ and ♀ SD rats.



Food consumption: A statistically significant reduction (7-14%) in food consumption was observed in both male and female animals from all treated groups, the effect being most pronounced in female rats. Effects were noted from Week 2 for females but did not become apparent in male rats until Week 20. In neither sex did the reduction in food consumption result in a decrease in terminal body weight.

Hematology: RBC levels were elevated in drug-treated females. *p<0.05

RBC x $10^3/mm^3$	Week 52	Week 78	Week 105
veh/15d	7.08	6.06	5.77
veh/30d	6.97	6.52	5.82
saline	6.97	6.75	6.35
15 mg/kg	7.32 (↑5%)*	6.64 (↑2%)*	6.66 (↑14%)*
10 mg/rat/30d	7.53 (↑8%)*	7.2 (↑10%)*	6.84 (↑18%)*
10 mg/rat/15d	7.7 (↑9%)*	7.35 (↑21%)*	6.78 (↑18%)*

There were no changes in hematology parameters that were considered to be related to treatment with ICI 182,780.
Minor differences of some parameters, occasionally statistically significant, were considered incidental and unrelated to treatment with ICI 182,780

Clinical chemistry: No clinical chemistry was included.

Organ weights: Organs were not weighed

Gross pathology:

	Males						Females					
	Veh/15d	Veh/30d	Saline/15d	15 mg/kg /30d	10 mg/kg /30d	10 mg/kg / 15d	Veh/15d	Veh/30d	Saline/15d	15 mg/kg /30d	10 mg/kg /30d	10 mg/kg / 15d
	I	II	III	IV	V	VI	I	II	III	IV	V	VI
n=	50	50	50	50	50	50	50	50	50	50	50	50
Injection site-cyst	36	34	1	39	41	49	45	41	2	40	44	45
Liver-enlargement	12	14	10	9	8	10	6	10	14	3	1	5
Pituitary-masses	17	12	14	9	11	14	34	38	35	20	15	13
Subcutaneous tissue-masses	8	13	6	10	13	11	30	29	29	5	8	5
-thickening	6	4	4	2	4	2	12	12	10	7	5	3
Bile duct-dilatation	2	4	2	3	5	5	1	1	1	6	5	5
Testes-pale areas	2	1	4	19	17	17						
-reduced size	4	4	6	19	18	19						
-soft texture	9	13	11	33	37	41						
Prostate-pale	26	21	32	21	23	26						
Ovarian-cyst							19	5	11	18	20	26
-mass							0	2	0	1	1	1
Uterus-cyst							16	12	14	0	0	0
-small							0	0	0	22	24	33
Vagina-pale material							13	15	12	9	7	6

- Presence of cysts at the injection sites in all groups, except saline, suggesting a vehicle effect.
- Visual inspection suggests that there was enlargement of the liver in both sexes from all drug treatment groups. The incidence of liver enlargement was lower in groups receiving the test article every 30 days (8♂/1♀) compared to animals receiving the drug every 15 days (10♂/5♀). A small increase in the incidence of dilatation of the extrahepatic bile duct was noted in drug-treated females.
- In drug-treated males, there was an increased incidence of pale areas, reduced size, and soft texture of the testes.
- In drug treated females, there was an increased incidence of grossly observable ovarian and uterine cysts, as well as small uteri. Conversely, there was a decreased incidence of subcutaneous masses or thickening and pituitary masses to levels comparable with the male incidence. The occurrence of pituitary masses is commonly higher in female than in male rats of the strain and age range of this study.

Histopathology: See tabular data on following pages.

Neoplastic:

	Males						Females						
	Veh/15d	Veh/30d	Saline/15d	15 mg/kg /30d	10 mg/kg /30 d	10 mg/kg / 15 d	Veh/15d	Veh/30d	Saline/15d	15 mg/kg /30d	10 mg/kg /30 d	10 mg/kg / 15 d	
	I	II	III	IV	V	VI	I	II	III	IV	V	VI	Findings
All tumors n=	50	50	50	50	50	50	50	50	50	50	50	50	
Animals with neoplasms	36	41	39	35	35	38	48	50	49	42	41	37	-↓ in ♀
>1 primary neoplasm	15	19	22	21	19	19	30	35	34	15	19	14	-some ♀ had > 1 primary tumor
Total primary tumors	55	71	73	69	67	66	115	120	119	60	65	53	-↓ in ♀
Ovaries n=							50	49	50	50	50	50	
Granulosa cell tumors [A]							0	0	1	1	2	7 (14%)	Typically nodular in appearance with solid masses of granulosa cells in a pseudofollicular pattern separated by areas of thecal-type cells.
Abnormal follicular development (*q/v*)							0	0	0	15	20	21	Granulosa cell layer showing ↑ cellularity with a florid proliferation and folding. Sometimes also proliferation of the ovarian interstitial cells.
Hyperplasia: follicular granulosa cells							0	0	0	0	0	4	↑ incidence w/ 10 mg/rat 15 days
Adenoma:Sertoliform tubular							2	2	1	0	0	0	
Testes n=	50	50	50	50	50	50							
Interstitial cell adenomas[B]	0	0	2	6	4	1							The low incidence in Group 6 may make finding equivocal for drug-effect.
Uterus n=							50	50	50				
Endometrial stromal polyps							4	4	5	0	0	0	
Endometrial adenocarcinomas								1					
Leiomyoma							1						
Leiomyosarcomas								1	1				
Mammary glands: n=							50	50	49	46	49	45	
Adenomas	0	0	1	1	0	2	10	7	9	0	0	0	↓ in tumor incidence
Adenocarcinomas	2	0	1	0	0	0	11	11	11	0	0	1	
fibroadenomas	0	0	1	0	0	0	18	20	23	0	0	1	
Pituitary gland tumors n=	50	50	50	50	50	49	50	50	50	50	50	50	Common tumors in elderly rats and frequent cause of death in this species. Most common in ♀s.
Adenomas	26	27	31	26	22	27	37	45	46	32	36	29	↓ incidence in drug-txn
Carcinomas	0	0	2	0	0	0	1	1	1	2	0	0	↓ incidence in drug-txn

Liver: n=	50	50	50	50	50	50	50	50	50	50	50	50	No txn effect
Adenomas	0	3	4	0	3	3	2	3	1	2	5	1	Appears more prevalent in Group 5
Carcinomas	0	2	3	3	1	1	0	0	0	0	0	0	
Lymphoid tissue (n=													.
Malignant lymphoma [C]	1	2	1	1	0	1	1	3	0	0	0	1	In both sexes and all doses
Hystiocytic sarcomata [C]	2	0	0	0	0	2	0	3	4	3	1	0	In both sexes and all doses
Endocrine organs n=													
Adrenal cortical or medullary carcinoma							2	0	0	0	1	1	
Adrenal cortical or medullary adenoma	1	0	0	0	1	0	3	1	2	1	0	0	
Thyroid follicular or C-cell carcinoma	1	3	2	3	3	4	1	1		2	2	1	
Thyroid follicular or C-cell adenoma	1	2	2	2	3	3	1	3	2	3	3	1	
Pancreatic islet carcinoma	0	1	2	2	3	0	2	1	0	2	2	0	
Pancreatic islet adenoma	2	2	2	4	0	3	3	0	1	3	0	2	
Subcutaneous tissue n=	6	9	3	5	5	3	5	3	5	8	4	3	
Fibroma	3	6	3	2	2	2	3	2	1	2	1	0	
Fibrosarcoma							0	0	0	1	1	1	

[A] Uncommon tumors. Background instances in this lab vary from 0/120 to 1/120 (0.2%) Study w/ same strain and source n= 4493 0.3%. Other strains 1-3%

[B] Background incidences in this lab: 1/60 to 6/120 (0.02-0.05)

[C] Comparison of the incidences in treated groups with the appropriate control groups (Groups 4 and 5 against Group 2 and Group 6 against Group 1) shows that that spontaneous pituitary tumor incidence in these groups was reduced to levels comparable to that seen in both control and treated male animals. The reduction in number of pituitary masses seen at necropsy (qv) was greater than that for histologically diagnosed pituitary tumours (above} suggesting tumours in drug-treated females were smaller than in untreated or male animals.

[D] Spontaneous tumors in aged rats and associated w/ death

Non-Neoplastic

	Males						Females						
	Veh/15d	Veh/30d	Saline/15d	15 mg/kg /30d	10 mg/kg /30 d	10 mg/kg / 15 d	Veh/15d	Veh/30d	Saline/15d	15 mg/kg /30d	10 mg/kg /30 d	10 mg/kg / 15 d	
	I	II	III	IV	V	VI	I	II	III	IV	V	VI	Finding
Ovaries n=							50	49	50	50	50	50	Normal rat ovarian structure in aged animals is typified, by a ↓ or absence of corpora lutea, by the presence of follicular cysts (small to grossly distended in size) and occasional sertoliform tubular hyperplasia in the interstitium.
Corporea lutea reduced or absent							40	35	37	50	50	50	Absent corpora lutea in drug-treated rats.
Prominent follicles							0	0	0	24	27	23	Follicles showed degrees of abnormal development, contained oocytes, and tended to include unusually prominent granulosa cell layers.Note: In anestrous rats of this age range, follicles of any stage of development sare rarely seen.
Abnormal follicular development *							0	0	0	15	20	21	Discussed inprevious table.
Hyperplasia: granulosa cells*							0	0	0	0	0	4	Discussed inprevious table.
Hyperplasia: sertoliform tubular							20	24	22	6	4	2	↓ incidence in treated animals
Hyperplasia: stromal							0	0	0	41	44	34	↑ incidence of unusual stromal hyperplasia not observed in untreated animals.
Cyst: follicular							21	5	10	21	23	28	↑ incidenceof follicular cysts in treated rats.
Cyst: hemorrhagic							0	1	1	3	4	6	Presence of hemorrhagic cysts in drug-treated rats.
Uterus n=							50	50	50	50	50	50	In aged ♀ rats, normal uteri show ↓ endometrial glands with occasional development of glandular cysts. Lumenal dilatation and stromal hypertrophy may be present.
Hypertrophy: Stromal							3	3	3	0	0	0	Absence of typical age-related changes.
Cyst: endometria;							10	10	13	0	0	0	Absence of typical age-related changes.
Dilatation: lumen							4	2	2	0	0	0	Absence of typical age-related changes.
Atrophy: Endometrial							0	0	1	50	50	50	↑ incidence in treated rats.
Atrophy: Myometrial							0	0	1	50	49	49	↑ incidence in treated rats.

Mammary Gland n=							50	50	49	46	49	45	
Hyperplasia							22	15	16	6	6	4	In treted ♀, the incidence of mammary hyperplasia was↓ and approached the incidence in ♂ animals.
Atrophy							0	0	0	19	17	8	In a some treated rats mammary glandular tissue was not discernible or was atrophic. These changes may be treatment related or a consequence of ↓ incidence of pituitary neoplasia. Note: Pituitary cells, normal or neoplastic, under the influence of estrogen produce prolactin levels that are implicated in mammary gland proliferation, an effect that can be inferred absent in treated rats..
Injection sites: n=	50	50	50	50	50	50	50	50	50	50	50	50	Cysts were absent in saline controls..
Cyst	43	43	0	37	43	48	49	43	0	42	47	47	Cysts were typically multiple and moderately large with thin fibrous walls and appeared to be empty. Commonly located in the adipose tissue adjacent to muscle blocks or in the connective tissue of the muscle fascia and often occurred in connective tissues immediately adjacent to nerve bundles located in the vicinity of the injection site.
Atrophy/degeneration Myofibers	15	4	3	12	10	20	3	3	1	3	6	11	Vvarying degrees of atrophy or degeneration of muscle fibers possibly due to the physical presence of the large cysts.
Inflammation: muscle	7	2	0	8	4	10	5	3	1	3	6	11	Mild cell infiltration that appeared more frequent in Group 6 {10 mg/rat every 15 days) ♀s.
Inflammation: Fat/connective tissue	8	6	1	8	8	8	5	5	1	7	7	22	Mild cell infiltration that appeared more frequent in Group 6 {10 mg/rat every 15 days) ♀s.
Liver n=	50	50	50	50	50	50	50	50	50	50	50	50	No treatment effect on the incidence of basophilic or eosinophilic hepatocellular foci.
Basophilic cell foci	6	4	4	0	1	0	8	12	8	11	9	7	↑ incidence of foci or areas of tension lipidosis in Group 6 (10 mg/rat every 15 days)..
Eosinophilic cell foci	1	4	3	8	8	9	6	4	5	6	9	3	
Eosin cells: cystic degeneration	4	4	1	5	8	6	0	0	1	5	2	0	
Clear cell foci	1	1	2	1	1	1	2	5	2	3	2	2	

Tension lipidosis	5	6	4	8	8	14	6	10	10	14	16	15	↑ incidence in drug-treated rats. This change is a specific focal lesion generally considered to be associated with prolonged tension by supporting ligaments acting on a particular subcapsular area of hepatocytes
Hyperplasia: bile duct	13	10	7	18	15	20	11	14	3	13	15	12	In a small number of treated animals, especially ♀, dilatation of the extrahepatic bile ducts was reported.
Bile ducts n=	50	50	50	50	50	50	50	50	50	50	50	50	
Dilatation	2	4	2	3	4	5	1	1	1	6	4	5	↑ incidence in treated rats.
Testes n=	50	50	50	50	50	50							
Atrophy: seminiferous tubules	8	8	12	46	45	50							↑ incidence in treated rats.
Hyperplasia: interstitial cells	0	0	0	1	2	0							↑ incidence in treated rats.
Inflammation: vascular	0	8	5	11	17	11							↑ incidence in treated rats.
Epididymis:	50	50	50	50	50	50							
Oligo/aspermia	6	8	7	40	42	50							↑ incidence in treated rats.
Endocrine tissues: Adrenals n=	50	50	50	50	50	50	50	50	50	50	50	50	
Hyperplasia: cortex	2	8	4	9	4	11	7	11	8	10	15	20	↑ incidence in in Group 6 (10 mg/rat/15 days). In ♀, ↑ in cystic degeneration (all treated groups).
Degeneration: cystic	3	6	5	3	4	4	28	24	20	33	36	33	
Pituitary n=	50	50	50	50	50	50	50	50	50	50	50	50	
Vacuolation: pars distalis	1	2	3	7	7	10	0	0	0	0	0	0	In ♂, ↑ incidence of vacuolation in the pars distalis.
Kidneys: n=	50	50	50	50	50	50	50	50	50	50	50	50	
Chronic progressive nephropathy	34	36	30	38	41	41	23	26	22	12	7	8	↑ incidence in treated ♂ rats while a ↓ incidence in ♀ rats.
Spleen n=	50	50	50	50	50	50	50	50	50	50	50	50	
Deposits: pigment	0	2	4	2	3	0	12	15	18	3	3	4	↓ in pigment deposition (hemosiderosis) in ♀ animals to levels comparable with ♂.
Skin n=	50	50	50	50	50	50	50	50	50	50	50	50	
Ulceration	21	17	18	19	15	28	21	17	6	31	28	25	
Joint: ulceration plantar	3	3	1	3	2	12	2	2	0	5	3	2	↑ incidence in Group 6 males (10 mg/rat every 15 days) compared to vehicle or saline controls and in all ♀ vehicle control and treated animals compared to saline controls.

Incidental changes:

- Endocrine tissues – adrenal cortical congestion and sinusoidal dilatation, thyroid C-cell proliferation, thyroid follicular cysts.
- Gastrointestinal tract – ulceration, primarily in the stomach, mucosal and submucosal inflammation.
- Liver –sinusoidal congestion, hepatocellular vacuolation, basophilic, eosinophilic and clear cell foci, foci or areas of necrosis.
- Lungs – focal aggregations of alveolar macrophages, congestion and edema.
- Urinary tract –interstitial inflammation, pelvic inflammation, pelvic dilatation, urinary bladder transitional epithelial hyperplasia..

- Heart – occasional instances of myocarditis, myocardial fibrosis, and atrial thrombosis

Lymphoid organs – plasmacytosis, sinus histiocytosis and lymphoid hyperplasia were present to varying degrees in many lymph nodes. Thymic atrophy associated with normal aging was seen in the majority of animals

Toxicokinetics:

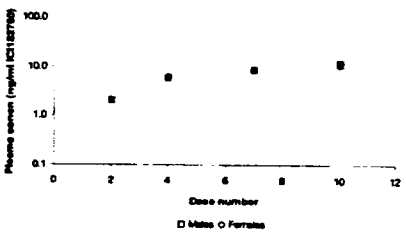
The following shows the ~ actual dose (mg/kg) administered to Groups V (10 mg/rat/30 days) and Group VI (10 mg/rat/15 days).

	Male					Female				
	BW (g)	**~Actual dose**	**BW (g)**	**~Actual dose**	**~Actual dose**	**BW (g)**	**~Actual dose**	**BW (g)**	**~Actual dose**	**~Actual dose**
Week	**10 mg/rat/ 30 days**	**mg/kg/ 30 days**	**10 mg/rat/ 15 days**	**mg/kg/ 15 days**	**mg/kg/ 30 days**	**10 mg/rat/ 30 days**	**mg/kg/ 30 days**	**10 mg/rat/ 15 days**	**mg/kg/ 15 days**	**mg/kg/ 30 days**
0	192.3	-	187.5	-	-	152.9	-	153.2	-	-
1	262.9	38	257.8	39	78	184.7	54	185.7	54	108
48	830.5	12	787.3	13	26	487.8	21	507.3	20	40
96	793	13	781.5	13	26	580.3	17	574.4	17	34
104	795.8	-	782.3	-	-	559.6	-	577.4	-	-

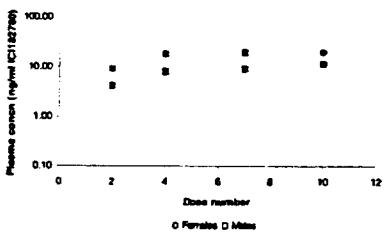
- Systemic exposure to ICI 182,780 was demonstrated in Group 4 (15 mg/kg/30days), Group 5 (10 mg/rat/30days) and Group 6 (10 mg/rat/15days) after a dosing period of ~12 months.

Mean trough plasma concentrations of ICI 182,780

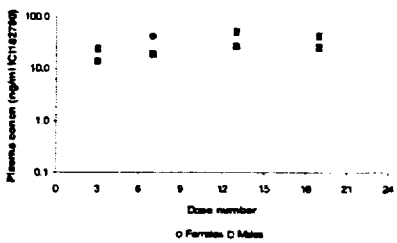
Group 4 (15 mg/kg/30 days)



Group 5 (10 mg/rat/30days)



Group 6 (10 mg/rat/15days)



- Trough plasma concentrations were monitored over the first 9 months of dosing in all groups (see Figures above) showing a steady increase up to 4 months, consistent with accumulation, followed by a flattening of the profile thereafter, suggesting that steady state pharmacokinetics had been achieved.

Group	4		5		6	
Dose	12		23		23	
Dose and frequency	15 mg/kg/30d		10 mg/rat/30d		10 mg/rat/15d	
Estimated actual dose (mg/kg/30 d)	15	15	12	21	26	34
Sex	♂	♀	♂	♀	♂	♀
C_{max} (ng/ml)	25	28.3	25.8	58.6	41	63.9
C_{max} .15d/30d ratio	1.6	2.3	1.6	1.1		
C_{max} .♀/♂ ratio		1.1		2.3		1.6
AUC (ng*d/ml)[&]	417	497	427	815	538	830
AUC-15d/30d ratio	1.3	1.7	1.3	1.0		
AUC -♀/♂ ratio		1.2		1.9		1.5
T_{max} (d)	2.0	2.0	2.0	2.0	2.0	4.0
$T_{½}$ (d)	23.5	28.9	25.7	21.5	NC	NC

[&] AUC was calculated over 30 days in Groups 4 and 5, and over 16 days in Group 6.

- The higher C_{max} values (1.6-2.3-fold) observed in Group 6 compared to Groups 4 and 5 may reflect drug accumulation due to more frequent dosing (every15 days).
- After ~ 12 months of dosing (i.e., dose 12 in Groups 4 and 5, and dose 23 in Group 6), C_{max} values for females in groups 5 and 6 were ~2-fold higher than males.
- Due to differences in the dosing criteria (mg/kg versus mg/animal) and the duration of the dosing interval (15 versus 30 days) comparison of exposure across the groups was not feasible.
- AUC values for female rats in groups 5 and 6 are ~2-fold higher than male values but there is no difference between the sexes for rats in Group 4. The Sponsor suggests that the sex difference resulted from females receiving relatively higher mg/kg dose in Groups 5 and 6 compared to male animals due to lower female body weights.
- If AUC values in the 3 dose groups are normalized for dose and body weight, the relative exposure between Groups 4 and 5 appears similar (see table below). Both groups received a similar dose level (~20 mg/kg for females and 11.9 mg/kg for males in Group 5 compared to 15 mg/kg in Group 4) over the same time interval of 30 days.

Table 1 **CTBR Project number 88579, AstraZeneca Reference number TCR2683. AUC values normalised for body weight, dose and time**

	Group 4 (15 mg/kg/30 days) Dose 12		Group 5 (10 mg/rat/30 days) Dose 12		Group 6 (10 mg/rat/15 days) Dose 23	
Parameter	Males	Females	Males	Females	Males	Females
Mean Weight (g)	823	501	843	500	795	513
Normalised Dose (mg/kg)	15.0	15.0	11.9	20.0	12.6	19.5
Observed AUC (ng.d/ml)	417	497	427	815	538	830
Normalised AUC (ng.d/ml/mg/kg)	27.8	33.1	35.9	40.8	85.4*	85.1*

*AUC values in Group 6 were normalised and then doubled in order to assess the exposure for 2 doses over a 30 day period

- Inspection of the plasma concentration-time profiles indicated that following the peak in Groups 4 and 5, ICI 182,780 declined monoexponentially with a long half-life (21.5 and 28.9 days). For purposes of calculating $AUC_{0\text{-}30d}$ it was assumed that steady state kinetics had been achieved prior to dosing at 12 months and the predicted concentrations at 30 days were therefore substituted as pre-dose values. For Group 6 the AUC was calculated from time 0 to 16 days; there were insufficient data points to calculate a half-life.
- No significant levels of sulphone and 17-ketone metabolites of ICI 182,780 were measured (below the assay limit of quantification of [redacted]ng/ml for sulphone and [redacted]ng/ml for 17-ketone).

Summary of individual study findings:
Adequacy of the carcinogenicity study and appropriateness of the test model:

- Despite the lower than expected survival rates for control males, there are an adequate number of animals that survived to the end of the study.
- The duration of the study (2 years) is appropriate.
- On July 28, 1998, the Sponsor obtained dose concurrence from the Executive CAC for the proposed carcinogenicity protocol.

Evaluation of tumor findings:
Administration of ICI 182,780 resulted in changes in the incidence (increased and decreased) of both neoplastic and non-neoplastic findings. Several of the changes in the ovaries, uterus, mammary glands, pituitary gland, and testes are considered to be related to the pharmacological activity of ICI 182,780.

ICI 182.780 increases the incidence of ovarian granulosa cell tumors and testicular interstital Leydig cell ademomas.

Ovaries: An increase in the incidence (14%) of ovarian granulosa cell tumors was recorded in the high dose female animals (7/50 rats at 10 mg/rat/15d). This was also associated with an increased incidence of hyperplasia of follicular granulosa cells in these animals. Also in the ovaries, there was an increase incidence of abnormal follicular development and a reduction in sertoliform tubular hyperplasia. Spontaneous incidence of granulosa cell tumors for this strain of rat is 0.06% (n=1729) (Giknis and Clifford, 2001 [redacted]. The conducting laboratory reports background instances varying from 0/120 to 1/120 (0.2%). Another study (n= 4493) with the same strain and source reports 0.3% (Gregson and Abbott, 1984).

Testes: There was an increase (2-12%) incidence of interstitial Leydig cell tumors (adenomas) in drug-treated animals. These tumors were present at a low incidence (4%) in the saline control group and absent in the vehicle control groups. The incidence in the high dose group was similar to controls (2%) while increased (8-12%) in the two lower dose groups. Spontaneous incidence for this tumor in this strain of rat is 2.35%.

ICI 182,780 decreased the incidence of uterine endometrial stromal polyps, mammary tumors (adenoma, fibroadenoma, adenocarcinoma) in females, and pituitary adenomas in females. Reductions in mammary gland and pituitary tumors may have contributed to the increase in longevity of animals administered ICI 182,780.

Carcinogenicity summary:
ICI 182,780 (fulvestrant-Faslodex) is a steroidal estrogen receptor antagonist. The compound is negative in *in vitro* and *in vivo* genotoxcity assays. The purpose of this study was to investigate the carcinogenic potential of ICI 182,780 following intramuscular injection to Sprague-Dawley rats once every 15 or 30 days over a minimum period of 104 consecutive weeks. According to the Sponsor, the highest dose level selected represent the maximum possible dose administerable by the intramuscular route based on strength of the formulation and injection volume.

Survival of drug-treated rats was increased compared to controls. It is noteworthy that survival rates for control males appear lower than expected. There were no treatment-related clinical signs, however a number of animals from all treated groups and from control animals in Groups 1 and 2 receiving vehicle, exhibited cysts at the injection sites. Overall body weights throughout the study were not affected by treatment even though there was a statistically significant reduction (7-14%) in food consumption in both male and female animals from all treated groups. Hematology parameters were unaffected by treatment.

Pharmacokinetic analysis showed systemic exposure to ICI 182,780 in drug-treated rats after a dosing period of ~ 12 months. Over the first 4 months of dosing, trough plasma concentrations increased steadily; subsenquently concentrations leveled off suggesting that steady state pharmacokinetics had been achieved. Similar to previous studies in rats (1 and 6 month), plasma concentrations of ICI 182,780 were higher in female rats compared to males in all groups. Plasma levels in Group 5 (10 mg/rat/30 d) and 6 (10 mg/rat/15 d) females were significantly greater than in males. This effect may result from higher mg/kg dose exposure due to lower body weights in females. Given the study design, in group 5 and 6 (10 mg/rat) the actual dose administered decreases with increasing weight. Thus, by dose 12/23, the actual dose administered to males (~13 mg/kg) is not significantly different than in Group 4 (15 mg/kg). Consequenlty, PK parameters are not different in males between Groups 4 and 5.

Administration of ICI 182,780 resulted in changes in the incidence (increased and decreased) of both neoplastic and non-neoplastic findings. ICI 182.780 increases the incidence of ovarian granulosa cell tumors and testicular interstital Leydig ademomas while decreasing the incidence of uterine endometrial stromal polyps, mammary tumors (adenoma, fibroadenoma, adenocarcinoma) in females, and pituitary adenomas in females. Reductions in mammary gland and pituitary tumors may have contributed to the increase in longevity of animals administered ICI 182,780.

Non-neoplastic changes included an increased prominence of ovarian follicles with the presence of abnormal follicles and granulosa cell proliferation in drug-treated groups. There was increased numbers of follicular cysts (also more hemorrhagic cysts) and increased interstitial cell stromal hyperplasia while age-related sertoliform tubular hyperplasia was decreased. There was an extensive uterine endometrial and myometrial atrophy and an extensive atrophy of mammary tissues in females. In males, testicular seminiferous tubular atrophy was associated with vascular inflammatory changes and epididimal oligo/aspermia.

Adrenocortical hyperplasia and cystic degeneration were seen primarily in high dose females. A decreased incidence of chronic progressive nephropathy was seen in females with a slight corresponding increase in males. There was evidence for a decreased splenic hemosiderosis in

females and a slight increase in the incidence of altered eosinophilic foci or areas of tension lipidosis in the liver of both males and females. The injection site exhibited multiple, thin walled cysts occasionally associated with minor degrees of local inflammation and degeneration or atrophy of skeletal muscle. Similar cystic structures were occasionally seen in perineural tissues of sciatic nerve distal to the injection sites.

Carcinogenicity conclusions:
The Sponsor concluded that ICI 182,780, administered intramuscularly to rats for 2 years at doses of 15 mg/kg/30 days, 10 mg/rat/30 days and 10 mg/rat/15 days, showed no evidence of direct carcinogenic activity. The Sponsor also suggests that induction of ovarian granulosa cell tumors and testicular Leydig cell tumors was consistent with the pharmacological activity of an anti-estrogen. The Sponsor notes that ICI 182,780-treated rats showed increased survival, "near abolition" of mammary tumors, and a reduction in the incidence of pituitary neoplasms.

On December 4, 2001, the results of this study were presented to the Executive Carcinogenicity Assessment Committee (Meeting minutes on Appendix A). After careful evaluation of the study, the Committee concluded that Fulvestrant increases the incidence of ovarian granulosa cell tumors in female rats, and the incidence of interstitial Leydig cell tumors in male rats. The increase incidence of granulosa and Leydig cell tumors should be included in the product labeling for fulvestrant.

The Committee also noted that while fulvestrant appears to have a negative profile for genotoxicity potential on a standard battery of tests, the Sponsor did not perform the defining studies to determine fulvestrant is not genotoxic. The Committee recommended that the Sponsor be asked to perform ^{32}P post-labeling study to determine if fulvestrant and/or its' metabolites may form adducts with cellular DNA. We agree that a ^{32}P post-labeling study will be valuable in assessing possible genotoxic potential of Fulvestrant. However, given that the current indication for Fulvestrant is
the request for this study maybe postponed. If the indication of Fulvestrant was to change to include any other population, the Sponsor is strongly recommended to conduct a ^{32}P post-labeling study to ensure that Fulvestrant is non-genotoxic.

Labeling Recommendations:
The Sponsor proposed the following carcinogenesis labeling:
Carcinogenesis:

Draft

FDA Recommendations:
A two-year carcinogenesis study was conducted in female and male rats, at intramuscular doses of 15 mg/kg/30 days, 10 mg/rat/30 days and 10 mg/rat/15 days. These doses correspond to approximately 1-, 3-, and 5-fold (in females) and 1.3-, 1.3-, and 1.6-fold (in males) the systemic exposure [$AUC_{0\text{-}30\ days}$] achieved in woman receiving the recommended dose of 250 mg/month). An increased incidence of benign ovarian granulosa cell tumors and testicular Leydig cell tumors was evident, in females dosed at 10 mg/rat/15 days and males dosed at 15 mg/rat/30 days, respectively. Induction of such tumors is consistent with the pharmacology-related endocrine feedback alterations in gonadotropin levels caused by anti-estrogen.

Addendum/appendix listing: CAC report:

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

Study title: ICI 182,780 : FEMALE FERTILITY AND DEVELOPMENTAL STUDY IN RATS: INTRAMUSCULAR ADMINISTRATION.

Key study findings:

- ICI 182,780 at doses ≥ 0.1 mg/kg/d had an adverse effect on female fertility and embryonic survival. At a dose of 2 mg/kg/d, following a 29 days off-dose recovery period, fertility and embryonic parameters were restored to values similar to the control group.
- A dose of 2 mg/kg/d administered during organogenesis resulted in an increased incidence of tarsal flexure. At 0.1 and 2 mg/kg/d there was an increase in litter incidence in non-ossification of the odontoid and ventral tubercule of the first cervical vertebra. At a dose of 0.001 mg/kg/d, there was no effect on female fertility, embryonic and fetal survival and development.

Study no.: TGR/2478.
Electronic NDA: \N_000\2001-03-28\pharmtox\tox\repro\tgr2478
Conducting laboratory and location: Zeneca Pharmaceuticals Safety of Medicines Department Cheshire, England
Date of study initiation: September 7, 1997
GLP/QA compliance: Yes
Drug, batch #, and % purity: C177/1, 96.2%
Formulation/vehicle:

Ingredients	Strength Placebo %/w/v	Strength 20 %w/v
ICI 182,780	-	2.0
Pluronic F127	1.0	1.0
Ethanol 96%	10.0	10.0
Water for injection Ph Eur	8.0	8.0
Propylene Glycol	to 100%	to 100%
Batch Reference Number	P/1203/48	P/1203/40

Methods:
Species/strain: AP rats (Alpk: AP$_f$SD strain, Wistar derived)
Doses employed: 0, 0.001, 0.1, and 2 mg/kg/d
Route of administration: IM
Study design: Fertility phase: Female rats from Groups I, II and III were dosed for 2 weeks prior to pairing, and through pairing to pregnancy day 7. Twenty- two Group I females and all of Group IV females were dosed for 3 weeks prior to a withdrawal period of 29 days until normal estrous cycling had resumed in the majority of the Group IV animals. This ensured that all female animals were dosed for at least 3 weeks. Necropsy/cesarean day 13
Teratology phase: daily dosing days 7-16 of gestation, necropsy/cesarean day 22

Group	Dose level (mg/kg/d)	# of animals (fertility phase)	#of animals (teratology phase)
I Control	0	44F+44M*	22F
II	0.001	22F+22M*	22F
III	0.1	22F+22M*	22F
IV	2	22F+22M*	22F

* Male rats remained undosed throughout the study.

Number/sex/group: 110 rats/sex for fertility study; 88 mated ♀ for teratology study.

Parameters and endpoints evaluated:

- clinical signs (daily),
- body weight (fertility phase: twice weekly until mated and then on days 1, 7, 10 and 13 of gestation; teratology phase: days 1, 7, 10, 13, 16, 19 and 22 of gestation; male rats twice weekly),
- food consumption (fertility phase: consecutive 7 days from the start of dosing until the rats were paired, or until the end of the dosing period in the withdrawal animals; teratology phase: day 7 to 14, and 14 to 21 of gestation)
- gross/ histopathology (Day 13 or 22),
- reproductive parameters (vaginal smears, mating, corpora lutea, implantations, uterus/ placental weight, early/ late resorptions, viable fetuses, sex, fetal weight, external malformations/ variations, visceral/ skeletal malformations
- pharmacokinetics

Statistical evaluations:The Jonckheere trend and the Cochran- Armitage trend test.

Results:

Mortality:

mg/kg/d	Control	0.001	0.1	2
FEMALES (fertility phase)	**44**	**22**	**22**	**22**
Number killed for scheduled necropsy	43	22	21	21
Number killed prematurely - for humane reasons	0	0	0	1
Number killed - reproductive tract examination	0	0	1	0
Number killed pregnant in harem	1	0	0	0
FEMALES (teratology phase)	**22**	**22**	**22**	**22**
Number killed for scheduled necropsy	22	22	22	18
Number killed - littered early	0	0	0	2
Number killed not pregnant	0	0	0	1
Number killed for humane reasons	0	0	0	1
Number of live fetuses on day 22	265	283	243	134

Clinical signs: Two females from the 2 mg/kg/day dose group (teratology phase) were killed on day 21 of gestation as they littered early. An additional female from the same dose group was sacrificed due to excessive discharge from the vagina.

Seventeen out of 22 females of the 2 mg/kg/day dose group, (teratology phase) had vaginal bleeding of which 7 animals had blood on their tray between days 17 and 22 of gestation.

Body weight: Fertility phase: Unremarkable
Teratology phase: At 2 mg/kg/d, there was a significant reduction in body weight gain during dosing period (↓(17%) and pregnancy as a whole.
At 0.1 mg/kg/d, body weight gain was reduced (↓20%) between days 19 to 22 of gestation.

Food consumption: Fertility phase: Unremarkable
Teratology phase: At 2 mg/kg/d, food consumption was significantly reduced (14%) between days 14 to 21 of gestation.

Estrous cycle:

Before dosing (14 d)	During dosing (14 d)	Control	0.001 mg/kg/d	0.1 mg/kg/d	2* mg/kg/d
Regular	Regular	36	16	0	0
Regular	Irregular	1	2	21	16
Irregular	Regular	3	3	0	0
Irregular	Irregular	4	1	1	6

*Dosing period was 21 d and not 14 d.

- ICI 182,780 disrupted the estrous cycle at ≥ 0.1 mg/kg/day with complete cessation at the high dose after two doses.
- Following a 3-week dosing period, animals at the 2 mg/kg/day dose level began to return to estrus within 17 days of withdrawal of the test compound.
- Females of the 2 mg/ kg/ day dose group were paired with untreated males on day 71 (equivalent to the 29th day post- withdrawal) of the study by which time 18/21 had returned to a regular estrous cycle.

Toxicokinetics:

Mean maternal plasma concentrations of ICI 182,780 were determined after administration of the final dose of 2 mg/ kg, on pregnancy day 16.

	Mean concentration ± SE ICI 182,780 ng/ml		
Dose	1 hour	2 hour	4 hour
2 mg/kg/d	156±17.7	225±56.7	153±36.8

Terminal and necroscopic evaluations:

Dams:

	Control*	0.001	0.1	2*
FEMALES (fertility phase)				
Number paired	44	22	22	21
Number mated	43	22	22	21
Number pregnant at scheduled day 13 necropsy				
Number with live embryos	37	19	0	18
Number with resorptions only	1	3	18	2
Number not pregnant at scheduled necropsy	6	0	4	1
Copulation index (%)	97.7	100	100	100
Fertility index (%)	88.3	100	81.8	95.2

* Control group includes withdrawal animals; 2 mg/kg/d group paired after withdrawal (29 days)

- Administration of ICI 182,780 (0.001 mg/kg/d) had no adverse effect on the ability of female animals to become pregnant.
- At 0.1 mg/kg/d, females were able to become pregnant but there were no live embryos at necropsy suggesting they were not able to maintain pregnancy.
- Female fertility was restored following withdrawal (29 days after receiving their last dose) of the test compound at 2 mg/kg/day.

Pregnancy and feto-placental unit assessment-fertility phase.

mg/kg	0	0.001	0.1[A]	2[B]
n=	37	19		18
Number of live fetuses/litter	16	16		15
Number of implants/dam	16	17		15
Number of corporea lutea/dam	17	17		17
Pre-implantation loss/dam	2	0		2
Post-implantation loss/dam	0	0		0

[A] All pregnant females had resorptions only at necropsy on day 13 of gestation.

[B] 2 mg/kg/d dose group ♀s, together with an additional 22 control ♀s, were dosed for a minimum of 3 weeks prior to a withdrawal period from the test compound. The withdrawal period continued until the majority of the 2 mg/kg/d dose group animals returned to regular estrus cycling, then they were paired 1 to 1 for mating with undosed male rats.

- Females of the 2 mg/kg/day dose level, following withdrawal of the test compound, had similar numbers of implantations, live fetuses, and post implantation loss compared to control group values.

Feto-placental unit assessment-teratology phase.

mg/kg	0	0.001	0.1	2
n=	22	22	22	18
Number of live fetuses/litter	12	13	12	6*
Post-implantation loss/dam	0	0	1	4*
Mean fetal bw/litter (g)	5.2	5	5.2	4.8*
Male proportion/litter	0.5	0.6	0.5	0.6*

* Significantly different from control

Necropsy observations

Dams:

- There were no compound related histological findings in the ovaries and uterus from all females involved in unsuccessful or infertile matings.

Unscheduled terminations

- One female (Group IV) was killed prematurely following vaginal bleeding. Tissue examinations did not reveal any additional reasons for mortality.

Teratology Phase
Offspring:
Fetal assessment

- There was a statistically significant increase in the number of fetuses and litters (22 fetuses from 12 litters) at the 2 mg/kg/day dose group with tarsal flexure.
- There were other major non-statistically significantly abnormalities observed in the 2 mg/kg/day dose group: cleft palate, umbilical hernia, oligodactyly of both the fore and hindpaws.
- The Sponsor reports that all other observations at the external/ visceral examination were either of low incidence and not dosage related or were of a type commonly observed in the strain of rat used on the study with an incidence similar to the control group.
- One fetus from the 0.001 mg/kg/d dose group exhibited fused 6th and 7th cervical centra at skeletal examination.
- At both 0.1 and 2 mg/kg/d, there was a statistically significant increase in litter and fetal incidence in non-ossification of the odontoid and ventral tubercule of the first cervical vertebra.
- At 2 mg/kg/d, there was a significantly increased incidence of incomplete ossification of the 6th sternebra at both the litter and fetal levels.
- There were significant increases in other skeletal observations at the fetal level across ICI 182,780 dose groups but did not appear to have a dose-related trend.

Summary of individual study findings:
Administration of ICI 182,780 did not affect the ability of females to achieve and maintain pregnancy at 0.001 mg/kg/d. At 0.1 mg/kg/d, females were able to become pregnant but there were no live embryos at necropsy suggesting that pregnancy could not be maintained. Following a 29 day withdrawal period from the test compound at a dose level of 2 mg/kg/d, female fertility and embryonic survival was restored to values similar to the control group. Administration of 2 mg/kg/d ICI 182,780 during the period of major organogenesis, resulted in maternal vaginal bleeding and a statistically significant increase in the incidence of tarsal flexure of the hindpaw in the fetuses. At both the 0.1 and 2 mg/kg/d doses, there was a statistically significant increase (litter incidence) in non-ossification of the odontoid and ventral tubercule of the first cervical vertebra. Thus, 0.001 mg/kg/d was a no effect dose level for female fertility and embryonic survival. This dose was also a no effect dose level for embryonic and fetal development when ICI 182,780 was administered during the period of major organogenesis.

Study title: ICI 182,780: TERATOLOGY STUDY IN RABBITS: INTRAMUSCULAR ADMINISTRATION.

Key study findings:

- ICI 182,780 (0.01, 0.1 or 0.25 mg/kg/d SA IM) administered to pregnant rabbits during the period of major organogenesis did not result in maternal toxicity.
- Increases in placental weight and post-implantation loss at 0.25 mg/kg/d were observed. There were no compound related effects on fetal development at any dose.

Study no.: TTB/665.
Conducting laboratory and location: Zeneca Pharmaceuticals Safety of Medicines Department Cheshire, England
Date of study initiation: April 14, 1997
GLP/QA compliance: Yes
Drug, batch #, and % purity: P/1203/40
Formulation/vehicle:

Ingredients	Strength Placebo %w/v	Strength 2.0 %w/v
ICI 182,780	-	2.0
Pluronic F127	1.0	1.0
Ethanol 96%	10.0	10.0
Water for injection Ph Eur	8.0	8.0
Propylene Glycol	to 100%	to 100%
Batch Reference Number	P/1203/48	P/1203/40

Methods:

Species/strain: New Zealand White Hybrid (HsdPoc) rabbits
Doses employed: 0, 0.01, 0.1 or 0.25 mg/kg/d ICI 182,780
Route of administration: IM
Study design: Daily dosing days 7-19 of pregnancy/cesarean day 29
Number/sex/group: 24 females/group
Parameters and endpoints evaluated: clinical signs (daily), body weight (days 1, 7, 10, 13, 16, 19, 22, 25 and 29 of pregnancy), food consumption (days 7 to 14, 14 to 21 and 21 to 28 of pregnancy), gross/ histopathology (Day 29), uterine examination (weight of intact uterus and contents, # and position of viable, dead and resorbing fetuses, individual fetal body weights and sexes , individual placental weights, empty uterus weight. number of corpora lutea), and fetal examination (gross soft tissue anomalies and sexed internally)
Statistical evaluations: Jonckheere trend test, non- parametric analysis of covariance, or the Cochran-Armitage test for trend.
Toxicokinetics Samples were taken from 9 pregnant animals in the 0.25 mg/kg/d group at 1, 2, and 4 h after dosing on day 19 of pregnancy. Plasma concentrations were determined using HPLC/[redacted] detection.

Results:

Mortality:

mg/kg/d	0	0.01	0.1	.25
Death/ sacrifice	0	1 (d24)[A]	1(d26 after abortion) 1 (d28)[A]	2[A, B] (d22-26) 3 (d21) non-pregnant (1 resorptions only at necropsy)

[A] These animals were sacrificed due to the extent of skin lesions around the injection sites.

[B] The cause of death in rabbit 17 probably related to the trichobeozar (hairball) in the stomach.

Clinical signs: Scabs and sores at the injection sites were observed in animals from all groups.

Body weight: Maternal body weight gain during pregnancy

(mg/kg)		0		0.01			0.1			0.25	
	Day	N	Median	N	Median	p	N	Median	p	N	Median p
Body wt. gain (g)	1-7	24	64	21	50		21	65		18	54
	7-10	24	-5	21	-12		21	-8		18	-28
	10-13	24	-8	21	-11		21	6		18	17
	13-16	24	48	20	56		21	109	c	18	130 c
	16-19	24	-14	20	-16		21	26		18	41 a
	19-22	24	-33	21	-69		21	-113	+	18	-72
	22-25	24	26	21	18		21	-86	c	18	-17 c
	25-29	24	34	21	43		21	42		18	12
whole preg.	1-29	24	130	21	46		21	61		18	76
dosing period	7-19	24	14	21	24		21	138 (↑9-fold)	b	18	156 c (↑11-fold)

Day=day of pregnancy
N=number of animals
a= p< 0.05, b = p< 0.01, c = p< 0.001 + signifies p< 0.01

- There was a statistically significant trend to increased maternal body weight gain at the 0.1 (9-fold) and 0.25 mg/kg/ (11-fold) dose levels during the dosing period (day 7-19).

Food consumption: There were no adverse changes in maternal food consumption.

Group : Dose 182.780 (mg/kg)		I		II			III			IV	
		0		0.01			0.1			0.25	
	Day	N	Median	N	Median	p	N	Median	p	N	Median p
Food cons. (g/an /day)	7-14	24	88	21	103		21	106		18	113
	14-21	24	80	21	79		21	82		18	80
	21-28	24	96	21	89		21	82		18	99

Histopathology (Dams): n=24 Figures in brackets represent the number of animals from which this tissue was examined microscopically.

LESIONS	GROUP	I 0 mg/kg /day	II 0.01 mg/kg /day	III 0.1 mg/kg /day	IV 0.25 mg/kg /day
GALL BLADDER:		(3)			(1)
Multifocal ulceration					1
IMPLANTATION OR INJECTION SITE:		(6)	(5)	(6)	(2)
Skin ulceration		3	4	6	2
Muscle necrosis		1	1	2	
Dermal inflammation		1	1		
Epidermal hyperkeratosis		1	1		
Granulation tissue		3	4	5	1
KIDNEYS:		(3)			(1)
Multifocal bilateral perivascular chronic inflammatory cell infiltration					1
Multifocal tubular dilatation		2			1
Intratubular microlithiasis		1			
LIVER:		(3)			(1)
Portal chronic inflammatory cell		2			
LIVER:		(3)			(1)
Diffuse fatty change		1			1
LUNGS:		(3)			(1)
Multifocal peribronchiolar chronic inflammatory cell infiltration					1
Focal alveolar haemorrhage					1
Alveolar septal congestion		1			
STOMACH:		(3)			(1)
Superficial oedema					1
Muscle necrosis					1

Figures in brackets represent the number of animals from which this tissue wa

- Eighteen animals had histological changes in the skin, in a spectrum of severity from inflammation with epidermal thickening to frank ulceration overlying granulation tissue and fibrous tissue. In 4 animals the underlying muscle was involved.
- One animal (0.25 mg/kg/d) had changes in the gallbladder (ulceration), and stomach (edema) typical of an animal in a moribund state.

Toxicokinetics: Mean maternal plasma concentrations of ICI 182,780 were determined after administration of the final dose of 0.25 mg/kg/d, on pregnancy day 19.

	Concentration ± SE ICI 182,780 (ng/ml)		
Dose	1 hour	2 hour	4 hour
0.25 mg/kg/d	23.7±4.22	25.4±3.11	13.8±1.26

Effects on pregnancy and the feto- placental unit:

mg/kg/d	0 (n=24)	0.01 (n-21)	0.1 (n=21)	0.25 (n=18)
# of live fetuses/litter	6	7	7	6
# of implant/dam	8	8	9	9
# of corporea lutea/dam	9	10	10	10
Pre-implantation loss/dam	1	1	1	1
Post-implantation loss/dam	1	1	1	2^A
Mean fetal bw/litter (g)	34.3	34.4	33	33.7
Mean placental bw/litter (g)	5.5	5.4	5.6	6.3^A

^A $p<0.05$

- There was an increased incidence of post- implantation loss at the highest dose (0.25 mg/kg/d), however, the number of live fetuses at this dose was similar to the control group.
- Placental weights in the 0.25 mg/kg/d were also significantly increased.

Effects on fetus:

Th Sponsor reported the following findings:

- Major external/visceral abnormalities observed in 6 fetuses, one from the control group, three (of which two were litter mates) from the 0.01 mg/kg/day dose group, and two from the 0.1 mg/kg/d dose group. No major external/visceral abnormalities were observed in the high dose.
- Three fetuses, 2 from the control group and 1 from 0.25 mg/kg/d dose group exhibited major defects of the vertebral column including additional vertebral arches and fused vertebral arches.
- There was a statistically significant increase in the number of fetuses and litters at the 0.25 mg/kg/d dose level with backwards displacement of the pelvic girdle (24 fetus in 13 litter in the 0.25 mg/kg dose group compared to 2 fetus in 1 litter in the control group).
- There was a significant increase in the number of fetuses at the hight dose showing normal length extra 13th ribs (80 fetus in 17 litter in the 0.25 mg/kg dose group compared to 106 fetus in 24 litter in the control group). There was also a significant increase in the number of fetuses exhibiting 27 pre-sacral vertebrae (24 fetus in 13 litter in the 0.25 mg/kg dose group compared to 19 fetus in 11 litter in the control group).

Note: The Sponsor did not evaluate the skeletons of fetuses from the lower two dose groups based on no skeletal changes in the 0.25 mg/kg/d dose level.

Summary of individual study findings:

ICI 182,780 (0.01, 0.1 or 0.25 mg/kg/d SA IM) administered to pregnant rabbits during the period of major organogenesis did not result in maternal toxicity. Nonetheless, there was a statistically significant increase in maternal body weight gain at the 0.1 and 0.25 mg/kg/d dose levels during the dosing period that may be related to the anti-estrogenic properties of ICI 182,780. At the high dose (0.25 mg/kg/d), there was a statistically significant increase in post-implantation loss. However, there was no significant change on the median number of live fetuses. Placental weights of the 0.25 mg/kg/d dose group were statistically significantly increased compared to the control group. A low incidence of a variety of external/visceral or skeletal abnormalities was observed in a number of fetuses from all groups. There was a significant increase in the number of fetuses and litters at the 0.25 mg/kg/d dose level with

backwards displacement of the pelvic girdle (24 fetus in 13 litter compared to 2 fetus in 1 litter in the control group). The Sponsor was considered this finding to be associated with the increase in the number of fetuses with extra 13th ribs and 27 pre- sacral vertebrae.

Study title: ICI 182,780: Post natal development toxicity study in rats.

Key study findings:

- **This study was submitted to IND [redacted] on November 20th, 2001. Since this study was submitted very late the review cycle for NDA 21,344, only a cursory review was feasible.**
- ICI 182,780 as a single IM dose of 15 mg/kg or two doses of 10 mg/rat (~38 mg/kg on day 2 or 25 mg/kg on day 17) to parent F0 female rats postpartum did not cause maternal toxicity and had no effect on pup body weight between days 2 to 22 post partum.
- Pup survival was lower after maternal treatment with ICI 182,870.
- In the pregnant Fl females, whose dams had been treated with 15 mg/kg during lactation, there was an increase in pre-implantation loss with concomitant reduction in the numbers of live fetuses.
- There was an effect on sexual maturation, as determined by an earlier age of vaginal opening, in female animals whose dams had been dosed with 10 mg/rat.
- Maternal drug treatment did not affect the fertility, sperm counts, or gonadal histopathology of the Fl males.

Study no.: TWR/3254
Volume #, and page #: Submitted to IND [redacted] (N-176) IT
Conducting laboratory and location: Zeneca Pharmaceuticals Safety of Medicines Department Cheshire, England
Date of study initiation: December 11, 2000
GLP compliance: Yes
QA reports: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: P/1359/26
Formulation/vehicle: ICI 182,780 LA/placebo

Methods:

Species/strain: Rat (Alpk: AP_fSD strain, Wistar derived)
Doses employed: 0, 15 mg/kg, 10 mg/rat
Route of administration: IM
Study design: The F0 generation of animals administered 15 mg/kg ICI 182,780 (LA IM) were dosed on day 2 post partum only, with a dose volume of 0.03 ml/100 g body weight.
The F0 generation control group animals and those animals administered 10 mg/rat ICI 182,780 (LA IM) were dose on days 2 and 17 post partum with a dose volume of 0.02 ml/rat (0.1 ml/hind limn)
F1 generation animals were not dosed.

Results:

Parent F0 females

Maternal performance: One female given placebo, and two females given 10 mg/rat on day 2 of lactation were killed prior to scheduled termination (day 22) following total litter loss on days 4, 3 and 8 respectively.

Clinical observations: Unremarkable (UR)

Bodyweight: There was no effect of treatment on maternal body weight during lactation.

BW (g)	Placebo	15 mg/kg	10 mg/rat
Day 1	262.7	259.8	261.0
Day 22	402.5	391.5	393.5

Note: Based on body weight, animals in the 10 mg/rat group received ~38 mg/kg on day 1 and ~25 mg/kg on day 22.

Food consumption: There was no effect of treatment on maternal food consumption during lactation.

Toxicokinetics: Plasma samples taken on day 22 postpartum, from F0 dams dosed with 15 mg/kg or 10 mg/rat (~25 mg/kg) during lactation were 4.09 ± 0.437 ng/ml and 26.5 ± 1.78 ng/ml, respectively.
These data show systemic exposure to ICI 182,780 in drug-treated F0 females.

Macroscopic findings: There were no macroscopic findings in the sacrificed animals with total litter loss (1 placebo, 2 high dose) to indicate any reason for the death of the pups in these females. The Sponsor considered the losses unrelated to treatment with ICI 182,780.
No findings were observed in females examined at scheduled termination on day 22 postpartum.

Gestation length: Gestation length (22 days) was the same for all parent F0 females.

F1 Litter data

F1 pup survival and litter size: There was no evidence for an effect of treatment of F0 dams on F1 litter size (live pups) from day 1 to day 22 post partum.

	Placebo	15 mg/kg	10 mg/rat
Proportion of pups born live (%)	285/286 (99.7%)	278/281 (98.9%)	313/313 (100%)
Proportion of litters with all pups born live	23/24	22/24	24/24

In the placebo group, 1 total litter loss occurred on day 4.
No total litter losses occurred in the 15 mg/kg treatment group.
In the 10 mg/rat group, two total litter losses occurred between days 3 and 8.

Excluding whole litter losses, pup survival was lower in the treated groups than the placebo group.

Excluding litter losses	Placebo	15 mg/kg	10 mg/rat
Proportion of pups surviving (%)	265/266 (99.7%)	265/276** (96.4%)	265/279** (95.5%)*
Proportion of litters with all pups surviving	22/23	18/24	15/22*

Including whole litter losses, pup survival was lower in the 10 mg/rat group compared with the placebo group.

Including litter losses	Placebo	15 mg/kg	10 mg/rat
Proportion of pups surviving (%)	265/279 (95.5%)	265/276** (96.4%)	265/309** (87.6%)*
Proportion of litters with all pups surviving	22/24	18/24	15/24*

Pup sex distribution: There was no effect of treatment of parent F0 females on F1 pup sex distribution.

Pup clinical observations: The number of F1 litters with pups found dead or missing, presumed dead, or killed for humane reasons, (i.e. pup mortality) was similar across the groups.

An increased incidence of litters with cold pups between days 1 and 3 postpartum was observed in the 10 mg/rat group. (29 observation on 7 litters compared to 21 observations in 2 litters in the placebo group)

Pup body weight: There was no effect of maternal treatment on pup body weight on day 3 postpartum through to day 22 postpartum.

Total Fl litter weight was unaffected by maternal F0 treatment with ICI 182,780.

F1 Animal data

Clinical observations: A total of 8, 7 and 20 pups in the placebo, 15 mg/kg and 10 mg/rat groups respectively, were observed as small up to day 25 postpartum.

Seven of those pups in the placebo group and two pups from the 10 mg/rat group were subsequently part of total litter losses on days 4 and 8 respectively.

A number of pups in one litter from the 10 mg/rat group were observed to be thin on day 5 postpartum.

Body weights: There was no effect of maternal treatment on the growth of the selected F1 animals from day 3 post partum to termination.

Surface righting: There was no effect of maternal treatment on the proportion of F1 pups able to move from a dorsal to a ventral position, on day 4 post partum.

Olfactory discrimination:	There was no effect of maternal treatment on the proportion of Fl pups able to select home bedding in preference to clean bedding, on day 9 postpartum.
Auditory startle response:	There was no effect of maternal treatment on selected F1 pups on startle amplitude. There was an increase in the time to maximum amplitude during repetition 31-40 for female selected F1 pups in the 10 mg/rat group compared with the placebo group when assessed on day 30 postpartum (21.9 ms compared to 19.4 ms in placebo controls). The Sponsor considered this difference incidental to treatment.
Placing response:	There was no effect of maternal treatment on the placing response of selected Fl pups when assessed on day 36 postpartum.
Pupillary reflex test:	There was no significant effect of maternal treatment in the pupillary reflex test of selected F1 pups when assessed on day 36 postpartum.
Tail flick response:	There was no effect of maternal treatment on the time to tail flick of selected F1 pups when assessed on day 36 postpartum.
Learning and memory:	There was a decrease in the percentage of successful trials with a cut-off time of 8 seconds only for selected F1 female pups from F0 dams treated with 10 mg/rat compared with placebo on day 28 (learning phase; 59.2% of successful trials compared to 70.8% in placebo controls). However, the Sponsor considered this difference not to be related to treatment. There was therefore, no effect of maternal F0 treatment on learning or memory, assessed using a Y shaped water maze, on days 28 and 31 postpartum respectively. The additional test for swimming speed in a straight channel showed the following differences compared with the placebo group. On day 28 postpartum, an increased time was recorded for selected F1 male pups, from F0 dams treated with ICI 182,780. On day 31 post partum, an increased time was recorded for selected F1 female pups from F0 dams treated with 10 mg/rat. In the absence of an effect at other time-points, or on performance in the Y maze, the Sponsor considered these differences in the straight channel to be incidental to treatment.
Motor activity:	There was no effect of maternal treatment on locomotor activity of selected F1 animals when assessed on day 57 postpartum. A statistically significant difference from control, for the 21-25 minute period, indicated increased activity in selected F1 females from the 10 mg/rat group (59.8 compared to 42.7 in placebo controls). The Sponsor considered this difference incidental to treatment.
Developmental landmarks:	Sexual maturation of the selected F1 animals was assessed from the age at which preputial separation in males and vaginal opening in females occurred

	Placebo	15 mg/kg	10 mg/rat
Preputial Separation (day)	43.8 ±1.9	43.1±1.5	43.2±1.8
Day of Vaginal Opening	32.6±1.5	31.9±2.2	31.3±2.1*

The age of vaginal opening was earlier in female animals, whose dams had been dosed with 10 mg/rat than in those dosed with placebo (31.3±2.1 compared to 32.6±1.5 days in placebo controls). The Sponsor considered this difference to be within the experimental error of a once daily observation. However, given the anti-estrogenic effects of ICI 182,780, a treatment effect may not be ruled out.

Reproductive performance: The estrous cycle was assessed for all selected F1 females for 2 weeks prior to mating. No effects on the estrous cycle of F1 females after treatment of their F0 dams with ICI 182,870 (LA IM injection) during lactation were observed.

There was no effect of maternal F0 treatment on pre-coital interval of selected Fl females. Almost all selected Fl animals mated within the first 4 days.

One selected F1 female (placebo group) and 2 selected F1 females, including one which failed to produce a positive smear (10 mg/rat group) failed to become pregnant.

Hence only one selected F1 male (placebo group) and 2 selected F1 males (10 mg/rat group) failed to sire a litter.

The fertility of selected F1 males and females was not affected by treatment of F0 females with ICI 182,870 (LA IM injection) during lactation.

Maternal body weight during gestation: There was no effect of treatment of F0 females on the body weight during gestation (days 1-14) of selected F1 females.

Uterine examination on day 14 of gestation: The number of corpora lutea, implantations, and intra-uterine deaths, and post-implantation loss was comparable for all groups of selected Fl females.

Pre-implantation loss (proportion of implants affected) was increased in both treated groups (15 mg/kg and 10 mg/rat), when compared with the placebo group. The increase was not dose-related.

	Placebo	15 mg/kg	10 mg/rat
Pre-implantation loss-Prop. of implants affected (%)	16/272 (6.3)	47/272 (18.4)	36/253 (12.5)
Post-implantation-Prop. of implants affected (%)	14/256 (5.5)	19/225 (10.5)	8/217 (8.5)

The number of live fetuses on day 14 of gestation was statistically significantly lower in 15 mg/kg group than in the placebo group.

	Placebo	15 mg/kg	10 mg/rat
No. of live fetuses	12.7±2.6	10.3±4.1*	11.6±4

Organ weights: There was no effect of maternal F0 treatment on the organs weighed from the selected F1 males.

Sperm data: There was no effect of maternal F0 treatment on the total number of sperm/cauda epididymis or the number of sperm/gram of tissue in the selected F1 males.

Toxicokinetics: Plasma samples, taken on day 22 postpartum, from F1 pups whose F0 dams had been dosed with 15 mg/kg or 10 mg/rat during lactation showed no systemic exposure to ICI 182,780.

Macroscopic findings: There were no macroscopic findings observed in the F1 males and females examined at scheduled termination on day 22 postpartum, or in the selected F1 males and females at termination.

Microscopic findings F1 males: No changes were observed in the testis or epididymis of selected F1 males.

Summary of individual study findings:

This study investigated possible adverse effects of ICI 182,780 on the lactating F0 female and on the development of the F1 offspring, following exposure of the F0 female during lactation. Selected F1 offspring were allowed to grow to adulthood and their physical development, sensory functions, reflexes, behavior, sexual maturation, and reproductive performance were assessed. Assessment of the estrous cycle in the F1 females and assessment of gonadal weights and histopathology plus sperm counts in the F1 males were also included.

Toxicokinetic analysis shows systemic ICI 182,780 exposure on day 22 postpartum, from F0 dams dosed with 15 mg/kg or 10 mg/rat (~25 mg/kg) during lactation. However, there is no measurable systemic exposure from F1 pups whose F0 dams had been drug-treated. Previous milk secretion studies in lactating rats given a single dose of 2 mg/kg of ICI 182,780 showed that the concentration of radioactivity was around twelve times higher in milk than in blood at most time-points examined (estimated drug exposure of ~10%). Thus, some drug exposure would have been expected in F1 offsprings. This difference may be explained because the ingestion of milk versus solid food was relatively less in day 22 postpartum rats compared with earlier in lactation. Also, the absolute oral bioavailability of ICI 182,780 in adult rats is less than 20% (Clarke, 1997).

During lactation, body weights and food consumption of the parent F0 females were comparable for all groups. One total litter loss in the placebo group and two in the 10 mg/rat group were considered incidental to treatment. There were no macroscopic findings in the parent females at scheduled termination on day 22 postpartum and no macroscopic findings to indicate any treatment-related effects on the 3 females that had total litter losses.

Pup survival (excluding whole litter losses) was lower after maternal F0 treatment with ICI 182,870 compared with placebo. The number of pups which died between days 2 and 5 was

increased in the treated groups compared with placebo (1, 10 and 13 for placebo, 15 mg/kg and 10 mg/rat respectively). Although the difference between the groups for the percentage of pups surviving was small (99.7%, 96.4% and 95.5% for placebo, 15 mg/kg and 10 mg/rat groups respectively) an effect of treatment with ICI 182,870 cannot be ruled out. Also, a number of individual pups in both ICI 182,870-treated groups were observed as 'small' and 'thin' between days 3 and 25 postpartum. This observation correlated with lower body weight of individual pups. Overall, there was no effect of maternal drug treatment on total F1 litter weight or pup body weight between days 2 to 22 postpartum.

There was no effect of maternal treatment with 15 mg/kg or 10 mg/rat on the physical development, sensory functions, reflexes, and behavior (learning and memory and locomotor activity) of the selected F1 animals. Similarly, there was no effect of maternal drug treatment on selected F1 female estrous cycle, pre-coital interval or fertility (ability to become pregnant). The number of corpora lutea, implantations, intra-uterine deaths, and post-implantation loss was comparable for all groups.

There was a slight effect of maternal drug treatment sexual maturation of the selected F1 animals. Even though three was no difference in time to preputial sepation, the age of vaginal opening was earlier in female animals, whose dams had been dosed with 10 mg/rat compared to placebo (31.3±2.1 compared to 32.6±1.5 days in placebo controls). The Sponsor considered this difference to be within the experimental error of a once daily observation. However, given the anti-estrogenic effects of ICI 182,780, a treatment effect may not be ruled out.

Pre-implantation loss in F1 female offsprings (proportion of implants affected) was increased, although not dose-dependently. The mean pre-implantation loss (as a percentage per litter) and the proportion of litters affected were not statistically significantly different from the placebo group. This increase in pre-implantation loss was sufficient to cause the number of live fetuses to be lower at 15 mg/kg than placebo. In contrast, there was no effect of maternal treatment with ICI 182,780 on selected F1 male organ weight, sperm count, or on testis and epididymis when examined microscopically.

ICI 182,780 as a single IM dose of 15 mg/kg or two doses of 10 mg/rat (~38 mg/kg on day 2 or 25 mg/kg on day 17) to parent F0 female rats postpartum did not cause maternal toxicity and had no effect on pup body weight between days 2 to 22 post partum. Nonetheless, pup survival was lower after maternal treatment with ICI 182,870. In the pregnant Fl females, whose dams had been treated with 15 mg/kg during lactation, there was an increase in pre-implantation loss with concomitant reduction in the numbers of live fetuses. Also, there was an effect on sexual maturation as determined by an earlier age of vaginal opening in female animals whose dams had been dosed with 10 mg/rat. Maternal drug treatment did not affect the fertility, sperm counts, or gonadal histopathology of the Fl males.

Reproductive and developmental toxicology summary:
In the rat, a sighting fertility study in females was done (TGR/2659) and followed by a female fertility and developmental study in rats (TGR/2478). In the sighting study, rats administered ICI 182,780 (0.01, 0.1 and 1 mg/kg/d SA IM) from 2 weeks prior to pairing, during mating and until day 7 of pregnancy survived to day 13 of gestation and no compound related changes were seen at maternal necropsy. ICI 182,780 was associated with a disruption to regular estrous cyclicity at doses greater than 0.1 mg/kg/d. At doses greater than 0.01 mg/kg/d, there was a dose-related reduction in female fertility and embryonic survival. No live embryos were found in animals dosed 1.0 mg/kg/d. For animals given 0.1 mg/kg/d there was a statistically significant reduction in the mean number of embryos and an associated elevation in the incidence of intrauterine deaths.

In the fertility phase of study TGR/2478, ICI 182,780 was administered to rats (0, 0.001 and 0.1 mg/kg/d IM SA) two weeks prior to mating and throughout mating to day 7 of gestation. To examine reversibility of fertility findings, rats were dose with 2 mg/kg/d ICI 182,780 for 3 weeks followed by a withdrawal period prior to mating. Males were not dosed. ICI 182,780 at doses greater than 0.1 mg/kg/d had an adverse effect on female fertility and embryonic survival. However, at a dose of 2 mg/kg/d, these effects were restored to values similar to the control group following a 29-day withdrawal period.

During the teratology phase of this study, ICI 182,780 (0.001, 0.1 and 2 mg/kg/d IM SA) was administered days 7 to 16 of pregnancy. A dose of 2 mg/kg/d administered during organogenesis resulted in an increased incidence of tarsal flexure of the hindpaw. At 0.1 and 2 mg/kg/d there was an increase in litter incidence in non-ossification of the odontoid and ventral tubercule of the first cervical vertebra. At a dose of 0.001 mg/kg/d, there was no effect on female fertility, embryonic and fetal survival and development.

A pre and post-natal sighting study in rats has also been done with ICI 182,780 (SA) (TWR/2968). All female rats dosed with ICI 182,780 (2 mg/kg/d SA IM) between day 7 and days 14 to 20 of gestation exhibited vaginal bleeding. There was also a delay and prolongation of parturition. The number of intra uterine deaths was increased in all groups dosed with ICI 182,780 with a consequent reduction in the number of live fetuses/pups. The incidence of fetal abnormalities was also increased in these groups compared to the controls.

A sighting teratology study in rabbits (TRB/718) was done to set doses for a full teratology study in the rabbit (TTB/665). In the sighting study, female rabbits were administered 0.1, 1, and 5 mg/kg/d ICI 182,780 SA IM during the period of major organogenesis (days 7 to 19 of pregnancy). For animals given 1 mg/kg/day there was a statistically significant reduction in maternal body weight gain, specifically between days 13 to 16 gestation. There was an overall reduction in maternal body weight gain in animals given 1 and 5 mg/kg/d. Blood on the tray was observed in 3/6 animals given 1 mg/kg/day and 5/6 animals in the 5 mg/kg/day dose group. Macroscopically, scab formation and ulceration of the skin were seen at the injection site of some animals from all groups. No live fetuses were obtained from females given 1 and 5 mg/kg/day ICI 182,780. The incidence of intra-uterine deaths was elevated in these groups. Thus, this sighting study showed that at IM doses of 1 mg/kg/day (SA) or greater, rabbits failed to maintain pregnancy. At the lowest dose tested (0.01 mg/kg/d), a slight decrease in the mean fetal weight and placental weight was seen compared to controls; however the mean litter weight was not

statistically different from controls. No external anomalies were detected in live fetuses obtained from dams given ICI 182,780.

In the definitive study (TTB/665), ICI 182,780 (0.01, 0.1 or 0.25 mg/kg/d SA IM) administered to pregnant rabbits during the period of major organogenesis did not result in maternal toxicity. Nonetheless, there was a statistically significant increase in maternal body weight gain at the 0.1 and 0.25 mg/kg/d dose levels during the dosing period that may be related to the anti-estrogenic properties of ICI 182,780. At the high dose (0.25 mg/kg/d), there was a statistically significant increase in post-implantation loss. However, there was no significant change on the median number of live fetuses. Increases in placental weight and post-implantation loss at 0.25 mg/kg/d were observed. A low incidence of a variety of external/visceral or skeletal abnormalities was observed in a number of fetuses from all groups. There was an increase in the number of fetuses and litters at the 0.25 mg/kg/d dose level with backwards displacement of the pelvic girdle. The Sponsor considered this finding to be associated with the increase in the number of fetuses with extra 13th ribs and 27 pre-sacral vertebrae. The Sponsor reports that "no effects on fetal development were observed in rabbits"

It is noteworthy that in the definitive study (TTB/665), administration of ICI 182,780 (0.01, 0.1 or 0.25 mg/kg/d SA IM) to pregnant rabbits did not result in any maternal toxicity. Also, the Sponsor did not evaluate the skeletons of fetuses from the lower two dose groups based on no skeletal changes in the 0.25 mg/kg/d dose level. Thus, given the lack of maternal toxicity at the highest dose (0.25 mg/kg/d) in the definitive study and the incomplete fetal assessment, this study is deemed not adequate to fully define possible adverse effects on developing fetuses.

A post natal development toxicity study in rats (TWR/3254) investigated possible adverse effects of ICI 182,780 on the lactating F0 female and on the development of the F1 offspring, following exposure of the F0 female during lactation. ICI 182,780 as a single IM dose of 15 mg/kg or two doses of 10 mg/rat (~38 mg/kg on day 2 or 25 mg/kg on day 17) to parent F0 female rats postpartum did not cause maternal toxicity and had no effect on pup body weight between days 2 to 22 post partum. Nonetheless, pup survival was lower after maternal treatment with ICI 182,870. In the pregnant Fl females, whose dams had been treated with 15 mg/kg during lactation, there was an increase in pre-implantation loss with concomitant reduction in the numbers of live fetuses. Also, there was an effect on sexual maturation as determined by an earlier age of vaginal opening in female animals whose dams had been dosed with 10 mg/rat. Maternal drug treatment did not affect the fertility, sperm counts, or gonadal histopathology of the Fl males.

Reproductive and developmental toxicology conclusions:

Rat studies: Tables shows findings in studies TGR/2659, TGR/2478, and TWR/2968.

mg/kg/d	.001	0.01	0.1	1	2*
$mg/m^2/d$	.006	.06	.6	6	12
Fertility Phase					
↓ maternal body weight				x	x
No live embryos				x	
↓ in the mean number of embryos/↓ embryonic survival		x	x	x	x
↑ incidence of intrauterine deaths			x	x	x
Disruption to regular estrous cyclicity.			x	x	
↓ in female fertility		x	x	x	
Delay and prolongation of parturition					x
Vaginal bleeding					x
Teratology Phase					
↑ incidence of tarsal flexure hindpaw					x
↑ litter incidence of non-ossification of the odontoid and ventral tubercule of the first cervical vertebra			x		x

* In the fertility phase, rats were dose with 2 mg/kg/d ICI 182,780 for 3 weeks followed by a withdrawal period prior to mating. Effects on fertility were restored to values similar to the control group following a 29-day withdrawal period.

NOAEL= 0.001 mg/kg/for female fertility, embryonic, and fetal survival and development in the rat.

Rabbit studies: Tables shows findings in studies TRB/718 and TTB/665*

mg/kg/d	.001	0.1	.25	1	5
$mg/m^2/d$	.012	1.2	3	12	60
Fertility Phase					
↓ maternal body weight				x	x
No live embryos				x	x
↑ incidence of intrauterine deaths/↑ post-implantation loss			x	x	x
↓ in female fertility				x	x
↑ placental weights			x		
Teratology Phase					
↓in the mean fetal weight		x			
↑ incidence of litters with backwards displacement of the pelvic girdle			x		
↑number of fetuses with extra 13th ribs and 27 pre- sacral vertebrae.			x		

* Given the lack of maternal toxicity at the highest dose (0.25 mg/kg/d) in the definitive study (TTB/665) and the incomplete fetal assessment, this study is deemed not adequate to fully define possible adverse effects on developing fetuses.

3 pages redacted from this section of the approval package consisted of draft labeling

VIII. SPECIAL TOXICOLOGY STUDIES:

Study title: ICI 182,780 : ACUTE INTRAMUSCULAR TOLERANCE IN RABBITS: COMPARATIVE STUDY.

Key study findings:

- ICI 182,780 administration resulted in the elevation of creatine kinase activity.
- Histopathological findings show muscle necrosis, myositis, and granulomata after administration of the ICI 183,780 formulation.
- Muscle necrosis was still present in 2/6 animals after 30 days, but had disappeared after 50 days.
- The formulation containing ICI 182,780 caused a greater degree of muscle necrosis, myositis, and granulomata compared to physiological saline or the reference compounds (Proluton Depot and Delestrogen) tested.

Study no: TIB/524.
Volume #, and page #: F:\N_000\2001-03-28\pharmtox\tox\Special\TIB/524
Conducting laboratory and location: ICI Pharmaceuticals Safety of Medicines Department Alderley Park Nacclesfield Cheshire SK10 4TG England
Date of study initiation: September 19, 1990
GLP compliance: Yes
QA reports: yes () no (x):
Drug, lot #, and % purity: Batch #: PH/6731/36; 98.5%
Formulation/vehicle:

Ingredients	Strength % w/v
ICI 182,780	5.0
Ethanol 96% v/v	10.0
Benzyl Alcohol	10.0
Benzyl Benzoate	15.0
Castor Oil	To 100
Batch number	PH/6731/36
Analytical Number	ADM 48015/90

Methods: New Zealand White rabbits (n=8/group; 2.5-3.4 kg) were given two 0.5 ml intramuscular (IM) injections (one on each side of the back) into the longitudinal muscles.

Dosing:

ICI 182,780: two 0.5 ml IM injections
Negative control: 0.9% w/v aqueous sodium chloride solution (batch # D755).
Reference article 1: Proluton Depot (batch # 92777).
Reference article 2: Delestrogen (batch # 8G36738).

Observations and times:
Clinical observations: All animals were examined for clinical signs of disease.
Body weights Day of injection and weekly thereafter.
Clinical pathology: Blood samples were taken from all rabbits in Group I (ICI 182,780 and physiological saline) once pre-study and on days 1 (6 hours

after dosing), 2 (24 hours after dosing), 3, 7, and 10. Samples for days 20, 30, 40, and 50 were not collected.

Blood chemistry: Plasma was visually checked for hemolysis and creatine kinase was measured in all samples.

Pathology: The muscle from each injection site was excised and preserved in 10% buffered formalin.

Results:

Clinical observations: Bruising of the injection site (Delestrogen) in one rabbit from day 2 to 8.

All the other injection sites appeared normal throughout the study.

Body weights: Several animals lost weight between week 2 and 3 but the Sponsor does not consider it to be treatment related.

Blood chemistry: Table shows mean activity of creatine kinase for animals in group I (ICI 182,780 and physiological saline).

ICI 182,780 and physiological saline	Pre-study	6 h post dose	24 h post dose	Day 3	Day 7	Day 10
Creatine kinase (IU/l)	666	14198 (↑21x)	11188 (↑17x)	5938 (↑9x)	733	548

Histopathology

DAY	Saline	ICI 182,780	Proluton Depot	Delestrogen
Necrosis				
Minimal	0/2	0/6	0/2	0/2
Mild	0/2	1/6	0/2	0/2
Moderate	0/2	1/6	0/2	0/2
Severe	0/2	3/6	0/2	0/2
Total	0/2	5/6	0/2	0/2
Myositis				
Minimal	2/2	0/6	1/2	1/2
Mild	0/2	2/6	1/2	0/2
Moderate	0/2	1/6	0/2	0/2
Severe	0/2	2/6	0/2	0/2
Total	2/2	5/6	2/2	1/2
Granulomata				
Minimal	0/2	0/6	0/2	0/2
Mild	0/2	3/6	1/2	1/2
Moderate	0/2	2/6	0/2	1/2
Total	0/2	5/6	1/2	2/2

DAY	Saline	ICI 182,780	Proluton Depot	Delestrogen
Necrosis				
Minimal	0/2	0/6	0/2	0/2
Mild	0/2	1/6	0/2	0/2
Moderate	0/2	2/6	0/2	0/2
Severe	0/2	1/6	0/2	0/2
Total	0/2	4/6	0/2	0/2
Myositis				
Minimal	0/2	0/6	0/2	1/2
Mild	0/2	2/6	0/2	0/2
Moderate	0/2	2/6	0/2	0/2
Severe	0/2	1/6	0/2	0/2
Total	0/2	5/6	0/2	1/2
Granulomata				
Minimal	0/2	1/6	1/2	2/2
Mild	0/2	4/6	1/2	0/2
Moderate	0/2	1/6	0/2	0/2
Total	0/2	6/6	2/2	2/2

	Saline	ICI 182,780	Proluton Depot	Delestrogen
Necrosis				
Minimal	0/2	0/6	0/2	0/2
Mild	0/2	0/6	0/2	0/2
Moderate	0/2	2/6	0/2	0/2
Severe	0/2	0/6	0/2	0/2
Total	0/2	2/6	0/2	0/2
Myositis				
Minimal	0/2	0/6	0/2	2/2
Mild	0/2	0/6	0/2	0/2
Moderate	0/2	2/6	0/2	0/2
Severe	0/2	4/6	0/2	0/2
Total	0/2	6/6	0/2	2/2
Granulomata				
Minimal	0/2	0/6	2/2	0/2
Mild	0/2	6/6	0/2	1/2
Moderate	0/2	0/6	0/2	0/2
Total	0/2	6/6	2/2	1/2

	Saline	ICI 182,780	Proluton Depot	Delestrogen
Necrosis				
Minimal	0/2	0/6	0/2	0/2
Mild	0/2	0/6	0/2	0/2
Moderate	0/2	0/6	0/2	0/2
Severe	0/2	0/6	0/2	0/2
Total	0/2	0/6	0/2	0/2
Myositis				
Minimal	1/2	2/6	0/2	1/2
Mild	0/2	0/6	0/2	0/2
Moderate	0/2	1/6	0/2	0/2
Severe	0/2	0/6	0/2	0/2
Total	1/2	3/6	0/2	1/2
Granulomata				
Minimal	0/2	2/6	1/2	2/2
Mild	0/2	1/6	0/2	0/2
Moderate	0/2	0/6	0/2	0/2
Total	0/2	3/6	1/2	2/2

- Areas of necrosis were seen only in sites where the formulation containing ICI 182,780 was injected.
- The incidence and severity of necrosis was reduced in the period from 10 to 30 days after injection, and necrosis was not seen 50 days after injection.
- Chronic myositis was seen after all treatments (but not at all sites), 10 days after injection. However, the severity was greatest in sites injected with ICI 182,780. At these sites, incidence and severity remained similar from 10 to 30 days after injection, but were reduced at 50 days.
- Proluton Depot sites showed minimal and mild myositis at 10 days, but none thereafter. Occasional sites injected with saline or Delestrogen showed minimal myositis at 20, 30 and 50 days.
- The Sponsor reports that chronic myositis was characterized by muscle fiber degeneration, with necrosis, regeneration, fibrosis, and inflammatory cell infiltration (mainly mononuclear). Areas of necrosis free of these other changes listed were classified separately as necrosis.
- Granulomata were observed in most sites injected with ICI 182,780, Proluton Depot, and Delestrogen. However, the incidence of granulomata was highest in sites injected with the ICI 182,780 formulation. The Sponsor reports that granulomata appeared as spaces surrounded by layers of inflammatory cells, mainly mononuclear. The presence of spaces, indicative of foreign material, was necessary for a diagnosis of granuloma. If inflammatory infiltration into adjacent tissue was extensive, concurrent diagnoses of granulomata and myositis were made.

Summary of individual study findings:
Intramuscular administration of ICI 182,780 and physiological saline to rabbits resulted in the elevation in activity of creatine kinase. Histopathological findings show muscle necrosis, myositis, and granulomata. Muscle necrosis was clearly related to injection of the formulation containing ICI 182,780. This change was still present in 2/6 animals after 30 days, but had disappeared after 50 days. The severity of myositis, an anticipated sequel to intramuscular injection, was markedly increased above control levels only at sites where the formulation containing ICI 182,780 had been administered. Incidence and severity of myositis at these sites were essentially similar at 10, 20 and 30 days, but had reduced at 50 days. Similarly, the incidence of granulomata was highest in sites injected with the ICI 182,780 formulation.

The formulation containing ICI 182,780 caused a greater degree of muscle necrosis, myositis, and granulomata compared to physiological saline or the reference compounds (Proluton Depot and Delestrogen) tested. The incidence and severity of muscle necrosis was reduced in the period from 10 to 30 days after injection, and necrosis was not seen 50 days after injection.

Study title: ICI 182,780 ASSESSMENT OF ANTIGENICITY IN THE GUINEA-PIG (ACTIVE ANAPHYLAXIS AND PCA TESTS)

Key study findings:

- Administration of ICI 182,780 at subcutaneous or intramuscular doses of 5 and 50 mg/kg produced no signs of active systemic anaphylaxis in guinea-pigs following intravenous challenge with 30 mg/kg ICI 182,780.
- There was no indication of the presence of IgG antibody in the PCA test using the sera of guinea-pigs sensitized with ICI 182,780.
- Under the conditions of this assay, ICI 182,780 had no antigenic potential.

Study no: TDG/189
Volume #, and page #: \N_000\2001-03-28\pharmtox\tox\Special\tdg/189
Conducting laboratory and location: [redacted]

Date of study initiation: January 15, 1997
GLP compliance: Yes
QA reports: yes (x) no ( ):
Drug, batch #: Short acting (SA): P/1203/40; Long acting (LA): P/1203/40
Formulation/vehicle:

Ingredients	SA formulation % w/v	Ingredients	LA formulation %w/v
ICI 182,780	2	ICI 182,780	5
Pluronic F127	1.0	Ethanol	10
Ethanol	10.0	Benzyl benzoate	10.0
Water	8.0	Benzyl Alcohol	15
Propylene Glycol	to 100%	Castor Oil	to 100%
Batch Reference Number	P/1203/40	Batch Reference Number	P\1203/36

Methods: Male Dunkin-Hartley guinea-pigs

Sensitization treatment:

Group	Treatment	Route	Dose (mg/kg)	Frequency	Animal numbers
1	0.9% w/v saline + FA	sc	-		1 to 15
2	Placebo (LA) + FA	sc	-	Once weekly in weeks	16 to 25
3	ICI 182,780 (LA) +FA	sc	50	1,3 and 5	26 to 30
4	ICI 182,780 (LA)	sc	5		31 to 35
5	ICI 182,780 (LA)	sc	50		36 to 40
6	ICI 182,780 (LA)	im	5	Once weekly for 4 weeks	41 to 45
7	ICI 182,780 (LA)	im	50		46 to 50
8	HSA	sc	5	Twice (week 3 and week 5)	51 to 55

FA Freund's adjuvant
LA long acting
HSA Human serum albumin

Challenge treatment: Passive Cutaneous Anaphylaxi

Group	Treatment	Route	Dose (mg/kg)	Frequency	Animal numbers
1	0.9% w/v saline + FA	sc	-		1 to 15
2	Placebo (LA) + FA	sc	-	Once weekly in weeks	16 to 25
3	ICI 182,780 (LA) +FA	sc	50	1,3 and 5	26 to 30
4	ICI 182,780 (LA)	sc	5		31 to 35
5	ICI 182,780 (LA)	sc	50		36 to 40
6	ICI 182,780 (LA)	im	5	Once weekly for 4 weeks	41 to 45
7	ICI 182,780 (LA)	im	50		46 to 50
8	HSA	sc	5	Twice (week 3 and week 5)	51 to 55

FA Freund's adjuvant
LA long acting
HSA Human serum albumin

Challenge treatment: Active anaphylactic reaction

Table 3 Challenge treatment

Recipient group	Intravenous challenge	Dose (mg/kg)	Animal numbers
1a	ICI 182,780 (SA)	30	1 to 5
1b	Placebo (SA)	-	6 to 10
1c	HSA	10	11 to 15
2a	ICI 182,780 (SA)	30	16 to 20
2b	Placebo (SA)	-	21 to 25
3 - 7	ICI 182,780 (SA)	30	26 to 50
8	HSA	10	51 to 55

SA short acting

Observations and times:

Passive Cutaneous Anaphylaxis:

Thirty minutes after challenge, each IV injection site was examined and scored based on the degree of blue observed in the area. The site receiving the most dilute serum that showed clear signs of blue (> 5 mm diameter blue spot) in two guinea-pigs was taken as the titer of that serum sample.

Active anaphylactic reaction

The animals were observed continuously for 30 minutes, then intermittently for 3 hours and signs of active anaphylaxis were recorded.

Results:

Active systemic anaphylaxis test:

- Animals sensitized with ICI 182,780 by the intramuscular (IM) or subcutaneous (SC) routes at doses of 5 and 50 mg/kg showed no signs of active anaphylaxis when challenged intravenously (IV) with 30 mg/kg ICI 182,780.
- Animals sensitized with 5 mg/kg HAS SC and challenged with 10 mg/kg HSA IV showed moderate to severe signs of anaphylaxis with 3/5 animals dying. The animals that died had enlarged pink lungs post mortem.
- Animals sensitized with placebo (LA) SC exhibited a marked vomiting motion approximately 2 minutes post-ICI 182,780 IV challenge. Since the reaction was not seen in other animals dosed with ICI 182,780, the sponsor did not consider it an indication of active anaphylaxis.

Passive cutaneous anaphylactic reaction-IgG_{1a} antibody titer:

- None of the sera of animals sensitized with ICI 182,780 either IM or SC, at doses of 5 and 50 mg/kg, produced a positive PCA response in this test.
- The sera of animals sensitized to 5 mg/kg HSA (positive control) produced a positive PCA response with a mean IgG_{1a} antibody titer of >55706 when challenged with HSA.

Summary of individual study findings:

Under the conditions of these assays, ICI 182,780 did not show antigenic potential in a study of active anaphylaxis and passive cutaneous anaphylaxis in the guinea pig.

Study title: ICI 182,780: ASSESSMENT OF ANTIGENICITY IN THE MOUSE (PASSIVE CUTANEOUS ANAPHYLAXIS TEST IN THE RAT)

Key study findings:

- Animals dosed with ICI 182,780 (5 and 50 mg/kg; IP and IM) produced no detectable IgE antibodies in the passive cutaneous anaphylaxis (PCA) test
- Under the conditions of this assay, ICI 182,78O showed no antigenic potential in the PCA test in the rat.

Study no: TDM/940
Volume #, and page #: \N_000\2001-03-28\pharmtox\tox\Special\tdm/940
Conducting laboratory and location: [redacted]

Date of study initiation: January 20, 1997
GLP compliance: Yes
QA reports: yes (x) no ( ):
Drug, batch #: Short acting (SA): P/1203/40; Long acting (LA): P/1203/40
Formulation/vehicle:

Ingredients	SA formulation % w/v	Ingredients	LA formulation %w/v
ICI 182,780	2	ICI 182,780	5
Pluronic F127	1.0	Ethanol	10
Ethanol	10.0	Benzyl benzoate	10.0
Water	8.0	Benzyl Alcohol	15
Propylene Glycol	to 100%	Castor Oil	to 100%
Batch Reference Number	P/1203/40	Batch Reference Number	P\1203/36

Methods:
In this study, ICR CD-1mice (n=40; 27-33 g and ~6 weeks of age) were exposed to ICI 182,780 by the intramuscular (IM) or intraperitoneal (IP) route at 5 and 50 mg/kg. After the sensitization period, the serum of each mouse was harvested and analyzed for IgE antibodies to the sensitizing article using the PCA test in the Wistar rat (n=21; 211-238 g and ~ 8 weeks of age).

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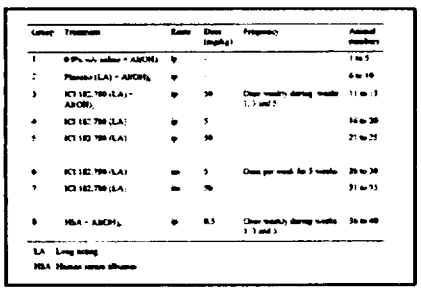
Dosing:



Group	Treatment	Route	Dose (mg/kg)	Frequency	Animal numbers
1	0.9% w/v saline + $Al(OH)_3$	ip	-		1 to 5
2	Placebo (LA) + $Al(OH)_3$	ip			6 to 10
3	ICI 182,780 (LA) + $Al(OH)_3$	ip	50	Once weekly during weeks 1, 3 and 5	11 to 15
4	ICI 182,780 (LA)	ip	5		16 to 20
5	ICI 182,780 (LA)	ip	50		21 to 25
6	ICI 182,780 (LA)	im	5	Once per week for 5 weeks	26 to 30
7	ICI 182,780 (LA)	im	50		31 to 35
8	HSA + $Al(OH)_3$	ip	0.5	Once weekly during weeks 1, 3 and 5	36 to 40

LA Long acting
HSA Human serum albumin

Sensitisation group sera	PCA Antigen challenge	Dose (mg/kg)
1	ICI 182,780 (SA)	50
	Placebo (SA)	-
	HSA	10
2	ICI 182,780 (SA)	50
	Placebo (SA)	-
3 to 7	ICI 182,780 (SA)	50
8	HSA	10

SA Short acting
HSA Human serum albumin

Observations and times:
Thirty minutes after challenge, each IV injection site was examined and scored based on the degree of blue observed in the area. The site receiving the most dilute serum that showed clear signs of blueing (> 5 mm diameter blue spot) in two rats was taken as the titer of that serum sample.

Results:

- ICI 182,780, administered either IP or IM at 5 and 50 mg/kg produced no detectable levels of IgE antibodies in this test.
- Sera from 4/5 mice sensitized with 0.5 mg/kg HSA exhibited detectable levels of IgE antibodies when challenged with HSA (10 mg/kg). The mean titer in this groups was of 8.0.

Summary of individual study findings:
Under the conditions of this assay, ICI 182,780 had no antigenic potential.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

In NDA 21-344, non-clinical toxicity studies were submitted to support the use of fulvestrant (ICI 182,780), by intramuscular administration (IM), in the treatment of locally advanced and metastatic breast cancer in post-menopausal women. These studies include acute (single dose) toxicity studies in rodents and multiple dose toxicity studies in rats and dogs of up to 6 and 12 months duration, respectively. Studies to determine the genotoxic, carcinogenic, and antigenic potential of fulvestrant as well as to assess intramuscular tolerance were also submitted.

Fulvestrant is a mixture of 2 diastereoisomers, ICI 182,780 Sulphoxide A (ICI 208,926) and ICI 182,780 Sulphoxide B (ICI 208,927) in the ratio of approximately 45 to 55. Both diasteroisomers are pharmacologically active and of similar potency. All doses used in toxicology studies were calculated on the quantity of active moiety administered. Different batches of fulvestrant, generally of a purity > 98.5%, were used in the toxicology studies. Also, 2 formulations were used, a short-acting (SA) intramuscular formulation of fulvestrant and a long-acting (LA) intramuscular formulation, the proposed commercial formulation.

Pharmacology studies show that fulvestrant binds to estrogen receptors in a competitive manner, with a high affinity comparable with that of estradiol. Also, the drug's mode of action leads to downregulation of estrogen receptor protein as shown *in vitro* when fulvestrant reduced ER protein levels after a 48-hour incubation with MCF-7:WS8, T47D:A18, and MCF-7:2A cells. Fulvestrant is a reversible inhibitor of the growth of estrogen-sensitive human breast cancer cells and tamoxifen-resistant cells *in vitro*. Fulvestrant blocks the tropic actions of endogenous and exogenous estrogens in rodents and monkeys, and of tamoxifen in the rat. In a series of *in vivo* xenograft studies, fuvestrant delayed the establishment of tumors from xenografts of human breast cancer MCF-7 cells in nude mice, inhibits the growth of established estrogen-sensitive xenografts and-inhibits the growth of tamoxifen-resistant breast tumors *in vivo*. It is important to note that *in vitro*, cultured MCF-7/LCC9 fulvestrant-resistant cells are also tamoxifen resistant. *In vivo*, fulvestrant-resistant tumors transplanted into castrated mice, showed cross resistance to tamoxifen. Slower tumor growth was shown with tamoxifen treatment in 6/25 mice.

Fulvestrant, administered IM, does not produce ataxia, has no CNS stimulant activity, has no pro-or anti-convulsant potential, and does not modify any other observable indices of CNS function. Also, the drug has no cardiovascular effects, as measured in anesthetized dogs and in a sheep Purkinje fiber preparation. Further, fulvestrant does not affect respiratory or renal function. Gastro-intestinal motiliy does not appear to be significantly affected although gastric emptying was reduced after administration of fulvestrant (5 mg/kg SC) in mice.

Exposure to fulvestrant in rats and dogs were compared to drug exposure in humans. PK values were obtained from the rat 6-month study (TPR/2042 -10 mg/rat/15 days dose group), 12-month dog study (TFD/913-40 mg/kg/28 days dose group), and the dose administered clinically to women (Study IL0020-250 mg/month).

	Rat (♂)	Rat (♀) [A]	Dog (both)	Human (♀) [B]
	10 mg/rat/15 days (~36 mg/kg/15 days)	10 mg/rat/15 days (~67 mg/kg/ 15days)	40 mg/kg/28 d	250 mg/montly
	216 mg/m^2/15 days	402 mg/m^2/15 days	800 mg/m^2/28 days	185 mg/m^2/month[C]
	432 mg/m^2/30 days	804 mg/m^2/30 days	800 mg/m^2/28 days	185 mg/m^2/month[C]
Dose ratio	2	4	4	1
AUC (ng.h/ml)	46,656	92,688	36,000	8887
AUC ratio	5.2	10.4	4.1	1
C_{max} (ng/ml)	105	372	88	9.7
C_{max} ratio	10.8	38	9.1	1

[A] Rats were administered fulvestrant on a 1 dose/15 days regimen and the pharmacokinetic parameters used were calculated over the penultimate dose interval (highest observed values). For comparison with dog and human data, the $AUC_{(0\text{-}15\ days)}$ was multiplied by 2 to estimate the 30 day exposure.

[B] Limited accumulation (x2.3) was demonstrated in this study; therefore, the $AUC_{(0\text{-}28\ days)}$ value obtained after the first dose was multiplied by a factor of 2.3 to represent the 0 to 28 day exposure at steady state. The exposure ratios ranged from 4 to 10 for AUC and 9 to 38 for C_{max}.

[C] Estimated dose for a 50 kg human.

The high doses used in the long-term studies in rats (10 mg/rat/15d for 6 months) and dogs (40 mg/kg/28d for 12 months), on a body surface area (refer to table) were approximately 4-fold higher than the proposed clinical dose of 250 mg/month. Drug exposure (AUC) ranged from 4-10 fold and C_{max} ranged from 9-38 fold higher in animals than the values attained in clinical testing.

Fulvestrant was investigated in rat and dog using a metabolically stable radiolabeled form to characterize distribution, elimination, and metabolism. Fulvestrant was well absorbed and widely distributed following IM administration. Fulvestrant is eliminated almost entirely in feces in rats and dogs. Metabolism was qualitatively similar in rats, dogs, and human.

In all the intramuscularly dosed toxicology studies, pharmacological effects upon the reproductive tract and other organs sensitive to hormones were observed. These effects are expected given fulvestrant's mechanism of action. In rats, following the administration of fulvestrant at doses of up to 10 mg/kg/day for one month or up to 10 mg/rat/15 days for 6 months, no other systemic toxicity was observed. In dogs administration of fulvestrant at up to 4 mg/kg/day for one month, 30 mg/kg/28 days for 6 months or 40 mg/kg/28 days for 12 months produced no other toxicologically important effects.

Pharmacological effects were observed in the female and male reproductive tract. In the female rat, atrophy of the uterus, cervix, and vagina with a loss of normal cyclical estrous activity was observed. In the ovary increased late stage and cystic Graafian follicles, loss of mature corpora lutea, and reduced vacuolation of the interstitial cells were also observed. There was some evidence of reversibility of these ovarian changes but not complete recovery at the end of the period studied. In dogs, atrophy of the uterus, cervix, and vagina has been seen after long-term dosing. Ovarian changes seen have included the development of multiple Graafian follicles, increased medium sized Graafian follicles, and a reduction in the number of active or regressing corpora lutea. An absence of clinical signs of estrous activity has also been recorded in the 12-month study with evidence of reversibility. Reversible elevations in plasma estradiol in females given fulvestrant have also been observed.

In male rats after 6 months dosing, a loss of spermatozoa from the seminiferous tubules with an accompanying dilatation, seminiferous tubular atrophy, and some associated degenerative changes in the epididymides were seen. Changes in the testes and epididymides were not recovered by the end of the recovery period. In male dogs administered fulvestrant for 1-month, Leydig cell hyperplasia was observed. However, no Leydig cell hyperplasia was seen after 6- or 12-months dosing. In the 12-month study, reversible elevations in plasma testosterone in males were seen but were not accompanied by any histological changes in the testes.

In female rats, fulvestrant (≥0.06 mg/m^2/day; ~ 100-fold lower than the human recommended dose based on body surface area; BSA) administered for 2 weeks prior to mating, through mating to day 7 of gestation, caused a reduction in female fertility and embryonic survival. No adverse effects on female fertility and embryonic survival were evident in female animals dosed at 0.006 mg/m^2/day (~ 1000-fold lower than the human recommended dose based on BSA). Restoration of female fertility to values similar to controls was evident following a 29-day withdrawal period after dosing at 12 mg/m^2/day (~ twice the human recommended dose). Further, a dose of 12 mg/m^2/day fulvestrant administered between day 7 and days 14 to 20 of gestation to rats (period of organogenesis) resulted in maternal vaginal bleeding, and delay and prolongation of parturition. In rabbits administered fulvestrant at levels of ≥3 mg/m^2/day from days 7 to 19 of pregnancy, there was an increase incidence of post-implantation loss.

Fulvestrant has been shown to cross the placenta following single intramuscular doses of 6.0 mg/m^2 in rats and 3 mg/m^2 in rabbits resulting in fetal tissue drug concentrations 2 hours after dosing of 76 and 97% compared to maternal plasma, respectively. Fulvestrant caused an increased incidence of fetal abnormalities in rats (tarsal flexure of the hindpaw at 12 mg/m^2/day IM; ~ twice the human recommended dose) and non-ossification of the odontoid and ventral tubercle of the first cervical vertebra at doses ≥ 0.6 mg/m^2/day (IM; ~ 10-fold lower than the human recommended dose) when administered during the period of organogenesis. Rabbits failed to maintain pregnancy when dosed with 12 mg/m^2/day fulvestrant (IM; twice the human recommended dose) during the period of organogenesis. Further, in rabbits dosed at 3 mg/m^2/day (half the human recommended dose), increases in placental weight and post-implantation loss were observed. Fulvestrant also caused an increased incidence of fetal abnormalities in rabbits (backwards displacement of the pelvic girdle, extra 13th ribs, and 27 pre-sacral vertebrae at 3 mg/m^2/day IM; half the human recommended dose) when administered during the period of organogenesis. This study in rabbits was considered inadequate to fully define possible adverse effects on fetal development due to the lack of maternal toxicity at the highest dose (3 mg/m^2/d) and an incomplete fetal assessment. Lastly, fulvestrant is found in rat milk at levels significantly higher than those in rat plasma (12-fold after administration of 12 mg/m^2 which is ~ twice the human recommended dose). The maximal drug exposure in pups from fulvestrant -treated lactating dams was estimated as 10.3% of the administered dose.

Fulvestrant showed no antigenic potential in a study of active anaphylaxis and passive cutaneous anaphylaxis in the guinea pig, and an assessment of antigenicity in the mouse, incorporating a passive cutaneous anaphylaxis test in the rat. Fulvestrant itself was not an irritant to rabbit skin, but the vehicle in the SA formulation was irritating. Fulvestrant showed no potential to cause contact sensitization.

Fulvestrant was not mutagenic or clastogenic in *in vitro* tests (Ames test for bacterial mutagenicity, L5178Y $TK^{+/-}$ mouse lymphoma mutation assay, chromosome aberrations in human lymphocytes) or *in vivo* micronucleus test in rat.

A two-year carcinogenesis study was conducted in female and male rats, at IM doses of 15 mg/kg/30 days, 10 mg/rat/30 days and 10 mg/rat/15 days fulvestrant. These doses correspond to approximately 1-, 3-, and 5-fold (in females) and 1.3-, 1.3-, and 1.6-fold (in males) the systemic exposure [$AUC_{0\text{-}30\ days}$] achieved in woman receiving the recommended dose of 250 mg/month). An increased incidence of benign ovarian granulosa cell tumors and testicular Leydig cell tumors was evident, in females dosed at 10 mg/rat/15 days and males dosed at 15 mg/rat/30 days, respectively. Induction of such tumors is consistent with the pharmacology-related endocrine feedback alterations in gonadotropin levels caused by anti-estrogen.

General Toxicology Issues:

The Sponsor's description of the mechanism of action of fulvestrant is supported by the data submitted. However, it is noteworthy that both *in vitro* (Brunner et al., 1997) and *in vivo* (Osborne et al. 1995), it has been shown that tumors which eventually develop resistance to fulvestrant will not subsequently respond to tamoxifen. Thus, a treatment sequence in which fulvestrant precedes tamoxifen may not be indicated. This information should be included in the label and has repercussions for indications in which patients may be treated first with fulvestrant.

In meeting to discuss the carcinogenic potential of Fulvestrant (December 4, 2001), the Executive Carcinogenicity Assessment Committee noted that while fulvestrant appears to have a negative profile for genotoxicity potential on a standard battery of tests, the Sponsor did not perform the defining studies to determine fulvestrant is not genotoxic. The Committee recommended that the Sponsor be asked to perform ^{32}P post-labeling study to determine if fulvestrant and/or its' metabolites may form adducts with cellular DNA. We agree that a ^{32}P post-labeling study will be valuable in assessing possible genotoxic potential of Fulvestrant. However, given that the current indication for Fulvestrant is [redacted] the request for this study maybe postponed. If the indication of Fulvestrant was to change to include any other population, the Sponsor is strongly recommended to conduct a ^{32}P post-labeling study to ensure that Fulvestrant is non-genotoxic.

Recommendations:

Recommendation on Approvability: The non-clinical studies submitted in NDA 21-344 adequately support the use of FASLODEX, by the intramuscular route, for the treatment of [redacted]

Draft

Labeling with basis for findings: Please refer to Appendix B

X. APPENDIX/ATTACHMENTS:

Appendix A

Addendum to review: CAC report –Meeting minutes

Executive CAC
Date of Meeting; December 4, 2001
Rat Carcinogenicity Study

Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Timothy McGovern, Ph.D., HFD-170, Alternate Member
David Morse, Ph.D. Supervisory Pharmacologist, HFD-150
Lilliam Rosario, Ph.D., Pharm-Tox Reviewer, HFD-150

Author of Draft: Lilliam Rosario, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA # 21,344
Drug Name: Faslodex (Fulvestrant; ICI 182,780
Sponsor: Astra Zeneca Pharmaceuticals

Mouse Carcinogenicity Study: Not conducted

Background

This 2-year carcinogenicity study in rats was submitted to NDA 21,344. This NDA proposes the use of ICI 182,780 (fulvestrant) for [redacted] DRAFT

The recommended dose of Faslodex is 250 mg to be administered intramuscularly (IM) monthly.

The Sponsor indicates fulvestrant is an antiestrogenic agent, which acts by downregulation of the estrogen receptor (ER). Fulvestrant binds ER in a competitive manner with a high affinity comparable to estradiol. Further, the Sponsor suggests that Fulvestrant is a non-agonist antiestrogen which blocks the uterotrophic action of estradiol in mice, rats and monkeys without itself having any partial agonist estrogen- like activity.

Genotoxicity
The mutagenic and clastogenic potential of ICI 182,780 has been studied in bacterial mutation assays in strains of *Salmonella typhimurium* and *Escherischia coli*, an *in vitro* cytogenetics assay in cultured human lymphocytes, a mouse lymphoma mutation assay, and an *in vivo* rat micronucleus test. ICI 182,780 has shown no evidence of genotoxic/clastogenic potential in this battery of tests.

Rat Carcinogenicity Study:

Study Design:

- Dose concurrence was obtained on July 28, 1998.
- The Sponsor selected the high dose level to represent the maximum possible dose by the IM route (maximum feasible dose).
- There were 6 groups (50 sex/group); Sprague Dawley rats [redacted]

Control-1 (C1):	Vehicle/15 days
Control-2 (C2):	Vehicle/30 days
Control-3 (C3):	Saline/15 days
Low Dose (LD):	15 mg/kg/30 days
Middle Dose (MD):	10 mg/rat/30 days
High Dose- (HD):	10 mg/rat/15 days

The following table shows the ~ actual dose (mg/kg) administered to Groups V (10 mg/rat/30days) and Group VI (10 mg/rat/15 days). For comparison purposes, these values have also been normalized for frequency of administration (from every 15 days to every 30 days)

		Group V 10 mg/rat/30 days		Group VI 10 mg/rat/15 days		
Sex	**Week**	**Body weight (g)**	**mg/kg/ 30 days**	**Body weight (g)**	**mg/kg/ 15 days**	**mg/kg/30 days**
Male	1	262.9	38	257.8	39	78
	96	793	13	781.5	13	26
Female	1	184.7	54	185.7	54	108
	96	580.3	17	574.4	17	34

Statistical Methods:

- All tests for tumor incidence were one-sided looking for an increase in response/incidence.
- The Haseman (1983) principle of statistical significance was adopted; a rare tumor (<1% spontaneous incidence) will be deemed statistically significant if $p<0.05$, and a common tumor shall be deemed significant if $p<0.01$.
- The statistical comparisons of interest were implemented using Peto's survival-adjusted trend test.
- Note that the significance values used by the Sponsor are in accordance with those employed by CDER when only a single carcinogenicity study is conducted. The probability levels for determining significance of tumor incidence has not been adjusted for multiple statistical comparisons as would be appropriate to maintain a constant error rate over multiple studies.

RAT TUMOR FINDINGS:

It appears that the IM administration of ICI 182,780 (fulvestrant) for 24 months increased the incidence of ovarian granulosa cell tumors and testicular Leydig cell tumors in female and male rats, respectively.

Ovaries:

- A 14% increase in the incidence of a rare ovarian granulosa cell tumors in the high dose female animals (7/50 rats at 10 mg/rat/15d; p=0.01887).
- Spontaneous incidence of granulosa cell tumors for this strain of rat is 0.06% (n=1729) (Giknis and Clifford, 2001 [redacted]).
- The conducting laboratory reports background instances varying from 0/120 to 1/120 (0.2%).
- Another study (n=4493) with the same strain and source reports 0.3% (Gregson and Abbott, 1984).

Testes:

- There was increase incidence (2-12%) of interstitial Leydig cell tumors (adenomas-common) in drug-treated animals.
- These tumors were present at a low incidence (4%) in the saline control group and absent in the vehicle control groups. The incidence in the high dose group was similar to controls (2%) while slightly increased (8-12%) in the two low dose groups.
- In Group 4 (15 mg/kg/30 days), interstitial cell tumors were increased significantly (p=0.01922)
- Spontaneous incidence for this strain of rat is 2.35% [redacted]

The reviewer proposed 3 questions for the EXEC CAC committee:

1. Are the survival rates observed in control and drug-treated groups adequate to determine the carcinogenic potential of ICI 182,780 (fulvestrant)?
   - Even though survival rates appear lower than expected for control males, the Committee agreed that the rate of mortality is adequate to determine the carcinogenic potential of ICI 182,780.

2. Does the Committee agree that administration of ICI 182,780 increases the incidence of granulosa cell tumors and interstitial Leydig cell tumors?
   The Committee
   - agreed that administration of ICI 182,780 increases the incidence of both granulosa cell tumors and interstitial Leydig cell tumors, in females and males, respectively.
   - recommended the statistical evaluation of these results take into consideration that only one carcinogenicity study was submitted.
   - recommended to carefully examine the pharmacological data submitted to support the claim that ICI 182,780 is a "non-agonist" antiestrogen. The increase incidence of interstitial Leydig cell tumors in males may suggest a drug-induced estrogenic effect.
   - noted that while the carcinogenicity study was acceptable, the Sponsor did not perform the defining studies to determine the compound is non-genotoxic. The Committee suggested that a ^{32}P post labeling study to determine whether ICI 182,780 induces DNA adduct may verify that indeed the drug is non-genotoxic.

3. Does the Committee agree that these findings should be included in the product labeling for ICI 182,780 (fulvestrant)?
   The Committee agreed that the increase incidence of both granulosa cell tumors and interstitial Leydig cell tumors, in females and males, respectively be included in the product labeling for ICI 182,780 (fulvestrant).

Additional comments from the Committee:

The Committee

- pointed out that, unlike tamoxifen, the incidence of liver tumors was not changed in ICI 182,780-treated rats.
- suggested that, since male rats in the high dose group lost weight, the mid-dose male group should be used for statistical comparisons.

Executive CAC Recommendations and Conclusions:

1) Fulvestrant increases the incidence of ovarian granulosa cell tumors in female rats, and the incidence of interstitial Leydig cell tumors in male rats.
2) The increase incidence of granulosa and Leydig cell tumors should be included in the product labeling for fulvestrant.
3) The Committee recommended that the Sponsor be asked to perform ^{32}P post-labeling study to determine if fulvestrant and/or its' metabolites may form adducts with cellular DNA.

/S/

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

cc:\

/Division File, HFD-150
/David Morse, Ph.D. Supervisory Pharmacologist, HFD-150
/Lilliam Rosario, Ph.D., Pharm-Tox Reviewer, HFD-150
/Amy Baird, HFD-150
/Adele Seifried, HFD-024

7 pages redacted from this section of the approval package consisted of draft labeling

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

/s/

Lilliam Rosario
1/10/02 05:21:21 PM
PHARMACOLOGIST

David Morse
1/17/02 02:50:18 PM
PHARMACOLOGIST